

Proj. No. 9310492-
PN- (2)

931-17-560-492

PROJECT STATEMENT

PD-AAC-812-B1

January 27, 1971

A. Project Summary

14p

1. Statistical

Project Title: Nutritional Improvement of Rice by Fortification with Synthetic Amino Acids and Vitamins

Extension

Principal Investigator: Stanley N. Gershoff
Associate Professor of Nutrition
Department of Nutrition
Harvard School of Public Health
Boston, Massachusetts 02115

Duration: RAC approved 6/30/68 to 6/30/70
extended to 6/30/71 (no extra funding)
Proposed Extension Period: 7/1/71 to 6/30/75

Previous Project Funding	\$213,722
Total Estimated Cost of Extension	\$405,181
Funding by Fiscal Years - FY-1971	92,347
FY-1972	100,210
FY-1973	104,114
FY-1974	108,510

931-17-560-492

CAD-2170

Project Manager: I. Hornstein, TA/N

2. Narrative

A promising means of improving protein nutrition in the developing world is by fortifying the food cereal grains of the country with those nutrients in which they are deficient. Rice nourishes more of mankind than any single food, but rice is low in protein content and deficient in several nutrients including the essential amino acids, lysine and threonine. For rice fortification programs to be introduced by governments on a national scale it is essential to show that rice fortification results in meaningful improvement of the health of people. This ongoing project in Thailand has as its objective to show the utility of rice fortification by evaluating the quantitative and qualitative effects on infants and young children of fortifying rice with lysine and threonine plus vitamins A and B and iron. Fortification is accomplished by adding to natural rice at the village mill, synthetic "rice fortified grains," (RFG) which simulate rice in shape, appearance, and cooking characteristics and incorporate the desired nutrients. The initial phases of this study were designed to evaluate the feasibility of initiating a full field study of the effectiveness of a rice amino acid fortification program in Thai villages. The results of this work have been most encouraging and this proposal requests funds for a full field study of rice fortification in Thailand.

B. Expanded Narrative Statement

1. Project Description and Background

This proposal has as its objectives the establishment of fortification levels and the evaluation of the quantitative and qualitative effect on infants and young children of fortifying rice with the limiting amino acids, lysine, and threonine. Fortification additives will also include vitamins A and B to offset the possibility of developing xerophthalmia or Beri-beri, iron will also be supplied since iron deficiency anemia is common. Fortificants will be added in the form of "rice fortified grains" (RFG) at the village mills. The required field studies will be conducted in 25 villages within less than an hours drive of Chiang Mai in northern Thailand. This area has been selected because villages whose inhabitants have the nutritional status characteristics necessary for the study are available. Further there is a medical school and two hospitals in Chiang Mai, and the relevant personnel in these institutions have assured their cooperation. The full assistance of the provincial health department has also been promised. The studies will be conducted in association with Dr. Amon Nondasuta and Mr. Uthai. The cooperation of these men has contributed much to the current success of the project.

1(a) Progress Report

The specific objectives, carried out in three phases can be summarized as follows:

Phase 1 was a survey phase conducted in Thailand to (a) determine the patterns of rice marketing and distribution; (b) ascertain the kind of fortification which would reach the greatest number of people; (c) collect information on the food habits of village populations who might be used for mass feeding trials in future research and; (d) to collect rice samples for use in Phase 2. This phase 1 involved visits to institutions, villages, day care centers, health centers, and conversations with essentially all professional and administrative personnel in Thailand who have authority for the conduct of nutrition programs in Thailand.

Phase 2 was designed to determine the amount of lysine and threonine which provide optimal growth stimulation when added to rat diets whose sole source of protein was provided by Thai Rice. These studies were conducted by Dr. D.M. Hegsted, professor of Nutrition at Harvard. The amount of fortificants added to the RFG has been based on the results of these investigations.

Phase 3 is a pilot study, essentially complete, where the feasibility of fortifying rice was evaluated. The original concept was to study pre-school children in closely controlled environments such as orphanages. However, the initial survey indicated that institutionalized Thai pre-school children received such excellent care that their use in supplementation studies was contraindicated. It was therefore decided to use all the pre-school children in each of 4 villages for the pilot study. The total population of each of the selected villages is approximately 650-800, about 60-80 in each village are pre-school children. There is one rice mill

in each village making the logistics of fortification fairly simple. The studies were centered around the day care centers in each village. The centers' major function (in the context of this study) is to serve as the operational base for the study. These centers are simple one-room thatch covered buildings measuring around 25 feet square, usually without walls. They serve as a place where village pre-school children from 2-6 years of age can be taken in the morning, left for the day, and picked up in the late afternoon and taken home.

These centers have from 10 to 90 children, depending on the size of the village, and acceptability of centers by the villagers. The centers are attended by one or two mothers who are given guidance by a supervisor trained by the Health Department. In the centers, the pre-school children get a variety of instructions and lunch. The food is brought in the morning from home with the child.

In the pilot study, the villages have been treated in the following ways:

- (a) Raw Control: No intervention except for surveys
- (b) Placebo Control: Day care center and RFG
- (c) Restricted Treatment: Day care center and RFG containing thiamine, vitamin A, and ferric phosphate.
- (d) Full Treatment: Day care center and RFG containing thiamine, vitamin A, ferric phosphate, lysine, and threonine.

Before feeding of the RFG was started, the pre-school children of the villages were given clinical examinations, had hand-wrist x-rays taken, had a fecal parasite examination made, and had blood and urine samples taken for hemoglobin, hematocrit, and urinary thiamine, and creatinine analysis. The data indicated that heights and weights of the pre-school children were considerably below the 50th percentile of the Iowa students. Growth in the village children was also considerably poorer than in the children of middle class Bangkok parents. Head circumference were also much smaller than of Iowa children of the same ages. About 25% of the children examined showed delayed bone growth.

Mean hemoglobin and hematocrit values appeared normal. This is probably related to the fact that intestinal parasites were detected in only about 10% of the children.

Experience to date have clearly shown the advantages of conducting a pilot study before launching into full field studies. Although conditions were almost ideal, problems were to more than justify the pilot study. The following are examples of difficulties:

- (a) To add the RFG proper feeders had to be designed and constructed, (this is a key part of the experiment). Such feeders have been developed satisfactorily. The millers in the villages have proven most cooperative. In fact, Representatives of the Division of Nutrition of the Thai ministry in each village each day checked that the RFG was being added properly to the rice as it was milled.

- (b) Problems rose in the field with the RFG. Under the environmental conditions in which they were stored, the grains caked badly. Eventually a satisfactory RFG was prepared by the Ajinomoto Co.
- (c) Having finally gotten the Ajinomoto Co. to prepare an apparently satisfactory RFG, the Thai government forbade the importation of these grains from Japan. Apparently there is a Thai law against importing rice. This problem took two months to resolve
- (d) Improvements were introduced in the methods for taking anthropometric measurements.
- (e) An improved clinical data sheet was developed which will permit all data to be put on IBM cards. Arrangements have been made in Bangkok for the necessary card punching.
- (f) The desirability of developing a program for measuring the effect of nutrition programs on mental development in conjunction with the field study became apparent. The feasibility of such a study was explored.

Progress reports are attached as Appendix 1.

2. Significance of Project to AID Objectives. These were adequately described in the initial project summary and are repeated here.

A prime objective of the War on Hunger is combatting malnutrition in the less developed areas of the world. One of the most effective means of improving the protein nutrition in the developing countries is by fortifying the food cereal grains of the country with those nutrients in which they are deficient. Rice nourishes more of mankind than any single food, but rice is low in protein content and deficient in two essential amino acids - lysine and threonine. Most of the rice eating populace have no additional source of protein in the diet which is markedly significant in the growth, health, and development of children during the weaning period and for several years after.

A "rice pill" has been developed recently which has the appearance of a grain of rice and can contain varying amounts of lysine, threonine as well as thiamin and Vitamin A and other nutrients. The incorporation of this "pill" with polished rice would appear to be an effective means of fortification. Before countries institute massive fortification programs, they must know the optimum levels of fortification required for all additives. Further, there should be evaluation of the nutritional effects under actual field conditions.

3. Relevance of Project to Existing Knowledge. These were adequately described in the initial project survey and are repeated here.

The fortification of cereal grains and rice with thiamin, vitamins, and other nutrients has been practiced for 20 years or more. Fortification with the essential amino acids has become economically possible only in the last few years. The price of amino acids has been a major deterrent to fortification, but lysine and threonine are now available at a price which will permit

their use in fortifying foods for mass distribution. A lack of appropriate techniques for incorporating the supplementary material, particularly in the case of rice, has also been a limiting factor. The development of the "rice pill" which maintains its structure throughout the various cooking procedures used with rice now provides a means of adding the amino acids, vitamins, and iron uniformly to polished rice.

The theoretical benefits of adding lysine and threonine to rice can be determined by the change in the amino acid spectrum of rice which results from the addition of the amino acids. Some limited animal trials have been conducted to verify these benefits and have shown that growth rate is increased markedly with the addition of lysine. They have also shown, however, that lysine alone will not sustain a continuous high growth rate curve.

These tests conducted at Kansas State University indicate that the addition of lysine may result in other ingredients becoming the limiting factor and thus affect the need for more fortification with several other nutrients. Similar studies at the British American Hospital in Lima, Peru with children gave similar results. The lysine and threonine can be beneficial but only when the entire amino acid spectrum and other supplementary ingredients are in balance to produce the quantity of nutrients needed.

This project proposes to establish levels and evaluate quantitatively and qualitatively the effect of fortifying rice with the limiting amino acids, lysine, and threonine, and other nutrients needed.

4. Proposed Work Plan

It is proposed that the 25 villages which will contain approximately 2,700 pre-school children in a total population of about 15,000 people be divided into 5 groups. The design will be as follows:

- 5 villages will receive day care centers and a placebo RFG;
- 5 villages will receive day care centers and an RFG containing thiamine, vitamin A, and Fe;
- 5 villages will receive day care centers and an RFG containing thiamine, vitamin A, Fe, lysine, and threonine;
- 5 villages will not receive day care centers but will receive the amino acid containing RFG;
- 5 villages will act as raw controls.

The villages used in this study all will have their rice entirely milled each in a single village mill to which an RFG dispenser will be attached. The RFG will be added to the milled rice at a level of 1%. The nutrients when used will be present in the RFG at the following levels:

L-lysine HCl	20%
L Threonine	10%
Thiamine	0.05%
Vitamin A	0.02%
FePO ₄	0.8%

In order to make sure that the biological value of the RFG, particularly of the amino acids, remains constant from batch to batch, its nutritional value will be checked regularly in the Boston laboratories. To insure that the RFG is being added properly at the mills the following checks will be employed. All mills will be inspected at least five times a week to make sure that the feeders are working properly and that the miller is adding the RFG to the milled rice. Records of the amount of rice milled and the amount of RFG used will be compared to see whether the RFG is being added at 1%. Urine analysis for thiamine and creatinine in randomly selected villages from villages receiving RFG with thiamine will be made.

The day care centers for pre-school children will be built to serve a variety of purposes. In villages receiving them, they provide immediate evidence of an intention to do something to benefit the village, and will help gain the confidence and cooperation of the people. The building of village day care centers appears to be a practical program in Thailand. The design of this study will help to evaluate the benefits to be derived from their use. By keeping careful daily attendance records in the day care centers, including reasons for absence, data can be obtained on the effect of rice fortification on morbidity in preschool children which would be difficult to obtain otherwise.

At the beginning of the full field study a map and full census of each village will be made. All pre-school children will be examined whether or not their parents elect to send them to the day care center. A family history will be obtained and an ICNND-type physical examination will be made. Biochemical analyses will be restricted to hemoglobin, hematocrit, and urinary thiamine and creatinine. Hemoglobin and hematocrit determinations will also be obtained on women of child bearing age.

The following anthropometric measurements will be made: weight, reclining length, hand-wrist x-rays, triceps, and subscapular skinfold thickness, and head, arm, and chest circumferences. In addition, all pre-school children will be examined for intestinal parasites, and records will be kept of birth weights and deaths in the villages. Anthropometric measurements will be made every six months, and a full examination once a year. The data obtained will be recorded on a card which includes a coding system for transcription of data to IBM machine sorted punch cards.

This study provides an ideal setting to show whether or not the anticipated improvement of nutritional status of village children receiving fortified rice is also associated with improvements in intellectual and psychological development. The development of appropriate tests which are "culture-specific" is a major undertaking. It will be necessary to identify a Thai psychologist who can work on the development of suitable tests for village children. Psychologists in Bangkok and Chiang Mai have been contacted who are very interested in generating such tests in Thailand. It is expected that it will take at least 2 years to develop tests of mental development and motor development, and performance for use in Thailand. This can be done while the fortification program is under way and the tests can be worked out

in villages comparable to the ones in the full field study. It is important to minimize outside influence in the fortification study villages until the initial objectives of the study have been realized. Only then will the testing of mental development be introduced.

5. Research Methodology

The data to be collected are basic to any evaluation of the fortification study which will be undertaken. The measurements to be made have been worked out technically and are commonly used in the evaluation of nutritional status.

6. Research Competence

The principal investigator of the proposed field study is Dr. Stanley Gershoff, Associate Professor of Nutrition, Harvard School of Public Health. Dr. Robert McGandy, also an Associate Professor of Nutrition at Harvard, will be closely associated with him in this study.

During the past 2½ years, a closely knit competent research team composed of Thai and American scientists has been formed. Their capabilities have been emphasized by Drs. Altschul, Forman, and Howe, who made an on-site visit to Chiang-Hi this past November.

Harvard University is also responsible for field studies in Tunisia involving the fortification of wheat flour with lysine. This backlog of experience in fortification field studies in two developing countries represents the greatest concentration of such talent in any one institution.

The facilities of the Department of Nutrition of the Harvard School of Public Health and the Division of Nutrition of the Thai Department of Health are available. These include excellent laboratory facilities including amino acid analyzers and facilities for doing animal feeding experiments.

7. Contributions to Institution Building

Most of the research in carrying out these field studies will be done by Thai investigators. The budget call for utilization of a full-time Thai physician, nutritionist, pediatrician, and dietitian, plus a part-time physician, radiologist, and a variety of local assistants, including nurses, midwife, etc.

Included in the budget for '71-72 are funds for training a Thai research fellow in Boston for one year.

In addition to developing a cadre of well trained nutritionists, the Day Care Centers that will be constructed as part of the field study will remain permanent establishments after the study is completed.

8. Utilization Plans

The results of these studies will be published. It is expected that this work will provide quantitative data concerning the health benefits

which can be derived from a rice fortification program. The data obtained will not be applicable to Thailand but also to other rice eating countries. Furthermore, the data will be of some importance to governments contemplating food fortification programs involving foods other than rice, and to governments interested in the development of day care center programs for pre-school children.

9. Budget Analysis

A detailed budget, July 1, 1971 to June 30, 1972, is presented in Appendix 2. It is somewhat surprising that 50% of Dr. Gershoff's salary and 25% of Dr. McGandy's salary, \$18,000 in all, is charged to this project. One would assume that they are full-time employees at the Harvard School of Public Health. Eight round-trips between Boston/Bangkok may be excessive. In other respects the budget seems reasonable. It is advisable to review the project at the end of three years, and decide at that time whether project continuance is merited.

10. Internal and External Reviews

The proposal has been reviewed by TA/N, and Drs. Aaron Altshul, H.L. Wilskie, John McKigney, George Graham, Richard Jansen, and Paul LaChance. In addition, a site visit was made by Drs. Martin Forman, Aaron Altshul, and Eugene Howe in November, 1970. The report of the on-site visit team emphasized not only the excellence of the technical aspects of the study but also the rapport existing between the Thai authorities, the villagers, and the Harvard team. The comments of the reviewers were favorable to moving ahead with the field study. Some suggestions were made concerning the composition of the fortification mix. These suggestions have been conveyed to Dr. Gershoff and it is anticipated that any necessary changes will be made.

11. Proposing Office General Evaluation

TA/N strongly recommends that the field study be funded. Fortification of cereal grains represent one solution to the key problem of supplying low cost, high protein foods. While this project has particular relevance to Thailand, it is equally important to all countries where rice is the major supplier of calories and protein. This test will help make it possible to evaluate the logistics of fortification and determine what may be expected to happen as a result of this type of intervention.

Budget:July 1, 1971 - June 30, 1972

a) Salaries and fringe benefits:

Harvard Nutritional Biochemist	50%	\$ 12,970
" Sr. Res. Asst.	25%	2,350
" Animal Technician	25%	1,945
" Secretary/data clerk	50%	4,435
Thai Physician-Nutritionist	\$300/mo	3,600
" Senior Nutritionist	"	3,600
" Pediatrician	200/mo	2,400
" Dietitian	80/mo	960
3 " Village workers	150/mo each	5,400
" Driver	40/mo	480
Temporary Thai Staffing:		
1 physician @ \$15/day, 100 days		1,500
1 radiologist \$ 5/child, 2700 children		13,500
Technician-stool examinations \$1/child (2700)		2,700
Local assistants in surveys (nurses, midwife, etc.)		
\$5/day, 200 days		1,000
Dietary and biochemical		
\$15/day, 100 days		1,500
\$ 5/day, 100 days		<u>1,000</u>
Total Salaries		\$ 59,340

b) Consultant fees: None

c) Non-expendable equipment: None

d) Expendable equipment and supplies:

Laboratory supplies-chemicals, reagents, glassware	2,000
Day Care Center housekeeping supplies	500
Animals & rations	<u>1,000</u>
Total supplies	3,500

e) Travel:

United States--Boston/Washington, 4 round trips	330
International--Boston/Bangkok, 6 RTs @ \$1310	7,860
Per diem Thailand, 150 days @ \$18	2,700
Thailand--Bangkok/study area travel expense	<u>1,200</u>

Total Travel	12,090
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f) Publication costs: None - Year 1

c) Other Direct Costs:

Local (in study area) transport	1,000	
Data Processing Services	<u>2,000</u>	
Total Other Direct Costs		<u>3,000</u>
Subtotal		77,930
h) Indirect costs @ 18.5% Off Campus rate		<u>14,417</u>
Grand Total Requested 7/1/71-6/30/72		92,347

Estimated cost per year and total cost of project:

	<u>Year 1</u>	<u>Year 2</u>	<u>Year 3</u>	<u>Year 4</u>
a) Salaries	59,340	62,300	65,415	68,685
b) Consultant fees	-0	-0	-0	-0
c) Equipment	-0	-0	-0	-0
d) Supplies	3,500	3,675	3,855	4,045
e) Travel	12,090	12,090	12,090	12,090
f) Publication costs	-0	500	500	750
g) Other direct costs	3,000	6,000	6,000	6,000
h) Indirect	14,417	15,645	16,254	16,940
Total/year	92,347	100,210	104,114	108,510

Total Cost of Project - 4 years - \$405,181.

Notes to Budget:

1. Categories a) salaries and d) supplies are increased approximately 5% in continuing years to reflect generally rising salary scales and costs.

2. In the second and succeeding years in category g) other direct costs, local transport remains \$1000 annually and data processing services increased to \$5000 annually.

Notes to Budget:

1. Categories a) Salaries, b) Consultant fees, and d) Supplies are increased approximately 5% in continuing years to reflect generally rising salary scales and costs.

2. In the second and succeeding years in Category g) Other Direct Costs local transport is reduced to \$2500 annually and Data Processing Services increased to \$5000 annually.

3. It should be emphasized that there is no item in the budget for purchase of the RFG pills because they are at this time provided free of charge by the Ajinomoto Company. If at any time in the future there should be a charge for these pills, a supplementary request would be necessary.

Other Research Support of Principal Investigator:

NIH research grant AM 03056-11, Metabolism in Vitamin B₆ Deficiency, 9/1/70-8/31/71, \$47,170; with five-year renewal application pending.

March 1, 1971

ADDENDUM TO PROJECT SUMMARY

for

Extension of Nutritional Improvement of Rice by Fortification
with Synthetic Amino Acids and Vitamins
On-going AID Harvard Contract AID/csd-2170

1. Importance of Fortification Studies

To effectively plan and carry out a nutrition research program relevant to the needs of the LDCs, the Office of Nutrition developed an "AID Nutrition Research Rationale and Program for the '70s." This incorporated not only our own concepts but the ideas of the TA/N Advisory Committee and other leading nutritionists. The "rationale" was reviewed, commented upon and concurred in by the International Committee of the Food and Nutrition Board of the National Academy of Science. The revised version was in turn discussed with and approved by RIGC's predecessor and by AID's Research Advisory Committee.

This study, once again, identified the development of low-cost, high-protein foods as a key research area requiring immediate attention. One solution the addition of limiting essential amino acids to cereal grains is a simple and relatively inexpensive method for making more of the protein already present in cereals available for use by the body. The "added" protein is introduced into the diet without changing food habits, functionality of the cereal or processing procedures.

Other expert nutrition groups that have struggled with the problem of increasing the protein content of the diets in the LDCs have also looked upon amino acid fortification of cereal grains as a most useful approach.

Thus, the report on the World Food Problem by the President's Science Advisory Committee (1967) specifically recommended fortification of Food for Peace cereal grains and of grain grown in the developing countries as a method for increasing the availability of high^{quality} protein in the food supply of the LDCs. And the Protein Advisory Group (PAG statement No. 9, January 1970) urged that large scale fortification studies be carried out in several of the LDCs in order to confirm, under field conditions, the excellent results obtained both in carefully controlled laboratory experiments with animals and clinical studies with humans.

The urgency of fortifying cereal grains will increase with time. Continued population growth insures that the available agricultural land will increasingly be turned to the production of maximum calories. A concomitant effort to maximize the nutritional value of grains with respect to protein, minerals and vitamins is inevitable. Some of this may be done by breeding.- All of this can be done by fortification. The two approaches are complimentary.

Accordingly A.I.D. has assumed the initiative in establishing a world-wide fortification research program involving wheat, rice, corn, and cassava.

2. Harvard-Thailand Rice Project

The Harvard-Thailand rice fortification project now being considered for extension was RAC approved in 1968. Rather than plunge directly into a full scale field study, a pilot project to pave the way for the field study was initiated. Approval of the rice fortification pilot project by the then equivalent of RIGC and by RAC included an awareness that a successful pilot project would lead to a full grown rice fortification field study.

The project since its inception has been carefully monitored by A.I.D. A thorough review of the project was held in Washington in early 1970. The senior members of the Harvard Staff working on this project met with TA/N, USDA, HEW, and industry representatives. The review concluded that the pilot project had successfully prepared the way for the next phase, but that an on-site monitoring visit in Thailand be made prior to proceeding with any full-scale field study. An on-site monitoring visit was made by a 3-man team during November 1970. The evaluation was most favorable and it was recommended that the field study be initiated.

The pilot project has clearly demonstrated that (a) rice fortification grains can be added in the crude village mills, (b) the fortified rice is acceptable to the local population, and (c) the Thai Health Officials are wholeheartedly in favor of the field study and the villagers involved in the study are most cooperative.

In accordance with the RIGC request that the budget and time scale be reviewed, a meeting has been held with Dr. Fred Stare, the director of the project. As a result of our discussions, both the budget and the time have been cut. (a) The proposed extension is for a 4 (instead of 5) year period. (b) The number of villages in the study remains unchanged at 25. (c) The new budget calls for a first year expenditure of \$92,347 (originally \$141,310) and a four year expenditure of \$405,181 (originally \$764,471 for a 5 year budget). ~~The new detailed budget is attached.~~

This drop in funding will prevent TA/N from obtaining the maximum amount of information originally hoped for but the essentials of the program remain intact.

3. Utilization Plans

The importance of improving the nutrition of populations outside normal marketing channels is recognized. An important component of this field study is the development of the methods for reaching people in rural villages.

It is also realized that synthetic rice fortified grains (RFG) developed for use in Thailand may require modifications for use in other rice eating areas. E.G., an altered mineral and vitamin mix may be required in regions where the nutritional deficits differ from those in Thailand; or the grain size and color of the RFG developed for Thailand may not be compatible with

the variety consumed in another country; cooking procedures or the texture of the cooked grain may also require modification of the RFG.

Therefore, during the life of the project, it is planned to hold in Thailand a workshop on rice fortification to be attended by appropriate people from other LDC rice eating countries. Consideration will be given to techniques, problems, costs, acceptability, and potential for adapting the Thai experience in other countries. Provision will also be made for providing technical assistance in rice fortification to interested countries.

Martin T. Forman/TH

Irwin Harnstein