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APPENDIX IV-b

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SUBJECT:

Progress Report -
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Research Contract AID/ta-C-1286
Clinical Assay of High Protein Foods

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Human testing of both food blends and the products of agricultural research is a necessity if appropriate recommendations for the use of these foods in human nutrition are to be made. This is especially so when the foods are destined to be consumed by infants and children, the group most at risk for developing malnutrition because of their higher nutrient requirements and lower digestive capacities. Such studies in the past have confirmed the high protein quality assayed in rats of such blends as Thriposha. On the other hand a study of three corn soy blends, all of apparently equal nutritional value based on animal studies, showed that only one was suitable for infants and children.

With the support of Contract ta-C-1286, the Nutrition Research Institute in Lima, Peru has continued work in the "Clinical Assay of High Protein Foods". This involves short-term balance studies to assess the tolerance, the digestibility of protein, fat and total energy content, and nitrogen balance. Where appropriate the effects of supplementation with the amino acid that is first-limiting for nitrogen utilization is carried out. Nitrogen balance, growth during more prolonged consumption and plasma amino acid values are used to assess the benefit of this supplementation.

This report summarizes work carried out from 1/1/80 to 12/31/80. Most of this work has previously been detailed in progress reports to AID during the year. These more detailed descriptions are found as appendices.

I. RESEARCH CARRIED OUT DIRECTLY UNDER CONTRACT ta-C-1286

A. Sorghum

Our original studies in nine children with two low protein-low lysine sorghums and two high protein-high lysine varieties as whole grain flours were detailed in a previous summary progress report. The protein quality and digestibility of all four sorghums was extremely poor. The conclusions were somewhat tentative, however, because an insufficient number of studies had been completed with one low protein-low lysine (954063) and one high protein-high lysine (IS 11758) variety. Additional whole grain flour was obtained and four additional infants age 8-30 months were entered in the study. Because of previously poor results the sorghum study diets provided 8% of energy as protein. Control diets (based on casein) provided the usual 6.4% of energy as protein. Carrying out these comparisons with study diets providing more protein than the control diet tended to underestimate the difference between the two diets but was felt necessary because of the poor protein quality of sorghum.

Nitrogen retention from the low protein-low lysine sorghum flour was 39% of the preceding casein control value and was associated with a slowing of weight gain or, in some cases, weight loss. Some of the poor nitrogen retention could be explained by poor digestibility but not all. Nitrogen retention from the high protein-high lysine sorghum flour was inferior, only 25% of the preceding casein control. Much, but not all, of the difference again owed to poor digestibility. Weight gain was similarly poor. An average of 20% of dietary energy was lost in the stool during all sorghum dietary periods.

The relative nutritional value of whole grain sorghum flour was compared to the other major staples.

Comparison of Various Results of Sorghum Studies
With Corresponding Data from Other Staple Foods

	Apparent N Balance		Stool Weight		Stool Energy
	Abs %	Ret %	Wet g/d	Dry g/d	Kcal/d
Sorghum[1]	46	14	223	38.9	183
Wheat[2]	81	20	95	13.3	60
Rice[3]	66	26	67	11.6	58
Potato[4]	66	34	165	20.3	78
Maize[5]	73	27	133	28.6	117
Casein[6]	81	38	94	16.0	69

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1 Mean values of all 26 sorghum periods

2 Studies of all-wheat pasta, J. Nutr. 109:1290, 1979.

3 Data from studies of two varieties of rice, J. Nutr. 108:1740, 1978.

4 Data from 9 balance studies with potato, J. Nutr. 110:1849, 1980.

5 Data from 6 studies with whole kernel normal maize, J. Nutr. 110:1061, 1980.

6 Data from the 11 preceding casein control periods of these studies.

Whole grain sorghum flour is clearly inferior to all. Extensive cooking (boiling for one hour or use of a pressure cooker) did not improve digestibility or nitrogen retention.

The value of "home pounded" and winnowed sorghum has been reported to be superior to whole grain sorghum in young men (1). Our evaluation of a blend containing extrusion cooked pearled sorghum (Thriposha) also suggested that processing could alter digestibility favorably. It is possible that the whole grain sorghum flour used in our studies was not representative of sorghum used in Asia and Africa. In a meeting at AID at which Dr. Lloyd W. Rooney of Texas A&M University reported on sorghum use in Mali and Upper Volta, it was pointed out that much of the sorghum used in these two countries is dehulled. In addition, cooking is often done in alkalai solutions rather than plain water. Plans for further studies of one high protein-high lysine and one low protein-low lysine pearled sorghum were discussed (see Section II, A., page 6).

Whole grain sorghum flour should never be distributed for human consumption by AID, even to those countries in which sorghum is currently eaten, since appropriate home processing is precluded. Whole grain sorghum that has not been milled might be appropriate in those countries in which sorghum is already eaten and processing understood; any of the other traditional staple foods would be preferable, if available. The introduction of sorghum into countries in which it is not already eaten and appropriate processing understood could be disastrous.

A complete report of these sorghum studies was submitted to AID on March 19, 1980 and is found as Appendix 1.

B. Maize

Short-term balance studies were carried out using endosperm and whole kernel maize meals from three different 3-way hybrids developed at Purdue University (normal, opaque-2 and sugary-2 opaque-2). The opaque (o_2) gene results in an increase in lysine and tryptophan contents while decreasing leucine concentration. The double mutant sugary-2 opaque-2 (su_2o_2) has more favorable kernel characteristics and a higher lysine content and PER in rats. In our studies energy digestibility from endosperm meals was seemingly lower than that of whole kernel meals, probably because of the increased amount of whole kernel meals needed to supply the 6.4% protein calories in each diet. Apparent nitrogen retention from whole kernel meals (29.5±7.5% of intake) was significantly higher than that from endosperm meals (20.9±7.5%) and seemed to relate possibly to better energy absorption, a better balance of absorbed amino acids or more available tryptophan. Samples for plasma free amino acids were obtained during the studies and have now been determined and the data analyzed.

Plasma free amino acids were determined before and 3 and 4 hours after the first meal of the 27th day of endosperm or whole kernel meal consumption. The three varieties of endosperm meals had been consumed in six different sequences during consecutive nine-day dietary periods by six subjects. The same protocol was followed for the three corresponding whole kernel meals. The plasma aminogram was expected to reflect the effects of 26 days of maize consumption and eight days of the last variety consumed, particularly the latter. The significantly poorer absorptions of nitrogen from the endosperm meals were reflected in lower fasting molar ratios of total essentials to total amino acids

(TEAA/TAA = 0.192 versus 0.229 for whole kernel). Fasting leucine/TEAA molar ratio was consistently elevated, notably after normal maize. With the exception of one very low (0.067) and one moderately low (0.120) lysine/TEAA ratio, fasting values did not suggest a first-limiting amino acid. Striking postprandial falls singled lysine out as first-limiting in five, possibly six studies, tryptophan in two studies (normal endosperm and $\underline{su}_2\underline{o}_2$ whole kernel) and probably isoleucine in one $\underline{o}_2$ whole kernel study. In two studies ($\underline{su}_2\underline{o}_2$ endosperm and normal whole kernel), lysine and tryptophan seemed equally limiting.

These plasma free amino acid studies suggest that lysine is probably the most common first-limiting factor when normal maize or $\underline{o}_2$ and $\underline{su}_2\underline{o}_2$ are consumed, but that tryptophan and perhaps occasionally isoleucine can also be first-limiting. The excess of leucine, particularly in normal maize, was invariably evident even in fasting plasma. The persistently normal methionine/TEAA ratios confirm the fact that this amino acid is not limiting in maize.

The complete report of these studies was not previously submitted to AID. It is found as Appendix 2 and was published in the Journal of Nutrition 110:1070-1075, 1980.

II. RESEARCH PLANNED OR UNDERWAY

A. Sorghum

Because of the question of whether pearling of sorghum would improve digestibility and nitrogen utilization further studies of sorghum are planned. Dr. Richard Jansen is preparing two samples of sorghum, one high protein-high lysine and one low protein-low lysine.

The samples will be studied in comparative short-term balance studies with a casein control diet. We are in contact with Dr. John D. Axtell concerning the optimal way of preparing the sorghum to be used in these studies. Recently completed studies in their laboratory using porcine pepsin *in vitro* showed that the digestibility of uncooked sorghum (78-100%) was substantially higher than that of cooked sorghum (45-55%) (2). Fermented products gave values of 65-86% compared with unfermented cooked gruels from the same flours (44-56%). Either fermentation or boiling in alkalai may be tried, adapting a traditional recipe to the requirements of diet preparation for the studies. The group at Purdue is continuing to look at other native-processed sorghum foods. As the *in vitro* and animal studies identify promising foods, these should be considered for tests in the Nutrition Research Institute.

B. Cereal-Legume Combinations

Cereal-legume combinations are widely consumed throughout the world. Although legumes represent an important source of protein, many are not easily digested and produce flatulence. In most countries one or more varieties are fed preferentially to children because they appear to be more readily digested. In Peru, "white" beans (Phaseolus vulgaris) and broad beans (Vicia faba) are the types most frequently used.

Studies are underway to determine how well combinations of pasta and beans are able to meet the protein requirements of small children. Because previous studies in our unit demonstrated that a mixture of pasta and casein in which each provided 50% of protein

supported nitrogen balance equal to that found when casein alone provided all protein, a control diet of casein and pasta has been used for comparison with pasta-broad bean and pasta-white bean diets in alternating nine-day dietary sequences. Each pasta-bean diet is preceded and followed by a control period. The order of the pasta-bean diets is reversed in half the children. These sequences are shown below.

Period	1	2	3	4	5
	Control	Pasta White Bean	Control	Pasta Broad Bean	Control
	Control	Pasta Broad Bean	Control	Pasta White Bean	Control

In all diets pasta provides 50% of protein. Either casein or beans completes the protein to provide 6.4% of total calories. In the case of beans the amount of protein provided in the diet has been increased to offset decreased digestibility so that the expected net absorbed protein will equal 50% of total protein intake.

To date six children have completed the study sequence. Nitrogen absorption from pasta-white beans has been 76±4%, from pasta-broad beans 78±6%, from control 82±3%. Corresponding apparent nitrogen retentions have been 31±6%, 28±7 and 30±9%, respectively. Energy losses in stool have been surprisingly low with the pasta bean diets, about 9±4% of intake (control about 7±2%). There have been no significant decreases of serum albumin concentration with any diet. Rates of weight gain have been greater during pasta-bean dietary periods than during control periods although the differences are not significant. We are reluctant to attach great significance to changes of body

weight occurring over such short periods of time. In the past, however, we have demonstrated marked decrements in rates of weight gain when dietary protein intake is inadequate, even during these relatively short periods of time. The excellent rates of weight gain seen with pasta-bean diets are reassuring in that sense.

Three additional children will enter the study to bring the total n to 9. Our preliminary impression is that pasta-bean combinations adequately support short-term nitrogen balance. If this conclusion is borne out when the short-term studies are completed, more prolonged (three month) consumption studies should be undertaken to confirm that these diets are adequate to support growth.

III. ADDITIONAL RESEARCH FACILITATED BY CONTRACT ta-C-1286

A. Nutritional Value of the White Potato for Infants and Small Children

The white potato is the fourth leading world food crop after wheat, rice and maize. In terms of energy produced per unit area of land, the potato exceeds all the cereals and stands only behind sugar cane. Protein yield per unit area is superior to the cereal grains and second only to soybeans. Despite this fact, the potato is popularly thought of only as a source of starch in the diet. In the United States, the potato contributes more than 2% of total dietary protein, compared with the 3% provided by fish and 6% supplied by eggs.

In the Andean region of South America, where the potato originated, approximately 50% of children under five years of age have moderate to severe forms of malnutrition. This prevalence owes primarily to early weaning and the lack of suitable weaning foods,

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both of which combine to initiate the vicious cycle of diarrhea, malabsorption and malnutrition.

1. Short-Term Balance Studies. To determine the potential of the potato as a food for weaned infants, studies were carried out in conjunction with the International Potato Center to evaluate clinical tolerance, overall digestibility and protein quality of increasing amounts of potatoes in the diet of human infants.

The digestibility and protein quality of the white potato were studied in 11 infants, ages 8-35 months, recovering from malnutrition. Seven nine-day dietary periods comprised the study. Digestibility was assessed by metabolic balance during the last six days of dietary periods in which potato provided 25%, 50% or 75% of dietary energy. Casein-based control dietary periods preceded and/or followed each potato dietary period. Fecal wet and dry weights, fecal energy, computed fecal carbohydrate and fecal fat during consumption of the 25% potato diet did not differ from control. Analysis of variance showed that consumption of increasing amounts of potatoes produced a significant linear increase in all parameters of digestibility except fecal fat. Fecal wet weight and energy content during the 75% potato period were more than double control values. Regression analysis of data from potato dietary periods showed fecal wet weight to be an excellent predictor of fecal dry weight ($r=0.904$), energy content ($r=0.785$) and computed fecal carbohydrate ($r=0.770$). Protein quality of potato was assessed with N balance studies during the dietary periods in which potato provided all dietary protein (~5% protein Kcal). Apparent N retention was 78% of that from an isonitrogenous casein

control diet. Decreased apparent N absorption was primarily responsible for the difference; the "biologic value" (percent of absorbed N that was retained) was similar for both diets.

A complete report of these studies was submitted to AID on February 29, 1980 and is found as Appendix 3. These studies were published in the Journal of Nutrition 110:1849-1857, 1980.

2. Prolonged Feeding Studies. Although short-term studies demonstrated that infants and children could consume diets in which 75% of energy and all protein was provided by the white potato, nitrogen balance was inferior to casein control at this level of protein intake. Potato intake was limited by the bulk of the diets and the poor digestibility of carbohydrates. In these more prolonged studies ten children were offered diets containing 50%, 75% and 84.2% of total energy as potato during a period of three months. Casein was added to the 50% and 75% diets to complete 8% of energy as protein (N x 6.25). Acceptability, tolerance, digestibility and growth of the children were analyzed. The 84.2% potato studies were aborted shortly after the beginning because of the excessively large volume. Acceptability and tolerance to the 50% potato diets were excellent but with the 75% potato diets were noted to decrease during the last days of the studies. Six of the eight children who completed the studies showed satisfactory weight gain and catch-up growth while maintaining normal serum albumin concentrations. In one other, weight gain was adequate but catch-up growth was not achieved; in the youngest child weight gain and linear growth were inadequate. Metabolic balances did not show improvement or deterioration of digestibility throughout the study. These studies

suggested that infants can consume up to 50 to 75% of energy and as much as 80% of their nitrogen requirements as potato if the remaining energy and nitrogen is provided by a non-bulky, easily digestible food.

A complete report of these studies was submitted to AID on December 30, 1980 and is found as Appendix 4 and has been submitted to the Journal of Nutrition for publication.

B. Quinua: Comparative Studies of Digestibility and Protein Quality of Quinua Grain and Flour

Quinua (Chenopodium quinoa) is a food native to the Andean region of South America that traditionally, with potatoes and corn, has been an important part of the diets for this population.

It is grown in cold weather and in altitudes that vary from 8,700 to 13,000 feet and for this reason competes little for land area with other food crops. The edible portion is the seed or grain, whose approximate composition is 58% starch, 5% sugar, 12 to 19% protein and 4-5% fat.

Most of the research done on this grain has been oriented to the analysis of its protein quality, leading to the conclusion that quinua could be a source of protein superior to most of the true cereals. Nevertheless there is little information in the human being about its digestibility, a factor that, based on studies with other staple foods, could be limiting in the utilization of nutrients.

In a previous study done with a quinua-oats diet we noticed that the germ of the quinua seed was recovered intact from the stools of the children that consumed the diet. Protein and fat digestibility was also found to be poor with this diet.

The present study was designed to analyze in human beings the digestibility and protein quality of the quinua grain, the form in which it is usually consumed, in comparison to quinua flour.

Six male infants consumed diets in which quinua grain or flour provided 6.4% protein energy. An isonitrogenous casein diet was used as a control. Apparent nitrogen retention from whole grains (28.9±9.9%) was inferior to that from flour (35.4±7.6%) but the difference was not statistically significant. Stool wet weight and energy loss, especially as carbohydrate, were greater during grain consumption. In general the results suggested that digestibility of the quinua seed was the factor limiting protein and energy utilization; milling improved digestibility significantly. Even properly milled quinua was somewhat inferior in digestibility to potato, wheat or rice.

A complete report of this work was submitted to AID on September 15, 1980 and is found as Appendix 5. These studies are in press in the Archivos Latinoamericanos de Nutricion.

C. Prolonged Consumption by Infants of Wheat-Based Diets With and Without Casein or Lysine Supplementation

Earlier short-term studies had demonstrated that recovering malnourished infants and small children could consume 75% of calorie requirements as 82% extraction wheat flour without deterioration in high digestibility of protein and energy of wheat. Nitrogen balances indicated that at this level protein needs of the children, represented by nitrogen retentions from an isonitrogenous casein diet, probably were not satisfied. Additional long-term (3+ months) studies in four similar infants and children have shown that a diet with 50% calories

and 80% protein from wheat (casein added so that protein calories = 8%) was consumed and digested efficiently and supported weight gain and linear growth in excess of that expected for age or "height age". Prolonged feeding of 75% of calories and 100% of protein from wheat was attempted in three infants and small children. In the oldest (age 15.5, height age 12.3 mos.) digestibility and growth were satisfactory. In one infant, despite good digestibility, weight gain and linear growth were inadequate and serum albumin fell to deficiency levels until 0.2% lysine was added to wheat flour. In the third infant weight gain was satisfactory but serum albumin fell to borderline (3.25 g/dl) levels and linear growth was inadequate. In six additional infants and children, the addition of lysine during the second of three months was associated with a significant increase in nitrogen retention and weight gain and stabilization of falling serum albumin levels. Withdrawal of lysine resulted in a significant decrease in the rate of weight gain, but had no effect on nitrogen retention or serum albumin levels. Linear growth was barely adequate during the three-month studies. The fall in serum cholesterol previously noted with wheat diets was seen only when dietary protein was inadequate. It is practically impossible for unsupplemented wheat to satisfy protein needs of infants and most small children.

A complete report of these studies was submitted to AID on November 30, 1980 and is found as Appendix 6. The paper has been submitted to the Journal of Nutrition for publication.

D. Plasma Free Amino Acid Levels of Infants and Children Consuming Wheat-Based Diets With and Without Supplemental Casein or Lysine

Plasma amino acid levels during previous short-term studies and from the studies outlined above have been determined and analyzed.

Fasting and postprandial plasma free amino acids were measured in infants and small children consuming five energy-sufficient wheat-based diets: (a) 44 to 50% of calories and all of 6.6% protein calories from wheat, (b) the same plus enough casein to make 8.0% protein calories, (c) 75% calories and all of 9.8% protein calories from wheat, (d) the same with 0.2% lysine added to the wheat, and (e) identical to diet c, but following diets c and d.

On the 9th day of diet a, which was clearly inadequate in available protein because of its severe lysine deficiency, 16 subjects demonstrated low fasting TEAA/TAA (total essential amino acid/total amino acid molar concentrations) ratios, with significant declines at 3 ($P<0.05$) and 4 hours ($P<0.01$) postprandially. Fasting molar fractions of each essential amino acid (EAA/TEAA) were not different from those of subjects consuming isonitrogenous milk protein diets, with the exception of slightly lower Lys/TEAA and higher Thr/TEAA. Postprandially there were significant ($P<0.001$) declines in Lys/TEAA and increases in Ile/TEAA, Tyr/TEAA and Phe/TEAA, as well as a modest ($P<0.05$) decline in Thr/TEAA. After 3+ months of diet b, four subjects had higher ($P<0.05$) fasting Lys/TEAA with marked ($P<0.01$) postprandial declines, much lower ($P<0.001$) fasting Thr/TEAA, and still significant ($P<0.05$ or <0.01) postprandial increases in Ile/TEAA, Thr/TEAA and Phe/TEAA.

After 28 to 89 days of diet c in ten subjects there was a significant ($P<0.001$) postprandial increase in TEAA/TAA; fasting Lys/TEAA

was lower than after diet a and its significant ($P<0.01$ and <0.001) postprandial decline reached lower levels; fasting Thr/TEAA was again elevated, with a modest ($P<0.05$) decline at three hours. There were significant ($P<0.01$ or <0.001) postprandial increases in Ile/TEAA, Tyr/TEAA and Phe/TEAA. When lysine was added in 7 of the 10 subjects (diet d), after 8 to 50 days fasting Lys/TEAA was significantly ($P<0.05$) higher, with no postprandial decline; fasting Thr/TEAA was lower ($P<0.02$). The only significant postprandial change was an increase ($P<0.01$) in Phe/TEAA. After 15 to 29 days of withdrawing the added lysine in 5 of the 7 subjects (diet e), the plasma amino acid changes were very similar to those after the identical diet c; fasting Lys/TEAA was lower, however, suggesting a greater depletion of body lysine pools.

Fasting plasma free amino acids tend to reflect the diet TEAA/TAA ratio, protein/calorie ratio, protein "status", and the first-limiting essential amino acid in the diet (after prolonged consumption). The second-limiting essential amino acid may actually be elevated. Postprandial declines of EAA/TEAA clearly identify the first-limiting essential amino acid; increases may be due to relative diet excess or delays in clearing from plasma.

A complete report of these studies is found as Appendix 7. The complete paper is currently under review by the Journal of Nutrition for consideration for publication.

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Nutritional Value of Normal, Opaque-2 and Sugary-2 Opaque-2 Maize Hybrids for Infants and Children. 2. Plasma Free Amino Acids[1]

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ABSTRACT Plasma free amino acids of convalescent malnourished infants and children were determined before and 3 and 4 hours after the first meal of the 27th day of maize endosperm (E) or whole kernel (WK) meal consumption. Three varieties of E—normal, opaque-2 (o_2) or sugary-2 opaque-2 (su_2o_2)—had been consumed in six different sequences during consecutive 9-day periods by six subjects; the same protocol was followed for the corresponding WK meals. Plasma aminogram was expected to reflect the effects of 26 days of maize consumption and 8 days of the last variety consumed, particularly the latter. The significantly poorer absorptions of nitrogen (N) from the E meals were reflected in lower fasting molar ratios of total essentials to total amino acids (TEAA/TAA = 0.192 versus 0.229 for WK). Fasting leucine/TEAA molar ratio was consistently elevated, notably after normal maize. With the exception of one very low (0.067) and one moderately low (0.120) lysine/TEAA ratio, fasting values did not suggest a first-limiting amino acid. Striking postprandial falls singled lysine out as first-limiting in five, possibly six, studies, tryptophan in two studies (normal E and su_2o_2 WK) and probably isoleucine in one o_2 WK study. In two studies (su_2o_2 E and normal WK), lysine and tryptophan seemed equally limiting. The improved lysine and tryptophan contents of o_2 and su_2o_2 maize still leave one or both of these, and possibly isoleucine, as potentially first-limiting for small children. J. Nutr. *110:* 1070–1075, 1980.

INDEXING KEY WORDS plasma free amino acids · opaque-2 maize · sugary-2 opaque-2 maize

The opaque-2 gene changes the distribution of endosperm proteins in maize, resulting in an increase in lysine and tryptophan and a decrease in leucine contents (1). The sugary-2 gene lowers lysine content but improves kernel vitreousness and density. The double-mutant sugary-2 opaque-2 (su_2o_2) has favorable kernel characteristics, a higher lysine content and a protein efficiency ratio (PER) in rats which is slightly higher than that of opaque-2 (o_2) in near-isogenic triple hybrids (2).

In a companion paper (3) we reported

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an evaluation, in eight convalescent malnourished infants and children, of the energy and protein digestibilities and protein quality of the above two hybrids and a near-isogenic normal triple hybrid, as the endosperm (E) and the whole kernel (WK) meals of each. Digestibility of energy from E meals was seemingly lower than that of WK meals, probably due to the greater percentage of calories from E meals (83.2, 91.2 and 82.1% for normal, o_2 and su_2o_2) than from WK meals (73.2, 68.2 and 67.2%) needed to make maize supply the 6.4% protein calories in each diet. Protein digestibility from WK meals (72.6 ±3.4% of intake) was significantly higher than that from E meals (66.8±7.6%). Apparent nitrogen (N) retention from WK meals (29.5±7.5% of intake) was significantly higher than from E meals (20.9± 7.5%). When related to presumed amounts of lysine absorbed, N retention from the WK meals was higher, suggesting that better energy absorption, a better balance of amino acids absorbed or more available tryptophan may have been responsible. Nitrogen retention from normal E meal (15.1±8.9%) was inferior to that from o_2 E meal (22.8±5.5%) and from su_2o_2 E meal (24.8±8.0%). For WK meals differences were less between varieties: normal, 26.8±4.6; o_2, 30.1±8.3; and su_2o_2, 31.6± 9.7% of intake.

With ordinary maize, Scrimshaw, Bressani et al. demonstrated that both lysine and tryptophan had to be supplemented to attain consistently improved N retentions in children (4, 5). Despite the increased lysine and tryptophan of opaque-2 containing maize, lysine is still first-limiting for animals (6) and for adult humans (7).

In the course of the studies summarized above, plasma samples were obtained at selected times for free amino acid determinations.

MATERIALS AND METHODS

In the evaluation of the three endosperm meals (normal, o_2 and su_2o_2), each of six children received consecutive diets based on each of the three meals in one of six different sequences. Maize provided all the 6.4% protein calories in isoenergetic, isonitrogenous diets of 100–125 kcal/kg/day. Each diet period lasted 9 days and the 27 days of maize diets were preceded and followed by a casein control diet. In this report, the diet sequences are identified by the maize meal fed during the last of the three 9-day periods, as this was likely to be the main determinant of the amino acid patterns found in plasma. For WK meals the same protocol was followed. Four children participated in both studies, two in the E meal studies only, and two in the WK meal studies only.

The children were 10–25 months of age, were free of apparent infection, had no evidence of malabsorption and were well advanced in their convalescence.

A sample of fasting (8 hours) plasma was obtained before the first meal of the last day of the third maize diet period in each sequence. This was expected to reflect the effect of 26 days of maize (E or WK) consumption and eight days of the particular meal by which the sequence was identified. Additional plasma samples were obtained 3 and 4 hours after the first meal. There should have been 12 sets of three samples, each of two sets corresponding to one of the six meals as last in sequence: normal E, o_2 E, su_2o_2 E, normal WK, o_2 WK and su_2o_2 WK. One of the normal E meal set of samples was not available for analysis.

Plasma free amino acid levels were determined by liquid column chromatography and photofluorometry (8). They were expressed as actual molar concentrations (μmole/liter) and as molar ratios: the ratio of the concentrations of total essential (and the semi-essentials cystine and tyrosine) amino acids to total amino acids (TEAA/TAA), and the ratio of the concentration of each essential amino acid to that of the total essentials (EAA/TEAA).

RESULTS

Table 1 summarizes the pertinent fasting plasma free amino acid levels and contrasts them with those previously found in infants and children consuming modified cow milk or casein as the sole source of protein, at 6.4–7.0% protein calories and at less than 6.4% protein calories (3). Subsequent

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TABLE 1

Fasting plasma free amino acids of infants and small children consuming milk or casein (8), maize endosperm or maize whole kernel meals as the sole source of dietary protein[1]

Protein source	TAA µmole/liter	TEAA/TAA	EAA/TEAA			
			Lys	Trp	Leu	Ile
% protein kcal						
Modified milk or casein 6.4–7.0% (N = 81)	2,633 ±439[2]	0.287 ±0.030	0.147 ±0.042	0.066 ±0.017	0.120 ±0.017	0.070 ±0.011
Modified milk or casein <6.4% (N = 8)	2,428 ±295	0.250 ±0.023	0.146 ±0.022	0.063 ±0.019	0.112 ±0.012	0.070 ±0.011
Endosperm meals—6.4%						
Normal (#568)	2,842	0.160	0.167	0.040	0.207	0.046
o_2 (#560)	2,602	0.256	0.150	0.058	0.147	0.058
o_2 (#567)	2,460	0.170	0.067	0.060	0.170	0.060
su_2o_2 (#549)	2,578	0.196	0.139	0.044	0.125	0.055
su_2o_2 (#553)	2,379	0.177	0.140	0 071	0.140	0.059
Whole kernel meals—6.4%						
Normal (#567)	2,857	0.204	0.120	0.051	0.153	0.057
Normal (#549)	2,616	0.229	0.150	0.072	0.149	0.062
o_2 (#565)	2,495	0.195	0.158	0.074	0.113	0.047
o_2 (#560)	2,361	0.253	0.132	0.077	0.136	0.060
su_2o_2 (#553)	1,845	0.255	0 136	0.072	0.136	0.055
su_2o_2 (#561)	2,793	0.236	0.150	0.092	0.098	0.050

[1] Number assigned to subject shown in parentheses. [2] Mean ±SD.

comparisons have been made with the lower level of milk protein intake because the significantly poorer absorptions of N from any of the maize meals than from milk protein made the "effective" intakes from maize more like those of the lower level.

Total free amino acid concentrations were all within two standard deviations (SD) of the milk protein values and seven of 11 were within one SD.

Four of five TEAA/TAA ratios from the E meals were more than two SD's below the mean for milk protein. The lowest ratio (0.160) corresponded to the diet period with the poorest nitrogen absorption (45% of intake) of the entire study. The next lowest (0.170) corresponded to the poorest absorption in all the o_2 E studies. The two other low values (0.196 and 0.177) corresponded to the two poorest absorptions of N from the su_2o_2 E meal. The fact that so many of the poorest absorptions were seen in the last of three consecutive periods of E meal consumption suggests that protein digestion tends to deteriorate during prolonged maize E meal consumption.

Only one of the six TEAA/TAA ratios from the WK studies was more than two SD's below the milk protein mean; this corresponded to the poorest N absorption of all the WK studies. The mean ratio for all the WK studies, 0.229, was higher but not significantly so ($0.05 < P < 0.1$) than that for all the E studies, 0.192.

When TEAA/TAA ratios are low, with normal TAA levels, the levels of the nonessential amino acids must obviously be higher. This was explained by higher alanine levels in all cases and by elevations of glycine, proline or asparagine-glutamine in some cases.

In six of 11 studies, plasma tyrosine was more than one SD below the milk protein mean.

Table 1 also lists the concentration of lysine, tryptophan, leucine and isoleucine as their molar fractions of TEAA. In only one E study was there a significantly low fasting lysine/TEAA ratio of 0.067, corresponding to the o_2 study with the poorest N absorption. In the WK studies, there was only one suggestively low ratio of 0.120.

In only one E study, with normal maize, was the tryptophan/TEAA ratio more than one SD below the mean for milk protein. In all the WK studies this ratio was within one SD of that mean. The mean for the WK studies was significantly ($P < 0.05$) higher than for the E studies.

In all five E studies, the leucine/TEAA ratio was more than one SD above the mean

for milk protein; in four of five it was more than two SD's above that mean. In two WK studies, the leucine/TEAA ratio was within one SD of the mean for milk protein; in four it was two SD's above that mean. The isoleucine/TEAA ratio was below the mean for milk protein in all five E and six WK studies. Both the one-half cystine/TEAA and methionine/TEAA ratios were normal to begin with and showed little or no change postprandially. The low tyrosine/TEAA ratios seen in the fasting state tended to correct themselves postprandially.

Figure 1 illustrates the postprandial changes in the EAA/TEAA ratios for lysine (Lys), tryptophan (Trp), leucine (Leu) and isoleucine (Ile) after the E meals. The lysine/TEAA ratio for the one subject consuming the normal E meal increased at 3 hours to more than 2 SD's above the mean for milk protein but fell at 4 hours to well below the fasting level. This same child, who during this diet period had the poorest absorptions and retentions of N of the entire study, also had the lowest fasting TEAA/TAA ratio and tryptophan/TEAA ratio. Postprandially, this last ratio fell to a level which we see only in kwashiorkor or in pellagra. He had the highest fasting leucine ratio and the lowest isoleucine ratio, neither one of which changed significantly at 3 and 4 hours. It seems likely that tryptophan and not lysine was first-limiting.

In one child consuming o_2 E meal, the very low fasting Lys/TEAA ratio fell even further postprandially, and his normal Trp/TEAA ratio also fell but not to the same degree. Fasting leucine, already high, rose to the highest level in the study. In the other child consuming o_2 E meal, Lys/TEAA dropped from a normal fasting level to 2 SD's below the mean for milk protein at 4 hours, while tryptophan remained unchanged at a normal ratio. The Ile/TEAA ratio fell more than one SD at 3 hours and recovered at 4 hours. For both of these children consuming o_2 E meal, lysine seems to have been clearly first-limiting.

After su_2o_2 E meals, the Lys/TEAA ratio fell slightly in one child and steeply in the other while changes in Trp/TEAA were modest. Their Leu/TEAA ratios were not

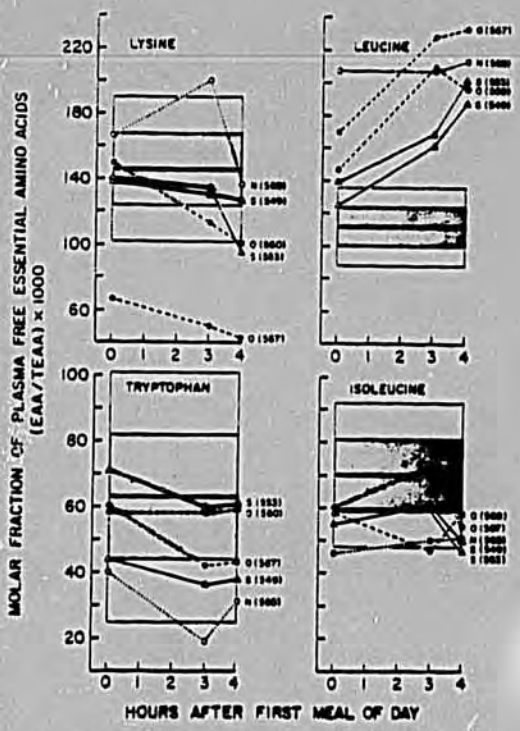

Fig. 1 Fasting and postprandial molar ratios (EAA/TEAA) for lysine, tryptophan, leucine and isoleucine in five children consuming normal (N), opaque-2 (O) or sugary-2 opaque-2 (S) endosperm maize meals. Mean±one and two standard deviations of milk protein values (8) are indicated by shaded and clear areas. Subject number in parenthesis after type of meal.

as high to begin with but did rise steeply particularly at 4 hours. For one child, lysine may have been first-limiting, but for the other, lysine and tryptophan may have been equally limiting.

Figure 2 illustrates the postprandial changes in EAA/TEAA ratios for the same four essential amino acids after the WK meals. For one child consuming normal WK meal, the relatively low fasting Lys/TEAA ratio fell steeply at 3 hours with some recovery at 4 hours. This is the same child with a very low fasting ratio after o_2 E meal and a further fall postprandially (fig. 1). He had a moderate postprandial fall in Trp/TEAA with a steep rise in Leu/TEAA at 3 hours. The other child consuming normal WK meal had a modest postprandial fall in lysine, tryptophan and isoleucine ratios and a more moderate rise

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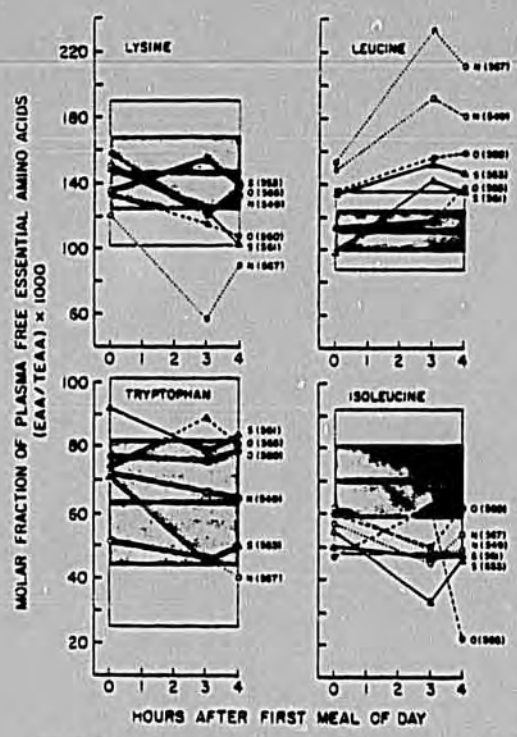

Fig. 2 Fasting and postprandial molar ratios (EAA/TEAA) for lysine, tryptophan, leucine and isoleucine in six children consuming normal (N), opaque-2 (O) or sugary-2 opaque-2 (S) whole kernel maize meals. Mean±one and two standard deviations of milk protein values (8) are indicated by shaded and clear areas. Subject number in parenthesis after type of meal.

in the already high Leu/TEAA ratio. For the first of these two children, lysine seemed clearly first-limiting; for the second, lysine and tryptophan and possibly isoleucine seemed equally limiting.

The two children consuming o_2 WK meal had modest postprandial falls in their normal Lys/TEAA ratios, no fall in Trp/TEAA ratios and modest increases in Leu/TEAA ratios. One had a moderate fall in Ile/TEAA at 3 hours and a recovery at 4 hours; the other had a steep fall in Ile/TEAA at 4 hours after a moderate increase at 3 hours. This may represent laboratory error or a true limitation of protein synthesis by isoleucine deficiency. There were minimal indications of lysine limitation and none at all for tryptophan.

One child had no postprandial fall of his normal Lys/TEAA ratio after su_2o_2 WK meal, the other a distinct drop to 2 SD's below the milk protein mean. The first, conversely, had a sharp drop in Trp/TEAA, and the second, an insignificant fall from a high normal fasting ratio. Child #553 also had a sharp 3-hour fall in Ile/TEAA which was not seen in #561.

DISCUSSION

Although the fasting TEAA/TAA ratio is a generally reliable indicator of protein intake or absorption and to a limited degree of protein quality (9), neither the fasting methionine/TEAA ratio (10) nor the fasting lysine/TEAA ratio (11) has proven to be an effective or consistent indicator of inadequacy. On the other hand, the postprandial changes in these two individual ratios have been shown to identify a deficiency of one of these two essential amino acids in the diet (10, 11).

Normal maize has been found to be almost equally limiting in lysine and tryptophan for small children: both amino acids had to be supplemented to obtain a consistent increase in N retention, with one producing no effect in some children if the other was not added (4, 5). In the same studies, the addition of methionine had an adverse effect on N retention, but the further addition of isoleucine had a supplementary effect. To our knowledge, similar studies have not been carried out in children consuming o_2 maize, and certainly not in children consuming su_2o_2 maize. Studies with o_2 in adults do suggest that lysine is still first-limiting (7).

In view of the above, it would not be surprising to find disparate results in the fasting and postprandial plasma amino acids of children consuming maize: this seems indeed to be the case, quite different from the experiences with soy and wheat proteins, both clearly limiting in a single amino acid. Because six different maize meals were studied and there were only two sets of plasma samples for each meal (only one for the normal E meal), the results found cannot clearly be attributed to each meal, as they might have been due to individual variations in amino acid requirements or absorption.

The fasting TEAA/TAA ratios consis-

tently identified poor N absorption, particularly from the E meals. Individual fasting EAA/TEAA ratios were only consistent in identifying the high leucine content of all the meals, particularly those of the normal maize, whether as the E meal or as the WK meal. In one case definitely, and possibly in another, the inadequacy of dietary lysine was evident from the fasting Lys/TEAA ratio. Interestingly, these two sets of samples were from the same child, once after consuming o_2 E meal for 8 days (which followed 18 days of normal and su_2o_2 E meals) and then after consuming normal WK meal for 8 days (after 18 days of o_2 and su_2o_2 WK). This may be an example of a higher individual requirement for lysine. Although the mean fasting Trp/TEAA ratio was significantly higher after the WK meals than after the E meals, both were within the normal range. Fasting Ile/TEAA ratios were moderately low, but never strikingly so. Thus, with only occasional exceptions, the fasting EAA/TEAA ratios were again ineffective in singling out essential amino acid inadequacies.

Postprandial changes, measured at 3 and 4 hours after the first meal of the day, were much more helpful. They identified lysine as clearly first-limiting in both children consuming o_2 E meal, one of two consuming su_2o_2 E meal, one of two consuming normal WK meal, and one of two consuming su_2o_2 WK meal. In one child consuming o_2 WK meal, lysine may also have been first-limiting. Tryptophan was clearly first-limiting in the one child consuming normal E meal and probably first-limiting for one of two consuming su_2o_2 WK meal. In one child consuming o_2 WK meal, if not a laboratory error, a steep fall in Ile/TEAA ratio at 4 hours may have identified isoleucine as first-limiting. In the remaining two studies, one with su_2o_2 E and one with normal WK meal, lysine and tryptophan may have been equally limiting.

These plasma free amino acid studies suggest that lysine is probably the most common first-limiting factor when normal maize or o_2 and su_2o_2 are consumed, but that tryptophan and perhaps occasionally isoleucine can also be first-limiting. The excess of leucine, particularly in normal maize, was invariably evident even in fasting plasma. The persistently normal methionine/TEAA ratios confirm the fact that this amino acid is not limiting in maize.

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Utilization of the Protein and Energy of the White Potato by Human Infants[1]

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ABSTRACT The digestibility and protein quality of the white potato were studied in 11 infants, ages 8–35 months, recovering from malnutrition. Seven 9-day dietary periods comprised the study. Digestibility was assessed by metabolic balance during the last 6 days of dietary periods in which potato provided 25%, 50% or 75% of dietary energy. Casein-based control dietary periods preceded and/or followed each potato dietary period. Fecal wet and dry weights, fecal energy, computed fecal carbohydrate and fecal fat during consumption of the 25% potato diet did not differ from control. Analysis of variance showed that consumption of increasing amounts of potatoes produced a significant linear increase in all parameters of digestibility except fecal fat. Fecal wet weight and energy content during the 75% potato period were more than double control values. Regression analysis of data from potato dietary periods showed fecal wet weight to be an excellent predictor of fecal dry weight ($r = 0.904$), energy content ($r = 0.785$) and computed fecal carbohydrate ($r = 0.770$). Protein quality of potato was assessed with N balance studies during the dietary periods in which potato provided all dietary protein (~5% protein kcal). Apparent N retention was 78% of that from an isonitrogenous casein control diet. Decreased apparent N absorption was primarily responsible for the difference; the "biologic value" (percent of absorbed N that was retained) was similar for both diets. J. Nutr. *110:* 1849–1857, 1980.

INDEXING KEY WORDS protein quality · potato · infant nutrition

The white potato is the fourth leading world food crop after wheat, rice and maize. In terms of energy produced per unit area of land, the potato exceeds all the cereals and stands only behind sugar cane. Protein yield per unit area is superior to the cereal grains and second only to soybeans. Despite this fact, the potato is popularly thought of only as a source of starch in the diet. In the United States, the potato contributes more than 2% of total dietary protein, compared with the 3% provided by fish and 6% supplied by eggs (1).

In the Andean region of South America, where the potato originated, approximately 50% of children under 5 years of age have moderate to severe forms of malnutrition (2). This prevalence is owed

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TABLE 1

Characteristics of subjects participating in the study

Patient No.	Age	Weight	Length	Serum albumin Initial	Serum albumin Final
	mos.	*kg*	*cm*	*g/dl*	
527	14	7.98	72.3	3.7	4.1
533[1]	35	11.10	85.0	4.0	3.9
536	15	8.21	71.7	3.6	3.8
538	15	8.21	70.0	3.9	4.3
542	13	8.75	72.0	4.0	4.6
548	12	7.62	68.7	3.8	3.7
550	17	6.79	65.0	4.2	3.9
554	10	7.49	69.5	4.0	4.0
558	8	4.93	61.5	3.8	3.9
571	15	7.25	68.3	4.5	4.2
579[1]	19	9.95	75.3	3.8	3.9

[1] These patients participated only in the protein quality studies.

primarily to early weaning and the lack of suitable weaning foods, both of which combine to initiate the vicious cycle of diarrhea, malabsorption and malnutrition. To determine the potential of the potato as a food for weaned infants, studies were carried out to evaluate clinical tolerance, overall digestibility and protein quality of increasing amounts of potatoes in the diet of human infants.

MATERIALS AND METHODS

Subjects. Eleven Peruvian male infants, age 8–35 months, who had been admitted to the Instituto de Investigacion Nutricional[3] for treatment of severe malnutrition participated in the study (table 1). All were well past the acute phase of treatment and fulfilled the following criteria: free of infection and diarrhea; no evidence of malabsorption in previous metabolic balance studies, adequate weight gain while consuming 100–125 kcal/kg body weight/day, and stable serum albumin concentration above 3.6 g/dl. Nine children participated in studies of both digestibility and protein quality. Two children (#'s 579 and 533) participated only in the studies of protein quality.

Diets. The potato has a natural variability in protein content that is dependent on factors such as variety, soil fertility and growing conditions (3). In an attempt to avoid the problem of variability, a single batch of oven-dried potatoes of approximately 80 kg was processed and provided by the Centro International de la Papa (International Potato Center) in Lima. Oven-dried potatoes were selected after a preliminary study showed no differences in digestibility when compared to fresh or lyophilized potatoes. Mariva potatoes (*Solanum Tuberosum ssp. andigena*) were cooked in boiling water for 30 minutes, peeled and cut. This material was dried in an oven at 40 ± 2° for 20 hours and milled in a hammer mill. Protein content ($N \times 6.25$) of the dehydrated material was 5.75 g/100 g.

Although nine studies had been planned, the quantity of potatoes provided proved to be sufficient to carry out only seven. Following the completion of these, a new batch of the same variety of potatoes was obtained. Analysis of the dehydrated material revealed a protein content of 9.13 g/100 g, considerably higher than the original lot used.

Studies of digestibility

Study diets. Three diets providing increasing amounts of potato were designed (table 2). The potato provided 25%, 50% or 75% of total energy in each diet. In the 25% and 50% diets, nitrogen (N) content was adjusted to 256.0 mg per 100 kcal (6.4% protein-energy) by the addition of casein. In the 75% diet, all N was provided by potato. A blend (80:20) of soybean-cottonseed oil was added to all three diets to provide 25% of energy as fat. The remaining energy was supplied by sucrose in the 25% and 50% diets.

The same percentages of dietary energy provided by potato were maintained with the second batch of potatoes in order to study digestibility. The percentages of energy as fat and sucrose were also unchanged. The 25% diet was adjusted as in the previous batch, to 256.0 mg N per 100 kcal (6.4% protein-energy) with casein. Because of the higher protein

[3] Protocols for these studies were approved by the Joint Committee on Human Investigation, The Johns Hopkins Medical Institutions and the corresponding committee of the Instituto de Investigacion Nutricional, Lima, Peru. Informed consent was obtained from the parent of all children participating in the studies.

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TABLE 2

Composition of study and control diets (per 100 kcal) used for the initial seven studies[1]

	25% Potato	50% Potato	Control 1	75% Potato	Control 2
Potato, *g*	7.29	14.58	—	21.87	—
N, *mg*	67.10	134.20	—	201.30	—
Energy, *kcal*	25.00	50.00	—	75.00	—
Ca Caseinate,[3] *g*	1.37	0.88	1.86	—	1.46
N, *mg*	188.90	121.80	256.00	—	201.30
Energy, *kcal*	5.10	3.26	6.90	—	5.40
Sucrose, *g*	11.20	5.40	8.50	—	8.70
Energy, *kcal*	44.90	21.70	34.00	—	34.80
Cornstarch, *g*	—	—	9.6	—	9.8
Energy, *kcal*	—	—	34.1	—	34.8
Soybean-cottonseed oil, *ml*	2.8	2.8	2.8	2.8	2.8
Energy, *kcal*	25.0	25.0	25.0	25.0	25.0
Totals					
N, *mg*	256.0	256.0	256.0	201.30	201.30
Protein, *(N × 6.25) % energy*	6.4	6.4	6.4	5.03	5.03
Energy, *kcal*	100.0	100.0	100.0	100.0	100.0

[1] Minerals were supplemented in all diets to provide intakes of 2 mEq Na/kg body weight/day using a mineral mixture without potassium of the following composition (w/w): NaCl, 30.701%; $MgSO_4 \cdot 7H_2O$, 22.804%; $CaHPO_4$, 41.615%; $FeC_6H_5O_7 \cdot 3H_2O$, 3.549%; $CuSO_4 \cdot 5H_2O$, 0.625%; $ZnSO_4 \cdot 7H_2O$, 0.355%; $MnSO_4 \cdot H_2O$, 0.277%; KI, 0.025% and NaF, 0.50%. [2] Vitamins were supplemented by a single daily dose of 0.6–1.2 ml of Poly-Vi-Sol (Mead Johnson, Evansville, IN). The composition (per ml) of this preparation is as follows: retinyl palmitate, 1,500 IU; ergocalciferol, 400 IU; Vitamin E, 5 IU; ascorbic acid, 35 mg; thiamin, 0.5 mg; riboflavin, 0.6 mg; niacin, 8 mg; vitamin B-6, 0.4 mg, and vitamin B-12, 1 μg. Patients also received a single dose of 191 mg choline, 180 mg myo-inositol, 200 μg folic acid and 500 mg PABA. [3] Casec, Mead Johnson, Evansville, IN. 1.88 g Casec was used to provide 1.6 g casein.

content of this batch, all N in the 50% (207 mg/100 kcal) and 75% (285 mg/100 kcal) diets was provided by the potato.

Control diets. Three control diets were prepared, providing 256.0, 201.3 and 285.2 mg of nitrogen from casein per 100 kcal. The same oil blend was used to provide 25% of energy as fat. The remaining energy of the control diets was derived from sucrose and cornstarch in nearly equal amounts.

All diets were supplemented with vitamins and minerals to meet or exceed U.S. Recommended Dietary Allowances (4). Sodium intake was fixed at 2 mEq/kg body weight/day. Potassium intakes varied from 6 to 10 mEq/kg body weight/day according to the amount of potato in the diet. Diets were prepared in a blenderized form at a concentration of approximately 0.67 kcal/ml. Diet analysis for N, Na and K was performed regularly throughout the studies to assure that intakes were as calculated. Total energy content of the diets was also determined regularly by bomb calorimetry (5).

Studies of protein quality

Accurate assessment of protein digestibility and utilization required that balance studies be carried out with diets providing only potato protein at marginal levels of intake. N balance while consuming the two diets in which potato provided approximately 5% protein energy was compared with that while consuming the isonitrogenous casein control diets.

Study design and analysis

Each 63-day study consisted of seven 9-day dietary periods in one of nine possible sequences (table 3). The use of three levels of potato intake allowed

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TABLE 3

Sequence of dietary periods[1]

A.	Control 2[2]	75% P	Control 2	Control 1	50% P	Control 1	25% P
	Control 2	75% P	Control 2	Control 1	25% P	Control 1	50% P
	Control 1	50% P	Control 2	75% P	Control 2	Control 1	25% P
	Control 1	50% P	Control 1	25% P	Control 2	75% P	Control 2
	Control 1	25% P	Control 1	50% P	Control 2	75% P	Control 2
	Control 1	25% P	Control 2	75% P	Control 2	Control 1	50% P
B.	Control 1	25% P	Control 3	75% P	Control 2	50% P	Control 2
	Control 3	75% P	Control 2	50% P	Control 2	Control 1	25% P
C.	Control 2	50% P	Control 2				

A. Sequence of dietary periods followed by the first seven patients. Composition of potato and control diets are shown in table 2. B. Sequences followed by two patients using the secon batch of potatoes. 25% P, control 1 and control 2 diets are the same as used with the first batch. 50% P diet is isonitrogenous (5.1% protein-energy) to the 75% P diet of the previous batch and used to assess protein quality. 75% P and control 3 were designed to evaluate energy digestibility. C. Sequence followed by two patients (#'s 579 and 533) to assess protein quality with the second batch of potatoes. Diets are as in (B.). [1] Underlined periods were used to assess protein quality. [2] This sequence was used two times. All others once.

assessment of tolerance to and digestibility of increasing amounts of the carbohydrate components of potato and permitted comparison of the three potato diets with their controls and with each other. Each potato dietary period was preceded and/or followed by an appropriate contorl diet. Because protein quality was to be assessed during the consumption of the 75% potato diet with the first batch and during the 50% potato diet with the second batch, each of these periods was always preceded and followed by an isonitrogenous control diet. Two additional patients participated only in the studies of protein quality with batch 2 and received only the 50% potato diet, with preceding and following corresponding control dietary periods.

All children consumed 100–125 kcal/kg body weight/day. This intake was constant for each child throughout the study and was recalculated at the beginning of each dietary period. Energy intake was based on that previously shown to maintain each child's rate of weight gain in an acceptable range (3–7 g/kg body weight/day). After a 5-day period of adaptation to each diet, a 6-day metabolic balance was carried out using methods standard for the unit (6). Urine and feces were collected separately in 3-day pooled collections. Serum total protein (Biuret) and albumin (acetate gel electrophoresis) concentrations were measured at the beginning of the study and at the end of each successive dietary period. Urinary and fecal N were determined by the micro-Kjeldahl method (7). Feces were weighed both wet and dry. Fecal fat was determined by the method of Van de Kamer et al (8). Total fecal energy content was measured by bomb calorimetry (5) and the carbohydrate content of stool was then computed using the formula:

$$\text{Fecal CHO g} = \frac{E - (N\,g \times 6.25 \times 5.65) - (\text{Fat}\,g \times 9.4)}{4.15}$$

where CHO = carbohydrate in grams, E = fecal energy by bomb calorimetry, N = fecal nitrogen in grams, fat = fecal fat in grams and 5.65, 9.4 and 4.15 are the accepted energy conversion factors for protein, fat and carbohydrate, respectively (9).

Fecal wet and dry weight, fecal fat and computed fecal carbohydrate were used to estimate apparent digestibility. All of these were first analyzed by a "t" test to ascertain if the value while consuming

TABLE 4

Summary of digestibility data at three levels of potato intake

	Casein control[1]	25% Potato-energy	50% Potato-energy	75% Potato-energy	Casein control[2]
	(n = 9)	(n = 9)	(n = 9)	(n = 9)	(n = 9)
Stool wet weight, *g/day*	85.3 ± 33.8	91.5 ± 32.5	148.2 ± 40.5[a]	238.5 ± 65.2[a]	108.1 ± 56.8
Stool dry weight, *g/day*	12.2 ± 3.6	12.8 ± 3.8	19.0 ± 4.6[a]	30.2 ± 6.9[a]	12.5 ± 4.3
Stool energy, *kcal/day*	48.9 ± 15.8	55.3 ± 19.4	73.6 ± 15.2[b]	108.9 ± 35.9[a]	50.4 ± 14.8
Stool fat, *% intake*	7.7 ± 2.5	5.9 ± 2.0	5.2 ± 4.0[c]	4.8 ± 1.5	8.4 ± 4.8
Stool CHO, *g/day*	4.1 ± 2.3	5.8 ± 3.8	10.3 ± 3.1[a]	16.9 ± 7.4[a]	5.4 ± 4.6

[1] Average value for all the corresponding control periods surrounding 25% and 50% potato. [2] Average value for all the corresponding control periods surrounding 75% potato. [a] Significantly different from corresponding control period by "t" test, $P < 0.001$. [b] Significantly different from corresponding control period by "t" test, $P < 0.01$. [c] Significantly different from corresponding control period by "t" test, $P < 0.05$.

potato minus the appropriate control value (or the average of both corresponding control values where there were two) differed significantly from zero. Further analysis was then carried out by analysis of variance blocking on subjects and treatments with orthogonal contrasts among the treatment means (10). This analysis was carried out in two ways: using the absolute treatment value or the treatment value minus the appropriate control value (or the average of the two surrounding control values). Least squares linear regression analysis was used to correlate fecal wet and dry weight with fecal energy content, fecal fat and computed fecal carbohydrates. This was first carried out separately for the potato and control diets. These relationships for both diets were then further compared by analysis of covariance (11). Values of P of less than 0.05 were accepted as significant. Apparent absorptions and retentions of nitrogen were calculated for the two potato dietary periods used to assess protein quality and were compared with the preceding and following corresponding control values by a two-tailed paired "t" test.

RESULTS

Digestibility. The diets were well tolerated. All children consumed the total volume planned for each diet, although in some cases the time to consume the 75% potato diet was noted to increase during the last days of this metabolic period. Digestibility data are summarized in table 4. Analysis by "t" test comparing the values of the control periods that preceded the 25% and 50% potato periods with those that followed, and comparisons of the preceding and following control periods of the 75% potato diet with each other, showed no statistical differences in any of the five parameters used to assess digestibility. Treatment values for the five parameters used to assess digestibility were further compared by "t" test to corresponding control values at 25%, 50% and 75%. During the consumption of the 25% potato diet, none of the parameters was found to differ significantly from control values. All parameters, except fecal fat, were significantly elevated relative to control during the 50% and 75% potato dietary periods. Fecal fat was significantly less than control values during the 50% and 75% potato dietary periods.

Further analysis by analysis of variance indicated that consumption of increasing amounts of potato produced a significant ($P < 0.005$) linear increase in all parameters except one: fecal fat. Fecal fat did not change significantly among the three levels of potato intake. Stool wet

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TABLE 5

Linear regression analysis and analysis of covariance of parameters of digestibility of the potato and casein control diets

	Regression equation	r	P
Stool wet weight (Y)			
Stool dry weight (x)			
Potato	Y = 7.86x − 2.48	0.904	<0.0001
Casein	Y = 8.26x − 6.34	0.719	<0.0001
Potato vs. casein			N.S.
Fecal energy (x)			
Potato	Y = 1.82x + 15.98	0.785	<0.0001
Casein	Y = 1.77x + 11.02	0.608	<0.0001
Potato vs. casein			N.S.
Fecal fat (x)			
Potato	—	0.034	N.S.
Casein	—	0.217	N.S.
Potato casein			—
Fecal CHO			
Potato	Y = 2.10x + 64.5	0.770	<0.001
Casein	Y = 2.10x + 58.32	0.501	<0.0002
Potato vs. casein			N.S.
Stool dry weight (Y)			
Fecal energy (x)			
Potato	Y = 0.24x + 1.86	0.892	<0.0001
Casein	Y = 0.22x + 1.40	0.904	<0.0001
Potato vs. casein			N.S.
Fecal fat (x)			
Potato	—	0.010	N.S.
Casein	Y = 1.77x + 8.68	0.342	<0.02
Potato vs. casein			
Fecal CHO (x)			
Potato	Y = 0.267 + 8.54	0.851	<0.001
Casein	Y = 0.28x + 7.45	0.756	<0.0001
Fecal energy (Y)			
Fecal fat (x)			
Potato		0.153	N.S.
Casein	Y = 11.24x + 25.05	0.534	<0.001
Potato vs. casein			—
Fecal CHO (x)			
Potato	Y = 1.14x − 27.31	0.967	<0.001
Casein	Y = 1.17x − 29.53	0.736	<0.0001
Potato vs. casein			N.S.

weight varied significantly among subjects ($P < 0.02$). Linear regression analysis of digestibility data from potato showed a significant correlation between fecal wet and dry weights (table 5). Both fecal wet and dry weights were good predictors of fecal energy and computed fecal carbohydrate. These last two parameters were also correlated with each other. There was no correlation between fecal fat and any of the other parameters. Regression analysis of data from all control diets showed similar relationships with the exception of fecal fat, which showed significant relationships with stool dry weight and fecal energy. Analysis of covariance determined that there were no differences in the corresponding significant regression lines for the two diets.

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TABLE 6

Summary of nitrogen balance studies

	5.03% Protein-energy ($n = 7$)			5.10% Protein-energy ($n = 4$)		
	Control 2	75% Potato	Control 2	Control 3	50% Potato	Control 3
Apparent N absorption, *% of intake*	82.9 ± 3.2	65.9 ± 3.7[a]	83.8 ± 4.5	80.5 ± 9.2	75.6 ± 6.7[b]	83.5 ± 6.9
Apparent N retention, *% of intake*	41.9 ± 5.5	33.9 ± 5.6[a]	46.1 ± 4.8	39.4 ± 8.5	34.3 ± 8.8	46.8 ± 4.3

[a] Differs from preceding and following control periods, $P < 0.02$. [b] Differs from following control period, $P < 0.02$. [c] Differs from following control period, $P < 0.05$.

Protein quality. Apparent absorption of nitrogen during the consumption of diets in which potato provided all nitrogen differed significantly from values obtained during corresponding control periods (table 6). In the first seven studies, apparent nitrogen absorption from the 75% potato energy diet was significantly less than that from the preceding and following control diets. In the four studies with the 50% potato-energy diet, a significant difference was found only between the treatment and the following control period. No significant difference between preceding and following control periods was found in either group.

Apparent retention of nitrogen from potato was inferior to casein. With the 75% potato-energy diet, the difference from the preceding and following controls was significant. In the four patients in whom protein quality was evaluated during the consumption of the 50% potato energy diet, apparent nitrogen retention was significantly less only when compared to the following casein control period. In both groups the mean apparent nitrogen retention during the following control periods was higher than that during the preceding control periods but the differences were not statistically significant. When the 11 studies in which potato provided all nitrogen were combined, apparent nitrogen retention was 78% of the mean of the casein control values.

DISCUSSION

These studies show that potatoes can be successfully used to supply a substantial part of the daily requirement for both protein and energy in the diets of infants and small children. The consumption of large amounts of potatoes may be limited by bulk and by carbohydrate digestibility. The increases in fecal weight, for example, with increasing amounts of potato are greater than we have previously reported for wheat pasta (12).

The analysis of digestibility was based on the assumption that the digestion and absorption of casein, vegetable oil and sucrose was almost complete or that it remained constant for each child throughout the study. Changes in fecal parameters used to assess digestibility could then be directly related to the amount of potato in the diet. Consumption of increasing amounts of potatoes was associated with a linear increase of stool wet weight, stool dry weight and losses of carbohydrates and energy in feces. Energy losses during the consumption of the 25%, 50% and 75% potato diets, expressed as a percentage of increase over control diets, were 13.1%, 50.1% and 116.1%, respectively. These represented 5.8%, 7% and 10.5% of the total energy intake for each diet, respectively and reflect indigestibility of carbohydrate. When viewed in terms of energy and osmotically induced water and electrolyte losses, these diet related changes could be considered of nutritional significance, especially in small children consuming considerable amounts of potato in their diets. Comparing the results of this study with those of our previous studies with wheat pasta and rice, carbohydrate digestibility of potato was found to be inferior to

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both (12, 13). Other investigators reached a similar conclusion when potato starch in the form of biscuits was fed to infants (14).

Fat digestibility was almost complete at all three levels of potato intake and showed no correlation with any of the other parameters used to assess digestibility. This is not surprising since virtually all fat was supplied by the addition of vegetable oil. As in previous studies done with staple foods, a strong linear correlation between fecal wet weight, dry weight and energy content was found (12, 13). These parameters, especially the wet weight of feces, can be very useful in estimating the overall digestibility of diets with considerable proportions of staple foods.

Apparent N absorption during the consumption of the 75% potato diet was 79% of the average of the casein control diets. This relatively poor apparent N absorption is similar to that obtained with rice (78% of that from casein), in which virtually 100% of the N is contained in poorly digestible protein bodies (15). Potato, in contrast, contains approximately half of its nitrogen in the form of water soluble compounds (16). The apparently superior digestibility found with the second batch of potatoes (92% of casein control) might have been due to the fact that approximately half of the increased nitrogen was in the form of free amino acids.

Potato protein is generally considered to be well balanced in amino acid composition, although there is evidence that sulfur-containing amino acids may be first limiting for human nutrition (17). Apparent retention of N during all 11 studies (in which potato provided all nitrogen 3.0–5.1% protein-energy) was, as might be expected, inferior to casein. The increase in apparent N retention during the control period following the study diet suggests compensatory retention of nitrogen, although when compared to the preceding control period, the difference was not statistically significant. Apparent retention of nitrogen seemed to be limited in large part by the amount of nitrogen absorbed. The "biologic value" of potato protein (apparent N retention as a percentage of absorbed nitrogen) was calculated to be 49% for potato protein (casein mean, 53%). Compared with previous studies carried out with wheat pasta and rice, potato protein quality expressed as a percentage of apparent N retention from casein (78%) was better than that of wheat pasta (51%) and similar to that of rice (76%).

Although the mean age of the children who participated in these studies is above 1 year, their lower "biological" age and their high protein and energy requirements for catch-up growth allow some generalization to the weaning age. A diet containing 75% of energy as potato is close to the maximum intake of this staple that can be tolerated by children. Based on bulk stool output and fecal energy losses, exceeding 50% of energy as potato may be impractical. The white potato could be a useful weaning food in developing countries at this level if adequate nitrogen content of the potato is assured.

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