Proposal for the
Support of a
RESEARCH PROJECT

HS-4 P

Submitted to :
Science Director
Office of Human Resources and Social Development
AGENCY FOR INTERNATIONAL DEVELOPMENT
Washington, D. C., 20523

1. From: Department of Research
British American Hospital
Apartado 2713
Lima, Peru

2. Title: LYSINE ENRICHMENT OF WHEAT FLOUR

3. Starting Date: July 1, 1967

Duration: 3 years

Principal Investigator: George G. Graham, M. D.

4. RESEARCH PLAN AND OBJECTIVES :

a. Background and Detailed Objectives :

The universal acceptability of wheat, its potential large-scale availability, its ready consumption in many forms, and its excellent digestibility, make this cereal deserve first consideration in programs designed to combat the malnutrition of large populations throughout the world. Its protein content is one of the highest among the common cereals but when we compare its essential amino acid composition with that of the commonly used reference proteins we invariably find a considerably lower content of lysine, both in absolute terms and in its relation to the other essentials. This does not mean that in actual practice wheat protein is always used only to the extent of its available lysine, with the consequent surplus of other essential amino acids being wasted. In many wheat-based diets there are commonly included protein sources with a surplus of lysine, particularly fish and legumes, which effectively complement wheat protein and make other amino acids, particularly the sulfur-containing ones, turn out to be first-limiting in the total diet. In many other situations, when wheat is likely to be the main if not the only source of dietary protein, this potential wastage of essential amino acids is a matter of real concern. This is particularly true of mass feeding programs based on wheat.

It is quite conceivable that adults and older children might consume in the normal course of events enough ordinary wheat flour to satisfy their minimum requirements of lysine, and this has indeed been demonstrated (1). In pre-school children, with a higher requirement of nitrogen and lysine per unit of body weight, this becomes considerably more difficult, and in the infant it is a practical impossibility.

A one year old child weighing 10 kg, requiring 90 mg of lysine (2) and

90 Calories/ kg of body weight/ day, and consuming as his only source of protein a wheat flour with 11 % protein and 2.5 g of lysine/ 100 g of protein, would have to consume 327 g of flour daily to satisfy his requirement of lysine. This amount of flour alone would provide him 3.6 g of protein and 118 Calories/ kg/ day. If he could consume such a diet, which is impossible, our experience tells us that he would not only become obese but that he might also develop a fattly liver and hypoalbuminemia. If the same child consumes as the only source of protein a wheat flour with 21 % protein and 2.67 g of lysine/100 g of protein, he would require only 160 g daily to satisfy his lysine requirement. This amount would provide him 3.4 g of protein and 58 Calories/kg/day, leaving room for a source of fat and some additional carbohydrate. Such a diet is readily conceivable, and although it might prove somewhat monotonous, it seems that the general availability of such a flour would be most useful as the major item of the diet for thousands of people.

It is evident that ordinary wheat flour cannot possibly be the exclusive or nearly exclusive source of protein in the diet of infants and young children without the addition of appropriate amounts of lysine, either as the amino acid alone, or in the form of a protein particularly rich in lysine such as fish flour. Even in the case of a high protein wheat flour the correction of the lysine deficiency would give it a considerably wider margin of safety and improve the utilization of its protein. The addition of a lysine-rich protein has the advantage of increasing the protein content of ordinary wheat flour, but in so doing attention must be given to the possibility of creating a deficiency of another essential amino acid or an amino acid imbalance. We have carried out extensive evaluations of wheat flour enriched with fish protein concentrates at the 3 % and 10 % levels. At the 3 % level lysine was still the first limiting amino acid and although the biological value, as determined in the human infant, was considerably increased over that of wheat flour alone, it was still below that of wheat flour enriched with 10 % fish protein concentrate, as

measured by weight gains and nitrogen retentions. At the 10% level these indices were definitely superior but serum albumin levels after prolonged feeding were significantly lower. This, plus the inability to correct significant hypoalbuminemia (3) were thought to be due to another amino acid deficiency or imbalance. In similar studies, enrichment with milk solids enhanced the biological value of wheat flour for the human infant but still left lysine as clearly first-limiting.

There has been a great deal of controversy in the literature over the lysine requirement of growing infants. Much of this has resulted from attempts at defining this in absolute terms. The best-known studies (2, 4) were carried out with different sources of amino acids, different levels of total protein in the diet, and what is probably most important, under different physiological states, the second set of studies having been carried out with infants who were on a lysine-deficient diet for a number of weeks prior to the study. As a result, this latter study probably over-estimated grossly the lysine requirement. More recent opinion suggests that estimates of absolute requirements have little practical meaning, and that these must be expressed in relation to the caloric content of the diet, its protein content and that of the other essential amino acids, and the physiological state of the individual. As a standard of reference for the balance among the essential amino acids, the pattern in human breast milk has recently been one of the two adopted as most useful (8) and for studies in the human infant is obviously the most logical standard of reference.

The purpose of this study is only one: to determine the optimum amount of lysine which must be added to wheat flours to make them most effective as the main or only source of protein in the diets of growing infants and pre-school children. We propose to study a particular wheat flour alone and at three different levels of lysine supplementation and will first discuss the rationale for these. It is understood, however, that as different wheat flours vary in

their protein content and amino acid composition, these same calculations would result in different levels of supplementation to achieve comparable results for different flours.

b. Concepts:

In the past we have found it impossible to support satisfactory growth in infants or very young children with wheat flour as the only source of protein. The availability of a wheat flour with approximately 21 % protein might make it possible for us to attempt these studies successfully, as it should permit us to increase the wheat protein content of the diet enough to obtain gains in weight and retentions of nitrogen comparable to those from casein diets or modified cow's milk. If a steady rate of growth is obtained, we can then measure other indicators of protein adequacy: serum proteins, plasma amino acids, liver histology and hematologic values, all of which tend to remain normal when growth is limited but which can be adversely affected when growth is supported by imbalanced proteins.

For the first level of supplementation, and using the pattern of human breast milk protein as the reference, we propose to add enough lysine to bring its content relative to the other essential amino acids up to that of the next-limiting essential amino acid. The following calculations are based on the amino acid composition of the wheat flour we propose to use, which has a 21 % protein content. In Table I are presented the essential amino acid compositions of human breast milk and of this flour. The first column shows the content of the 10 essential amino acids in human breast milk expressed as grams of each in 100 grams of protein. The total content of these is 48.8 grams in 100 grams of protein. The second column shows the content of the same 10 essential amino acids expressed as milligrams of each in 1 gram of total essential amino acids. The third column gives the content of the same amino acids in the wheat flour protein, as grams of each in 100 grams of protein. The total content

of the 10 is only 33.77 grams/100 grams of protein. The fourth column gives the content of the same amino acids as milligrams of each in 1 gram of total essential amino acids. The fifth column expresses the relative content of each essential amino acid in the wheat flour protein (column IV) as a percentage of its relative content in human breast milk protein (column II). For this purpose the two aromatic amino acids and the two sulfur-containing amino acids have been considered as single ones. Histidine, although essential for young infants, has not been considered.

It will be seen that the relative value for lysine in the wheat flour is 61 % of that in human breast milk. The next limiting amino acid, based on these data, is threonine, with 88 % of the relative content. Although this amino acid has been shown to be consistently the second limiting for the rat, similar evidence for the human is inconclusive and often contradictory. It is to be expected that these studies will help clarify this point. In order to bring the relative content of lysine up to that of threonine we would have to add 1.16 grams of lysine to 100 grams of wheat flour protein, the equivalent of approximately 0.24 % supplementation of the wheat flour, assuming a 21 % protein content. Lest this level seem high, we must remember that if the protein content were only one half of the given value, as in most ordinary wheat flours, this would amount to 0.12 % supplementation. As a result of the proposed level of addition, the lysine content of the wheat protein would then be 3.8 grams/100 grams of protein, or 110 milligrams of lysine/gram of essential amino acids. Assuming a protein intake of 2 grams per kg of body weight per day, an infant consuming this enriched wheat protein as the only protein in the diet would theoretically receive 76 mg of lysine/kg/day, which is just below the lower limit estimated by Snyderman et al (2). With the unenriched wheat flour, a child with the same protein intake would be receiving only 53.4 mg lysine/kg/day.

The second level of supplementation was chosen on the basis of the

content of lysine relative to the other essential amino acids in human breast milk. In the table we can see that this is 129 mg of lysine/gram of essential amino acids. To reach this figure we would have to add 1.94 grams of lysine to 100 grams of wheat protein, or approximately 0.4 grams/100 grams of wheat flour. For a 10.5 % wheat flour of similar amino acid composition this would represent 0.2 % supplementation. If we again assume an intake of 2 grams of enriched wheat protein/kg of body weight/day, such an infant would then be receiving 90 mg of lysine/kg/day, which is the average requirement estimated by Snyderman et al. While at the first level of supplementation lysine and threonine would presumably be equally limiting, at this second level threonine should appear as first limiting, if indeed it is second limiting in wheat for the human. The plasma amino acids after an appropriate length of time on this diet might well confirm this, as they have in the rat studies of McLaughlan (5).

Referring again to the table, we see that the lysine content of human breast milk is 6.3 grams/100 grams of protein, while that of the wheat flour is 2.67 grams/100 grams. Our final level of supplementation, by adding 3.87 grams of lysine to 100 grams of the wheat protein, should make its content almost exactly that in human breast milk protein. This would be accomplished by 0.81 % lysine supplementation of the 21 % wheat flour, and is the approximate equivalent of 0.4 % enrichment of ordinary flour. An infant consuming 2 grams of this enriched wheat protein/kg of body weight/day would be receiving approximately 126 mg of lysine/kg/day, a figure above the maximum reported by Snyderman et al (2) but well below that suggested by Albanese (4). This level of supplementation is only a fraction higher than the one used by Bressani et al (6), which was based on the original FAO reference protein. As his diets included a significant amount of wheat gluten, which is particularly low in lysine, it probably did not represent an excess, as it well might in our studies, almost certainly bringing out clearly biochemical and clinical signs of deficiency of the

next limiting amino acid when the diet provides a moderate or low intake of protein and a generous supply of energy.

c. Methods of Procedure :

The use of a dietary protein source for large-scale consumption by infants and small children requires that in the presence of adequate amounts of other nutrients it be able to support normal growth for indefinite periods of time at a level of approximately 8 % of total calories, maintaining normal levels of serum proteins, plasma amino acids, liver lipids and hematologic constants. Although it is recognized that it is possible to satisfy these requirements by providing considerably higher intakes of inferior proteins, this entails a high renal solute load and the consequent risk of embarassing water and electrolyte homeostasis under stress. In order to establish this for wheat flour enriched with lysine, we propose to compare the rates of weight gain, nitrogen retentions, stability of serum proteins and other biochemical measures when casein is the source of dietary protein, with similar measures when the various enriched flours are the source of protein in the diet of normal or convalescent children. When these criteria are satisfied by one or more of these flours, they will then be given as the only source of protein, at similar calorie/protein ratios for prolonged periods of time, following the rates of growth in weight, height and bone age, serum proteins, plasma amino acids, liver histology, and the excretions of creatinine and hydroxyproline. These children will be supervised closely for any indication of nutritional inadequacy or toxicity. If these results are satisfactory, the corresponding flours will then be tried in the initial nutritional treatment of infants with severe marasmus or kwashiorkor, measuring their ability to correct different forms of depletion. Finally they will be tried in the diet of very young infants, whose amino acid requirements are different from those of older subjects.

Severely malnourished infants, almost all marasmic and many with superimposed kwashiorkor, are admitted to our hospital for treatment and study. They are hospitalized in the Pediatric Unit until infections and the acute electrolyte disturbances are brought under control, when they are transferred to the Metabolic Unit for study. For long-term observation on a particular diet they are transferred to the Convalescent Unit. Once their acute nutritional disturbance is brought under control they become available for studies of protein quality.

In 4 recovered infants we will determine the minimum protein and calorie intake from a casein formula (simulating human breast milk in its fat and carbohydrate contents) which will consistently maintain a steady rate of weight gain and normal levels of serum proteins. A metabolic collection of at least 6 days will then be carried out to estimate nitrogen absorption and retention, as well as mineral and fat balances. Each infant will then be given one of the 4 wheat flours under study as the only source of protein in the diet at isonitrogenous and isocaloric levels, recalculated daily on the basis of body weight, for a period of one month. By using isonitrogenous diets for each subject we eliminate the possibility that any effect of lysine supplementation might have been produced by non-essential nitrogen. Intakes of the major minerals will also be kept constant, and complete mineral and vitamin mixtures will be given daily. During this time the balance studies will be repeated, the rates of weight gain noted, and the evolution of serum proteins followed. Plasma amino acids will be measured at the beginning and end of the same period, after which the casein formula will again be given and balance studies repeated. Experience has shown that increased rates of weight gain and nitrogen retention from casein or milk in the period immediately following a test protein are indicative of inadequacy, which might otherwise not have been apparent. Following a two week period on casein, the remaining three wheat flours will be given in the same manner, with intervening periods of casein, repeating the same studies during each different diet period.

In this manner each subject will receive all four products, but in different sequence (a, b, c, d - b, d, a, c - c, a, d, b - d, c, b, a). If this scheme fails to bring out clear-cut differences, an additional four subjects will be studied in the same manner. If, as expected, the unenriched wheat flour results in very poor weight gain and retention of nitrogen in all four subjects, a separate study with at least two subjects will be carried out at various different higher levels of protein intake. The level at which these parameters equal the same for the casein diets will be used for long-term studies with unenriched wheat, with particular attention to renal concentrating ability and its ability to handle the higher solute load.

The enriched wheat flours which produce satisfactory results in the above comparative studies will then be given as the only source of protein, at similar protein/calorie ratios, for periods of between 4 and 6 months to a minimum of two infants who have been pretreated with milk, noting rates of growth and the previously listed measures. With advancing age and state of recovery the caloric requirement diminishes steadily, so that if the protein/caloric ratio is kept constant, the protein intake per unit of body weight will also be decreased. Liver biopsies will be carried out at the beginning and end of each long-term study, while plasma amino acids will be measured at monthly intervals. The niacin content of the vitamin mixture which we have been using is low, allowing for detection of marginal tryptophan intakes through the measurement of the excretions of niacin metabolites and pyridones. If biochemical or clinical evidence of niacin inadequacy becomes apparent, its intake will promptly be increased.

If long-term results confirm the nutritional value, at practical levels of intake, of one or more of the enriched flours, they will then be used as the only source of dietary protein in the initial treatment of two or more marasmic infants, two or more cases of kwashiorkor, and two or more infants under six months of age.

We estimate that 4 subjects will be necessary for the comparative studies, a maximum of 10 for the long-term studies, and probably 6 for the initial treatment with the flour which proves most satisfactory. If any of the 4 products proves obviously unsatisfactory in the comparative studies, it will naturally not be carried on to the long-term studies, with the exception of the unenriched wheat, which will be tried at higher levels of protein intake.

The wheat flour will be mixed dry with the other ingredients of the diet, water will be added to provide an adequate but not excessive intake, and the blended mixture will be cooked slowly for at least 5 minutes after reaching the point of boiling. Enough cane sugar and cottonseed oil will be added to the flour to achieve the desired caloric intake, which will usually be between 100 and 125 Calories/kg/day, maintaining the same proportion of fat and carbohydrate as in human breast milk. The liquid mixture will always be fed from a nursing bottle, assuring a minimum of spillage and an accurate estimate of the amounts consumed.

d. Discussion :

The studies with the unenriched wheat flour should serve to confirm the fact that at the usual level of casein or modified cow's milk protein which is necessary for continued growth and normal serum proteins in our subjects, and' which is usually 2.0 grams of protein/kg/day, unenriched wheat flour, because of its lysine deficiency, will not support adequate growth at a level of caloric intake which has previously been shown to be adequate. We will be interested in knowing if such a diet, continued for some days or weeks, results in other detectable manifestations of protein inadequacy and a marked fall in plasma lysine levels. Also, if by increasing the level of unenriched wheat protein intake, satisfactory growth is obtained without detectable adverse effects.

The first level of supplementation should result in weight gains and nitrogen retentions which are slightly inferior to those from casein and might reveal a similar but moderate fall in lysine and threonine of plasma. At the second level, and particularly at the highest level, we might see superior weight gain and nitrogen retention, but with a more marked fall in plasma threonine, possibly accompanied by hepatic steatosis and hypoalbuminemia, particularly after long-term feeding.

On the basis of our experience with other protein sources, a prediction of the results which might be obtained in the initial treatment of severely malnourished subjects is difficult.

The studies carried out by Bressani et al (6) with a mixture of wheat flour and gluten at a level of protein intake which is virtually the same as the one we will most likely be using in most subjects, demonstrated that lysine supplementation at levels close to our highest proposed level produced nitrogen retentions similar to those from milk. Further supplementation with 1 to 5 of the next limiting amino acids demonstrated no consistent further improvement, suggesting that at least during their short periods of study the deficiency of the next limiting amino acids did not become manifest. We anticipate that more prolonged feeding of a similar diet will demonstrate whether supplementation with the first-limiting amino acid beyond the level of the next one will bring out biochemical or clinical signs of its deficiency. This may also become apparent during the repletion of a severely malnourished infant. The study of Barness et al (7) was complicated by the fact that variable levels of calorie and protein intakes were used in different subjects and by the fact that during many of the studies potassium was the first-limiting nutrient, making it difficult to draw valid conclusions about the lysine effect. The level of lysine supplementation which they used was similar to our second level and when adequate potassium was given, a significant improvement in nitrogen retention was apparent.

We expect that the present study will allow us to draw valid conclusions as to the amount of lysine which can be added to wheat flour in order to obtain the maximum possible improvement in its protein quality without danger of creating a significant deficiency of other amino acids.

References

1) Widdowson, E. M. and R. A. McCance. Studies on the nutritive value of bread and on the effect of variations in the extraction rate of flour on the growth of undernourished children. Medical Research Council Special Report No. 287. Her Majesty's Stationery Office, London, 1954.

2) Snyderman, S. E., P. M. Norton, D. I. Fowler and L. E. Holt, Jr. The essential amino acid requirements of infants: Lysine. Amer. J. Dis. Child. 97:175, 1959.

3) Graham, G. G., J. M. Baertl and A. Cordano. Dietary protein quality in infants and children. I. Evaluation in rapidly growing infants and children of fish protein concentrate alone and in combination with wheat. Amer. J. Dis. Child. 110:248, 1965.

4) Albanese, A. A. Protein and amino acid requirements of children. In: Protein and Amino Acid Nutrition, ed., A. A. Albanese. Academic Press, Inc., New York, 1959, p. 444.

5) McLaughlan, J. M. Blood amino acid studies. V. Determination of the limiting amino acid in diets. Canad. J. Biochem. 42:1353, 1964.

6) Bressani, R., D. L. Wilson, M. Behar and N. S. Scrimshaw. Supplementation of cereal proteins with amino acids. III. Effect of amino acid supplementation of wheat flour as measured by nitrogen retention in young children. J. Nutrition 70:176, 1960.

7) Barness, L. A., R. Kaye and A. Valyasevi. Lysine and potassium supplementation of wheat protein. Amer. J. Clin. Nutr. 9:331, 1961.

8) FAO Nutrition Meetings Reports, Series no. 37. Protein Requirements. Report of a joint FAO/WHO Expert group. Rome, Italy, 1965, p. 37.

Table 1

Comparison of the patterns of essential amino acids in human breast milk and in a 21 % protein wheat flour.

	Human Breast Milk		Wheat Flour		Relation
	I	II	III	IV	V
	g/100g Prot.	mg/g EAA	g/100g P	mg/gEAA	100(IV/II)
Isoleucine	6.4	131	4.22	125	95 %
Leucine	8.9	183	6.51	193	105 %
Lysine	6.3	129	2.67	79	61 %
Aromatics	10.1	207	8.25	244	118 %
Phenylal.	(4.6)	(94)	(4.8)	(142)	(151%)
Tyrosine	(5.5)	(113)	(3.45)	(102)	(90%)
S-containing	4.3	88	3.62	107	121 %
Cystine	(2.1)	(43)	(2.13)	(63)	(146%)
Methionine	(2.2)	(45)	(1.49)	(44)	(98%)
Threonine	4.6	94	2.8	83	88 %
Tryptophan	1.6	33	1.2	36	109 %
Valine	6.6	135	4.5	133	98 %
Total	48.8	1000	33.77	1000	

5. AVAILABLE FACILITIES

a. British American Hospital

(i) Pediatric Unit and general hospital services

(ii) 12-bed air conditioned metabolic ward

(iii) Diet kitchen

(iv) Biochemistry laboratory

(v) Nutrition and Pediatric library

(vi) Offices

b. Convalescent Unit - Peru

(i) 28-bed capacity at our disposal

(ii) Diet kitchen

(iii) X-ray and routine laboratory

c. Rat colony

(i) Operated jointly with Agrarian University

d. Baltimore City Hospitals

(i) Amino acid analyzer (Moore-Stein) - Phoenix

6. PERSONNEL

HS-4 P

a. George G. Graham, M. D. - Principal Investigator - Soc. Sec. #167-20-2720

Born October 4, 1923, Hackensack, N. J., U. S. Citizen
A. B. (1940) M. D. (1945), University of Pennsylvania
Internship - British American Hospital, 1946-47
Research Resident Pediatrics, Hospital of the U. of Pa., 1951
Resident, Pediatrics, Baltimore City Hospital, 1955-56
Director of Research, British American Hospital, 1960-
Associate Professor of Pediatrics, Johns Hopkins, 1965-
Associate Chief Pediatrician, Baltimore City Hospitals, 1965-
American Board of Pediatrics, 1958; American Academy of Pediatrics, 1965; Society for Pediatric Research, 1965; American Institute of Nutrition, 1964; American Society for Clinical Nutrition 1964; Committee on Amino Acids, FNB, NRC, 1966

b. Angel Cordano, M. D. - Chief Pediatrician

Born June 9, 1929, Genoa, Italy, Citizen of Peru
M. D. (1954) San Marcos University, Lima, Peru
Pediatric Resident, Harper Hospital, Detroit, 1958-60
American Board of Pediatrics, 1960; American Academy of Pediatrics, 1960
Department of Research, British American Hospital, 1960-

c. Gladys Acevedo, M. D. - Pediatrician, Convalescent Unit

Born June 25, 1930, Lima, Peru, Citizen of Peru
M. D. (1958) San Marcos University, Lima, Peru
Interne and Resident, Pediatrics, Crawford Long Hospital, Atlanta 1959-62
Fellow, Pediatric Hematology, Cincinnati Children's Hosp. 1962-63

Department of Research, British American Hospital, 1963 -

d. Robert P. Placko - Biochemist in chief, Baltimore Lab. SS #288-22-2802

Born May 5, 1927, Cleveland, Ohio - U. S. Citizen

Cleveland Clinic - Microchemistry Laboratory 1947-60

Chief Biochemist - British American Hospital, Research Laboratory 1960-66

Research Associate - Johns Hopkins University 1966-

e. Pertinent Publications

1. Graham, G. G.: Infant feeding in Peru. Quart. Rev. Ped. 10: 240, 1955.
2. Graham, G. G. and Morales, E.: Studies in infantile malnutrition. I. Nature of the problem in Peru. J. Nutrition 79: 479, 1963.
3. Graham, G. G.; Cordano, A. and Baertl, J. M.: Studies in infantile malnutrition. II. The effect of protein and calorie intake on weight gain. J. Nutrition 81: 249, 1963.
4. Graham, G. G.; Cordano, A. and Baertl, J. M.: Studies in infantile malnutrition. III. The effect of protein and calorie intake on nitrogen retention. J. Nutrition 84: 71, 1964.
5. Graham, G. G.; Cordano, A. and Baertl, J. M.: Studies in infantile malnutrition. IV. The effect of protein and calorie intake on serum proteins. Amer. J. Clin. Nutrition 18: 11, 1966
6. Graham, G. G.; Cordano, A. and Baertl, J. M.: Studies in infantile malnutrition. V. The effect of dietary protein source on serum proteins. Amer. J. Clin. Nutrition 18: 16, 1966.
7. Graham, G. G.: Infantile diarrhea and malnutrition. Bull. Pan Amer. Health Org. 60: 46, 1966.
8. Cordano, A.; Baertl, J. M. and Graham, G. G.: Copper deficiency in infancy. Pediatrics 34: 324, 1964.
9. Cordano, A.; Baertl, J. M. and Graham, G. G.: Growth sequences during recovery from infantile malnutrition. J. Pediatrics 63: 698, 1963.

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11. Cordano, A.; Placko, R. P. and Graham, G. G.: Hypocupremia and neutropenia in copper deficiency. Blood 28:280, 1966.

12. Graham, G. G.: Growth during recovery from infantile malnutrition. J. Amer. Med. Wom. Assoc. 21:736, 1966.

13. Cordano, A. and Graham, G. G.: Copper deficiency complicating severe intestinal malabsorption. Pediatrics 38: 596, 1966.

14. Graham, G. G.: Johns Hopkins Conjoint Clinic on Vitamins. J. Chron. Dis. 19: 1967, 1966.

7. BUDGET ESTIMATES FOR ALL YEARS OF SUPPORT REQUESTED FROM AID

DIRECT COST IN DOLLARS

Category	1st year	2nd year	3rd year
Personnel Salaries	22,100	30,694	32,229
Equipment	11,500	1,500	1,500
Supplies	3,000	3,000	3,000
Travel-foreign	900	900	900
Hospitalization Costs	2,400	2,400	2,400
Subject Costs	600	600	600
Publication Costs	300	300	300
All other direct	400	400	400
Administration	1,200	1,200	1,200
TOTALS	42,400	40,994	42,529
TOTAL FOR ENTIRE PROPOSED PROJECT PERIOD			$125,923

Research Support from Other Sources: Active

British American Hospital - General Support	30,000/year
Convalescent Unit - General Support	22,000/year
Various - supplies	6,000/year
NIH - AM-09137 - Research grant to 12/31/67	45,000/year
NIH - AM-09980 - Research grant to 8/31/71	90,000/year
AID/csd-1433 - Research contract to 6/30/69	57,000/year

8. BUDGET CATEGORIES FOR FIRST YEAR (DIRECT COSTS) IN DOLLARS

Description		Amt. req. from AID
Personnel	%time/effort on project	
George G. Graham, M. D., Principal Investigator	10%	
Angel Cordano, M. D., Chief Pediatrician	10%	
Additional Pediatrician	100%	12,000
Robert P. Placko, Biochemist	25%	2,400
Reg. Nurse, Convalescent Unit - 6 mo.†	100%	1,400
Reg. Nurse, Metabolic Unit - 6 mo.†	100%	1,200
Nurse aides (2) - 6 mo.†	100%	1,000
Dietitian - 6 mo.†	100%	1,300
Asst. Dietitian - 6 mo.†	100%	400
Secretary - 6 mo.†	100%	900
Asst. Biochemist - 6 mo.†	100%	1,500
Equipment		
Phoenix M-7800 Automatic Amino Acid Microanalyzer		11,500
Supplies		
Diet ingredients		600
Glassware, reagents, replacement parts for analyzer		2,400
Travel - foreign		
Principal Investigator to Peru - fare and per diem		900
Hospitalization costs - subjects - British American Hospital and Convalescent Unit		2,400
Subject costs - transportation between units		600
Publication costs		300
Books, journals, correspondence and other minor costs		400
Administrative costs, British American Hospital		1,200
TOTAL		$42,400

† These persons are presently supported by NIH-AM-09137 and will continue to be until 12/31/67

9. OTHER RESEARCH PROJECTS CURRENTLY UNDERTAKEN BY PRINCIPAL INVESTIGATOR

a. Infantile diarrhea and malnutrition
Sponsored by: NIH, USPHS
Amount and years of support: 90,000/year until 8/31/71

b. Critical evaluation of new protein sources
Sponsored by: AID
Amount and years of support: 57,000/year until 6/30/69

10. OTHER SPONSORS

None

George G. Graham, M. D.
Principal Investigator
Director of Research

Sister M. Venard
Administrator

British American Hospital
December 19, 1966