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This study was carried out under a Participating Agency Service Agreement (PASA) between the Office of Health, Agency for International Development, and the Nutrition Program, Center for Disease Control, Department of Health, Education and Welfare, Atlanta, Georgia, 30333.

Individual contracts were established between the Center for Disease Control and the three contractors: Christian Medical College, Ludhiana, Punjab, India; University of Rochester School of Medicine; and Pahlavi University School of Medicine, Shiraz, Iran.

REPORT SUMMARY

A.1. Project Title: Measurement of nutrient loss from the intestinal tract due to malabsorption.

Contract Numbers: PASA RA(HA) 9-69 (HSM 110-69-435, HSM 110-69-425, HSM 110-69-427)

2. Principal Investigators:

a. Betty Cowan, M.D.
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3. Contract Period: 30 June 1969 to 30 June 1973

4. Period covered by Report: 30 June 1969 to 30 June 1973

5. Total AID Funding to Date: $176,228

6. Total Expenditures and Obligations: $166,228

7. Total Obligations for Current Year: $10,000

8. Estimated future Expenditures: None

B. Summary of Accomplishments

Detailed studies of nutriture and absorption were carried out on representative populations of rural villages in three countries of the developing world. A moderately high prevalence of certain measures of malabsorption was found, with considerable geographic and seasonal variation in their nature and magnitude. In none of the populations was significant malnutrition present. To delineate the degree of malabsorption and its relationship to nutritional health, more thorough and carefully controlled studies, over a longer period of time, must be conducted in an appropriate field research facility.

ANNUAL RESEARCH REPORT (FINAL)

Measurement of Nutrient Loss from the Intestinal Tract Due to Malabsorption

A. General Background

According to many experts, intestinal malabsorption is relatively common in developing countries, especially in hot climates, although there is increasing evidence that it may also occur in some temperate climates. Severe and widespread malabsorption may be considered a form of starvation; a type in which a patient may starve although his gastro-intestinal tract contains food. Even minor malabsorption may result in malnutrition, which in turn affects productivity, resistance to infectious diseases, growth, and possibly even mental development. Tropical sprue, a syndrome characterized by steatorrhea, weight loss, and megaloblastic anemia, has been described extensively in the West Indies, India, and Southeast Asia. In Africa, pancreatic insufficiency with calcium deposits in the pancreas has been reported as a frequent cause of steatorrhea. Intestinal parasitism with such pathogens as *Strongiloides stercoralis* and *Giardia* is believed to contribute to malabsorption. Protein deficiency may result in a failure of transport processes across the intestinal mucosa, creating the clinical syndrome of malabsorption.

In 1965, the Agency for International Development, recognizing malnutrition to be an obstacle to social and economic development, placed the *War on Hunger* at the top of its program priorities. As

an outgrowth of this policy, it became important to assess quantitatively the contributory role of malabsorption to malnutrition. The Agency undertook to develop a research protocol for the study of the magnitude of nutrient losses due to intestinal malabsorption. Previous work on this subject is summarized in the attached bibliography (see Appendix A). However, prior research had been focused on individuals or groups of individuals, rather than on entire communities. These earlier studies had indicated that for unknown reasons the degree of malabsorption varies from region to region. It was decided that a project should be designed to be carried out simultaneously in several places, representing the major environmental, cultural and dietary divisions of the developing world.

Finances eventually permitted the selection of three sites for the study: Puerto Rico (contractor - University of Rochester) representing the Caribbean area and a rice-beans-fish dietary pattern; southern Iran (contractor - University of Pennsylvania for Pahlavi University at Shiraz) representing the Middle East and a diet based primarily on wheat in the form of unleavened bread and lamb; and the Punjab region of India (contractor - Ludhiana Christian Medical College Board) representing a wheat-pulses-milk dietary pattern. Originally, East Pakistan (now Bangladesh) was chosen as the third site because it represented an Asian rice culture, but since the initial contractor (SEATO Cholera Laboratory in Dacca) proved unable to carry out the work, a contract was negotiated with Ludhiana Christian Medical College.

After numerous consultations with experts on gastrointestinal physiology, a protocol was developed which established the following methodologies for determining intestinal malabsorption:

Protein. Any amount of stool nitrogen in excess of 2g per 24 hours was deemed to represent either malabsorption or excessive excretion through the gastrointestinal tract. Both stool and urine were collected in order to document complete nitrogen balance (see Appendix B).

Fats. Fat absorption was assessed by measuring fecal fat. Excretion of more than 6g daily was abnormal, when the diet contained 80g of fat (see Appendix B).

Carbohydrates. Carbohydrate absorption was measured by ascertaining levels of D-xylose in the urine after oral ingestion of 25g.

Calories. In Shiraz and Ludhiana bomb calorimetry was performed on food and stools.

Other nutrients. Absorption of certain other nutrients was assessed, and jejunal biopsies made, where possible.

B. Statement of Project Objectives as Stated in the Contract

The objective of the project, as stated in the contracts, was to define quantitatively the magnitude of the waste of nutrient materials in the intestinal tract of randomly selected, apparently normal individuals of all ages and both sexes living in Puerto Rico, Southern

Iran, and the Punjab region of India. It was subsequently mutually agreed to restrict subjects to adults over the age of 15 and to eliminate pregnant and lactating women as well as individuals presenting obvious signs of disease. Although not written into the contracts, AID had two ultimate objectives: 1) to extrapolate the findings from the study sites in Puerto Rico, Iran, and India to a worldwide basis; and 2) to place a monetary value on nutrient losses due to malabsorption.

C. Continued Relevance of Objectives

1. Contract Objectives

The contract objective of measuring the magnitude of nutrient losses due to intestinal malabsorption remained relevant and required no basic modification throughout the life of the project.

2. Unwritten AID Objectives

While it was originally thought that findings arrived at in the three contrasting cultural and ecological sites of Puerto Rico, southern Iran, and the Punjab, India, might provide a basis for estimating worldwide nutrient losses due to intestinal malabsorption, it has become evident that this was not feasible. Seasonal, geographic, and individual differences were found to produce much greater variations in results than expected. Thus, to obtain findings that would be capable of extrapolation would require such a multiplicity of studies as to make the project prohibitively expensive. Moreover, the investigators encountered difficulties in obtaining truly representative population samples.

The problem of financially quantifying nutrient losses due to malabsorption was found to be even more difficult. It is almost impossible to place a dollar price tag on a few grams of excess fecal nitrogen or fat, given the variation in cost of the dietary protein or fat in the original intake. Moreover, the three studies revealed considerable lack of agreement among scientists as to the norms for nutrient absorption, given differences in climate, altitude, genotype and dietary habits.

D. Accomplishments to Date

1. Findings

In Puerto Rico, the Punjab, and southern Iran it was found that the populations examined had a high rate of certain abnormalities related to intestinal absorption. The prevalence of all measures of malabsorption was given as 22 percent in Puerto Rico, 44 percent in the Punjab, and 33 percent in southern Iran. These rates point to a substantial public health problem which needs to be broken down into its components. True, the visible consequences of these anomalies were not always obvious and in each location there were only a few extreme cases of overt sprue presenting the classic symptoms. But according to at least one previous study (at Lalton Kalan in India), there is evidence that additional stresses, such as pregnancy or lactation, may aggravate the situation to a notable extent. It is very likely that other stresses such as intensive physical activity or infectious diseases may also have

major consequences for the health of individuals with malabsorption and, as a result, for the socioeconomic development of the community. This is particularly true in view of the fact that these studies happen to have been made in areas where malnutrition is not severe.

In the Punjab a high prevalence of excess fecal fat was found, but otherwise the investigators did not demonstrate significant losses in protein or calories due to malabsorption.

A challenging result, however, concerned the malabsorption of certain minerals. The Shiraz team stressed that losses of zinc, calcium, iron, and probably other minerals appear to be linked with the presence of phytates in the bread prepared in the surrounding rural areas. Because phytates are found in the seed of all cereals and because cereals are the most promising resource available to win the War on Hunger, the problem of reducing phytate effect without unduly reducing the amount of protein and vitamins in the germ, will present an important challenge in the immediate future.

The importance of seasonal variations in intestinal malabsorption was raised by the investigators. The Puerto Rico research proved that certain abnormalities were twice as prevalent in the winter as in the summer. It should be remembered that foods consumed vary throughout the year, sometimes resulting in changes in intestinal content which may affect absorption as much as the phytates do. The AID-sponsored

research concerned malabsorption from whatever cause, not only from abnormalities of the gastrointestinal tract. A change in a nutrient, making it nonabsorbable, is relevant to the question of wastage. These findings convinced the investigators of the importance of longitudinal studies to sharpen our knowledge of seasonal variations.

All the investigators faced, at one time or another, the problem of "what is normal," for without a clear definition of normality, abnormality cannot be measured. A Caribbean norm may not be an Iranian norm. What is the range of normality for excretion of D-xylose in the urine, or of protein and fat in the stools in relation to dietary intake? It was clear that great uncertainty exists on these matters, and that local norms from the ethnic and geographical standpoint must be ascertained.

The question of comparability of results also proved to be very troublesome, despite the considerable efforts made at the start to insure methodologic comparability. Change of leadership in midstream and the transfer of contract from SEATO Cholera Laboratory to Ludhiana did not help, but other factors also tended to offset the comparability of results. It is necessary for each investigator to adapt to local circumstances, but at the same time to maintain a minimal core of comparability. Every instrument, even when carefully calibrated, has its own "bias" which may be important when dealing with very minute quantities. To obviate this pitfall, some of the group, at the last

general conference, stressed the desirability of doing all tests at a central laboratory, on specimens shipped by air, after uniform collection, processing and packaging in the field.

2. Interpretation of Data and Supporting Evidence

All the investigators found absorptive abnormalities in the populations studied.

a. Puerto Rico

Dr. Klipstein (Principal Investigator) stated that the findings of his investigation indicate that nearly one-half of the study sample demonstrated at least one abnormality of intestinal function. Multiple absorptive abnormalities were present in 6 percent of that population. Projecting this prevalence of 6 percent to the total population of Puerto Rico results in an estimate of 168,000 Puerto Ricans who may have similar dysfunction. However, these malabsorptive findings resulted in nutritional deficiencies in only a small number of people, probably because of the fact that their dietary intake was reasonably adequate, so that mildly impaired intestinal absorption was insufficient to cause a net nutritional deficit. (In contrast, similar investigations conducted in the Dominican Republic by this investigator have shown that the same degree of malabsorption when combined with marginal dietary intake, is associated with nutritional deficiencies.)

b. Southern Iran

Dr. Nasr (Principal Investigator) found that 32 percent of the subjects showed one of several symptoms or signs, suggestive of

malabsorption. Moreover, nearly 50 percent of the subjects showed negative nitrogen balances during the several days under study. Small intestine suction biopsies showed 49 percent to be within normal limits, 28 percent to have some degree of villous atrophy, and 9 percent to be insufficient for diagnosis.

The probable cases of malabsorption were divided into the following abnormalities.

TABLE 1

Summary of Findings
Shiraz 1970-1972

Abnormalities	No. of Cases	Percent
Steatorrhea	3	3
Fecal nitrogen loss	12	12
Both of the above	2	2
Abnormal vitamin B_{12} absorption	12	12
D-xylose malabsorption	3	3
	32	32
No evidence of malabsorption	68	68
Total	100	100

The 100 cases had been selected among adults over 15 years of age. Pregnant or lactating women, and persons with fever or serious disease were excluded.

This investigator documented other findings as possibly indicative of malabsorption.

TABLE 2

Indirect Evidence Suggestive of Malabsorption
Shiraz 1970-1972

Nutrient in Blood	No. of Subjects	Critical Levels
Iron	14	< 70 mcg %
Calcium	13	< 8.6mg %
Zinc	13	< 75 mcg %
Carotene	52	< 60 mcg %
Cholesterol	29	<150 mg %
Albumin	35	< 3.5g %

Despite the frequent findings of structural abnormalities, described as spine-like patterns, Dr. Nasr concluded that there is little malabsorption of major nutrients in the rural population of southern Iran. It is possible that the relatively high percentage of plasma deficiencies in albumin and carotene could be due to inadequate intake from the diet. The most significant manifestation of malabsorption in this population appears to be that of certain minerals, which may be due to the high content of phytate in the village bread. Dr. Reinhold, collaborating with Dr. Nasr, emphasized this aspect of their research. "Consumption of large amounts of phytate in the form of unleavened whole meal bread by the rural population of Iran interferes extensively with the utilization of mineral nutrients, including calcium, iron, zinc, probably magnesium, and phosphorus. Preliminary experiments indicate that the phytate of village bread is more potent in its effect upon calcium and zinc metabolism than is pure sodium phytate. The prevalence of rickets and osteomalacia, retardation of growth and sexual development, and iron deficiency anemia are direct effects of high phytate values.

Practical methods for overcoming the harmful effects of phytate are proposed.... High phytate consumption may be a major cause of malnutrition in developing countries."

Three points come to mind for discussion of this hypothesis.

a. Zinc, in this case, is not unabsorbed because of a lesion of the intestinal wall; it is unabsorbed because it has been made less absorbable.

b. Obviously, the infinitesimal amount of zinc needed for adequate nutrition is so small and the cost so minimal that the magnitude of the waste, if any, and whatever its cause, is also insignificant.

c. Any practical method devised to combat this malnutrition must take care not to deprive the consumer of the protein located in the wheat kernal along with the phytates. This remark applies also to other minerals such as iron and calcium (rickets is prevalent around Shiraz). Hypogonadal dwarfism is common in this region and can be corrected by the administration of zinc, along with a better general diet. The Shiraz investigators also stressed the potential role of yeast which, if used in large quantities, could destroy some of the phytic acid in wheat flour. However, the rural Iranians prefer unleavened bread.

The cost of zinc deficiency could best be expressed in terms of diminished productivity in the population at risk. Such a consideration is relevant to the general purpose of AID and speaks for the development of a research program designed to assess this problem.

The prevalence of iron deficiency anemia is high in Iran, with 30 percent of the population investigated showing levels of hemoglobin below 12.5g/100ml, in spite of an altitude of 1,700m. (Equivalent to about 12.0g/100ml at sea level.)

c. Punjab (India)

Dr. Cowan (Principal Investigator) worked in a region characterized by two distinct socioeconomic groups: one, a "good" income group which owned small plots of land, a few buffaloes, and had access to agricultural machinery and to the high yield wheat of the "Green Revolution." The other, a low income group with no property and little money (less than $4 a month per capita) to buy the food which they cannot grow. Sixty individuals were chosen for study from the first group and 38 from the second. Dr. Cowan alluded to a study made in 1968 at Lalton Kalan, a village 7 miles from Ludhiana where a rather startling prevalence of steatorrhea (41 percent) previously had been observed, although only 2 percent had clinical sprue and none were severely anemic. In the present research, sprue was not observed, but as before, there was a high prevalence of fecal fat excretion of 6.0g or greater, per 24 hours. Forty-three percent of the study population had steatorrhea, as defined by this level of excretion. It was more common among men than women and among the more affluent social group.

Nitrogen absorption was studied with a technique recommended by Drs. Anderson and Reinhold on 11 individuals of the first group and

on all 38 of the second group, making a total of 49 subjects. The usual home diets of these people, as estimated by dietary history, were in general inadequate, except for males in the good income group who consumed more than 50g of protein daily. Among females, protein intake was between 30 and 50g per day. Against the background of this customary dietary intake, the following experimental results were found. Nitrogen balance was negative in only one of the 49 subjects; all the others were in strong positive balance. Fecal excretion was within normal limits in all but two subjects, one of whom excreted 2.65g and the other 2.63g per 24 hours. Thus no significant malabsorption of nitrogen was demonstrated. The reasons for the predominantly positive nitrogen balances remain unexplained.

Calorie loss was studied by bomb calorimetry of both the diet and the stools in 38 individuals from the poor income group. Six of these subjects had fecal calorie loss greater than 10 percent of intake. There was no correlation between bomb calorimetry results and fecal fat excretion, nor D-xylose excretion.

D-xylose excretion was abnormal (5.0g or less in the urine 5 hours after ingestion of 25.0g) in 58 percent of subjects. Again, this laboratory abnormality was more common among men than women, and among the more affluent than the poorer class.

Vitamin B_{12} absorption was abnormal in one subject. Serum folate levels were low (less than 5ngm/ml) in 24 of the 60 in the more

affluent group. There was no correlation between folate levels and other measures of malabsorption.

3. Research Design

In view of the findings discussed above, it was agreed by all concerned that the protocol for any future studies along this line should be modified to include the following:

a. Establishment of normal ranges for each nutrient, in each region.

b. Longitudinal studies to determine seasonal variations, and changes over time, in relation to health status.

c. Centralization of laboratory analyses to insure comparability of data.

In addition, the investigators felt that consideration ought to be given to a number of other possible alterations. For instance, would it be wise at the outset to select, through village screening with D-xylose and beta-carotene tests, a subsample of people who are "abnormal," as suggested by Dr. Anderson? Should the bomb calorimetry method be used in all locations? What further steps could be taken to insure comparability of methodology?

The unwritten AID concept of assessing the financial value of nutrient waste due to malabsorption should be abandoned as being impossible to measure with significant accuracy in the present state of our knowledge. Yet, the probability that intestinal malabsorption may be a serious public health problem in many developing countries is

sufficient justification for continuing to explore the complex components of this problem in the hope of finding a method of prevention.

It is suggested that a new project be established, the characteristics of which would be that all laboratory investigation be done in the same facility, that the methods for population sampling and for collection of body fluids be identical and be strictly adhered to, and that the various local studies all operate under the direction of a central research team. One of the first tasks of the project would be to establish in each locality norms for the values of the most important nutrients. The main task would be to proceed in each location with longitudinal studies carried out at least twice a year for a minimum of 5 years.

At the same time, a study of the dietary, economic and social changes occurring in the selected locations would be recorded. Such an effort would be costly but would be crucial toward our understanding of nutrition and health, as they relate to national development.

E. Dissemination and Utilization of Research Results

No effort has yet been made to disseminate the results because a consensus first had to be reached on the relevance of the objectives, the standardization of methodology, and the significance of the findings. This now has been achieved, and the investigators undoubtedly will publish their individual findings in professional journals. The experience of the past 4 years has shown that the road to the original objectives is far more difficult than had at first been suspected. Suggested steps

for future efforts are outlined in section D3, but cannot be taken under the present contract.

F. Statement of Expenditures and Obligations

These are outlined in the following table:

Fiscal Year	Consultants	Travel Domestic	Travel Inter-National	Contract Services	Equipment	Printing	Total
1969			$ 2,377	$ 65,481			$ 67,858
1970			2,227	82,420			84,647
1971	$2,062	$ 606	70		$1,469		4,207
1972	1,500	650	7,366				9,516
1973	2,400	2,400	4,700			$500	10,000
Totals	$5,962	$3,656	$16,740	$147,901	$1,469	$500	$176,228

G. Work Plan and Budget Forecast for Coming Year

The work under this project has been completed.

H. Appendices (attached)

A. Bibliography

B. Research Protocol

C. Agenda of Concluding Workshop of Investigators and Consultants Washington, D.C., 12-14 September 1972

D. Individual Research Reports from:

1. Ludhiana

2. Puerto Rico

3. Shiraz

APPENDIX A

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APPENDIX B

Protocol

MEASUREMENT OF NUTRIENT WASTAGE FROM MALABSORPTION IN RURAL INHABITANTS

Introduction:

The following protocol has been devised as the result of consultation with several authorities and as the result of a meeting of all concerned held in Shiraz, Iran, May 2-3,1970.

It is essential that each Center follow the basic features of this protocol in similar fashion. As noted previously, modifications and extended research beyond this are expected and welcomed but are not part of the A.I.D. requirements in the basic contract.

Patient Selection:

In each of the three areas to be surveyed, one hundred people of both sexes between the ages of 16 and 50 will be selected at random from a rural area. They must be all of the same socio-economic status and carrying on their normal rural occupations. Persons should be omitted from the study who have evidence of chronic or acute illness, such as any chronic infections, hepatic, cardiac or renal disease or any acute febrile illness. Also excluded are women currently pregnant or recently delivered, and any person recently or currently being treated with vitamins or antibiotics over more than 10 days' time. The persons studied therefore will be representative of the average population. They will be admitted to the hospital in groups for study.

Investigative Procedures:

On the first day (Day 0) each person will have a complete history and physical exam. The history should provide dietary information, such as frequency of meat consumption, types of cereal grains eaten, and kinds of vegetables and fruit usually consumed. Samples for the initial laboratory studies are obtained on Day 0 or the morning of Day 1, and should include the following: complete blood count including blood film, urinalysis, stool guaic test, examination of the stool for ova and parasites, bacterial cultures of blood, urine and stool, total serum protein, albumin, cholesterol, calcium, carotene, blood urea nitrogen, glucose, prothrombin time, serum glutamic oxaloacetic transaminase (SGOT), and alkaline phosphatase. Approximately 15 cc of blood should be obtained, and serum being placed in a folate free tube("Vacutainer," Becton Dickinson and Co., Rutherford, N.J.) for subsequent analysis of serum folate, serum

B_{12}, serum iron and total iron binding capacity. After separating serum from cells, freeze for future analysis. If possible a bone marrow aspiration should be done, the smears being stained with Wright's stain. If not possible to do on all subjects obtain a representative sample.

Intestinal absorption tests are to be started on the day following admission (Day 1) and the test diet begun.

At the beginning of Day 1, the patient is started on the standard diet. This should contain 80 gm of protein and at least 80 gm of fat. A sample diet must be analyzed for nitrogen and fat. At the same time a carmine red stool marker, 1.2 gm, is given by mouth and stool sampling begun. The diet should contain at least 2000 calories and the same diet given every day.

Cobalt^{60}-B_{12}, 0.5 mcg, is given by mouth as the first step of either the hepatic uptake or fecal excretion test for B_{12} absorption. Prior to administration of the isotope, background counts are obtained over the liver, if the hepatic uptake test is used.

Day 2: Breakfast is omitted and a d-xylose test is performed by oral administration of 25 gm d-xylose. Urine is collected for 5 hours, during which time the subject is kept well hydrated by the hourly intake of 100-200 cc water. Urinary excretion of d-xylose is measured by the photometric method of Roe and Rice.

The remainder of the study period is occupied by measurement of fat and nitrogen balance. As soon as the carmine red marker is seen in the stool, complete collections of stool are made. Each stool is placed in the collection container until the second marker (see below) appears. The pool is mixed, weighed, and aliquots taken for nitrogen and fat determination. After six days a second marker is given by mouth. As soon as this appears in the stool, the collection, representing that stool formed during the 6 day test diet, is stopped.

In addition to stool collection, daily 24-hour urine should be obtained for nitrogen in order to have a complete nitrogen balance. This will provide information as to protein utilization as well as absorption or loss.

Interpretation: 1) Fat absorption is assessed from measurement of fecal fat. Any excretion in excess of 6 gm daily is abnormal when the diet contains at least 80 gm fat. 2) Protein wastage is assessed by making the assumption that up to 2.0 gm of stool nitrogen per day is normal. Any amount in excess of 2.0 gm is deemed to represent either malabsorption or excessive excretion into the gut. The number of grams of nitrogen multiplied by 6.25 represents

the amount of protein which is either not absorbed or lost.

Day 8: Hepatic uptake of Co^{60}-B_{12} is measured using surface scintillation counting.

Day 9 (or when stool collection is completed): Jejunal Biopsy. (This is delayed until the end of the study in order to avoid possible bleeding which would introduce an error by contributing to fecal nitrogen. Furthermore, it provides for poor cooperation at the outset by giving an unpleasant procedure).

For jejunal biopsy the Crosby capsule is ideal, as it may be passed on the end of a 100 cm length of pliable tubing (intramedic polyethylene tubing, PC 200, Adams) and without recourse to a fluoroscopic screen. After swallowing the capsule, the patient is placed on his right side until bile is seen to drain from the tubing. He is then placed on his back and when 90 - 100 cm of tubing have passed, a flat plate x-ray of the abdomen will localize the tube at the duodeno-jejunal angle, once the tube is gently filled with hypaque dye. About 20 cc of suction on the tube will fire the capsule, which is then removed and opened. Obtaining the specimen should take 4 hours. The specimen may then be gently sectioned with a razor blade and a portion immediately wrapped in wax paper, placed in a plastic test tube in dry ice and quick frozen by pouring acetone around the tube. This portion is then stored at 20° for subsequent analysis of disaccharide enzymes. The other portion is gently spread, villi upwards, on a piece of filter paper or blotting paper and immersed in 10% buffered formalin for subsequent histologic preparation (hematoxylin and eosin) and examination. If a dissecting microscope is available, the specimen may be examined grossly and classified as fingers, leaves, convolutions, or flat.

The subject may then be discharged to his village after being placed into one of three categories for daily treatment: * (1) placebo; (2) 5 mg of folic acid twice daily with 1000 mcg B_{12} subcutaneously on discharge and every two months; or (3) tetracycline, 250 mg twice daily. A worker should visit the village weekly to ascertain that treatment is progressing as scheduled. At the end of six months appropriate subjects are brought back to the research ward and re-studied.

*For the Puerto Rico and Ludhiana groups only. The Iranian patients will not be treated.

APPENDIX C

CONCLUDING WORKSHOP OF THE TRIPARTITE MALABSORPTION STUDY
12-14 September 1972
Washington, D.C.

The central purpose of this final workshop is to present and discuss critically the final data and conclusions from the three studies, in order to answer, insofar as possible, the prime question which had been asked. The question: "What is the best quantitative estimate of nutrient loss, among typical villagers of a developing country, due to malabsorption from whatever cause?"

A. Day 1

The activities of the first day, 12 September, are to be informal, attended mainly by the central staff and the project directors from Ludhiana and Shiraz. These sessions will be devoted to becoming acquainted with the final results of these two studies, which have not yet been received, and to preliminary discussion of certain key problems. The final report of the Puerto Rico study has already been sent to you.

During the second and third days, it is hoped that all consultants and investigators will be able to attend.

B. Day 2

The following considerations suggest themselves as agenda items for 13 September. Some pertinent questions and comments are listed which have a bearing upon the validity and significance of the study results.

1. Selection of study sample

a. Site - (How did the nutritional status of the villages selected in the three studies compare? Was severe malnutrition prevalent?)

b. Demographic characteristics - (The Shiraz protocol specified 50 as the upper age limit. Since malabsorption appears to be more prevalent among older persons, should the data on persons over 50 be analyzed separately? The protocol also specified that all persons studied "must be of the same socioeconomic status." It also specified however that they be "representative of the average population," and in fact the study subjects appear to have been from varying socioeconomic levels, as is the case in many villages. Since in Ludhiana malabsorption is more prevalent among the more affluent, it appears that subjects must also be stratified by socioeconomic level.

c. Sample size - (The two previous points suggest that the original sample number of 100 will be inadequate for sub-analysis of certain data by age and socioeconomic status.)

d. Health characteristics - (Were persons with "hepatic, cardiac or renal diseases" consistently excluded, as stipulated in the protocol, as well as persons with acute febrile disease or recent pregnancy?)

2. Methodology of conducting study

a. Diet - (With marked differences in estimated home diet of villagers, was the study diet consistently fixed at the 80 gm. level for protein; for fat? Differences in nitrogen absorption were observed in Puerto Rico subjects at two different protein levels of protein intake. Should the study diet conform more closely to the usual home diet; how long does it take to "adapt" to a marked increase in dietary protein or fat? How consistently was the study diet actually consumed by the subjects? Were leftovers carefully accounted for? Should study diets have been chemically analyzed, as well as calculated from food tables, for protein and fat? Were alternative methods available for measuring nitrogen balance which would have been more valid, and still feasible?)

b. Collection of urine and stool samples - (Were adequate precautions uniformly taken to ensure total collection; to prevent significant chemical change prior to analysis? Were same sequences consistently followed in collecting the various specimens?)

c. Laboratory determinations

Battery of screening tests - (Were abnormal results, such as elevated SGOT, used for exclusion of subjects due to disease? Was it possible and feasible to obtain a bone marrow specimen on a subsample?)

Nitrogen and fat determinations - (Were in fact identical Kjeldahl and van der Kamer procedures carried out by each laboratory, so that results from each study can be grouped?)

Determinations in general - (What precautions were taken for purity and stability of reagents? What procedures were employed for quality control within each laboratory; between laboratories? What control measures might have been taken; should be taken in such collaborative studies in the future?

Were intestinal biopsies generally feasible; did their results correlate with functional absorption results?)

3. Post-study follow-up of subjects - (Were the Ludhiana and Puerto Rico subjects actually given alternative treatment regimens and regularly followed as specified in the protocol?)

4. Data processing and analysis - (Were similar, and satisfactory, methods employed for looking at the data? Were standard "dummy" tables used for display of the various parameters measured in all studies?)

C. Day 3

On the final day, following a thorough and penetrating discussion of these and other problems brought up during the second day, suggestions will be discussed regarding how best to consolidate the significant results of the three studies into a final, composite report.

The shortcomings of these studies will be specified, as well as the strengths, and from their consideration should emerge helpful suggestions for possible future studies of malabsorption as it may relate to significant wastage of food in the developing world.

FINAL REPORT

1.	Title.	Measurements of Nutrient loss from Intestinal Malabsorption.
2.	Contract Number.	HSM 110-70-352.
3.	Issuing Office.	Health Services and Mental Health Administration, 5600 Fishers Lane, Rockville, Maryland. 20852.
4.	Contractor.	Ludhiana Christian Medical College Board, U.S.A., Inc., Room 250, 475 Riverside Drive, New York, N.Y. 10027.
5.	Place of Performance.	Christian Medical College and Brown Memorial Hospital, Ludhiana, Punjab, India.
6.	Appropriation 7500399.	Allowance: 0-6999 00 931 761. (RFP HSM 110-NP-34(0)).
7.	Contract Amount.	$23,820.00
8.	Sponsor.	Nutrition Programme, NCDC.
9.	Authorised Individual.	Charles Reynolds, Executive Secretary.
10.	Chief Local Investigator in Punjab.	Dr. Betty Cowan, Christian Medical College, Ludhiana, Punjab, India.

While "classical Tropical Sprue", with an easily recognisable clinical picture of an emaciated individual showing multiple nutritional deficiencies and, frequently, a severe megaloblastic anaemia, and with a history of passing frequent bulky frothy foul-smelling stools is occasionally seen in North India, we encounter much more frequently, patients with anaemia for which there is no obvious cause and they too may have deficiency signs but less than half give a history of diarrhoea. This led us to suspect that symptomless malabsorption due to small intestinal incompetence might be widely prevalent and an improtant cause of the unexplained anaemia frequently seen. We investigated 45 patients admitted to the wards of Brown Memorial Hospital on account of anaemia and thorough investigation failed to reveal any cause for the anaemia but, when the small intestinal functionswas studied, it was found that 41 had significant steatorrhoea, excreting an average of more than 7 gms. of fat daily on a three day collection of stool while eating a diet containing 80 gms. of fat. The interesting feature was that 18 of the 41 patients gave no history of diarrhoea and in fact most were constipated. All responded to treatment with folic acid and / or iron; approximately half of those who attended for follow up remained well after discontinuing treatment three months after the haemoglobin levels were normal, the remainder relapsed and presented with a clinical picture identical to the initial one three months after discontinuing treatment. The steatorrhoea was accompanied in most cases by abnormal absorption of D-Xylose (5 gm. dose) and jejunal mucosal biopsies. (1) These findings led us to survey a village of Lalton Kalan (2) and there we found that 87 of the 165 subjects who gave us a satisfactory 24 hour sample of stool had steatorrhoea, excreting more than 7 gms. of fat. This was, of course, a single 24 hour sample and therefore less satisfactory in terms of obtaining an incidence of steatorrhoea than in the hospital study.

The same village, Lalton Kalan, was chosen for the present study. It is a typical Punjab village 7 miles from the city of Ludhiana and most people are landowners, each owning a small piece of land, several buffaloes whose milk has a very high fat content, and sharing at least one major piece of machinery with other farmers. Approximately two thirds of the villagers are in this socio-economic group but the remainder, who live in a corner of the village, are labourers and their low daily wage means that their dietary intake is less good, particularly in terms of milk and milk products, taken liberally by those who own buffaloes, but too expensive for those who must buy milk. Lalton Kalan has shared in the recent green revolution and there is little doubt that the income of the villager has increased considerably, particularly that of those with food grains to sell, but the poorer people have to pay more for the staple foods and probably there has been no improvement in their dietary intake. This is obvious, when one observes the "vulnerable" groups, particularly of the poor class, the pregnant women, and the children just after breast-feeding has finished. The multiple nutritional deficiencies exhibited by these two groups, include severe anaemia, protein deficiency with oedema, browning of the usually glossy black hair and pigmentation of the skin. The Chief Minister of Punjab has recently made the surprising statement that, although there are many wealthy Punjabis, the average per capita monthly income is Rs. 85.00 and 42% of the population have a per capita income of less than Rs. 27.00 (less than $4). It is therefore of prime importance to determine whether or not any of the available food is rendered unavailable for nutrition by incompetence of the small intestine, and in particular, whether this abnormality remains undetected since it is symptomless.

Selection of patients for the study

The total population of the village is approximately 3,000 and during the previous study (2) it was found that there were 1,440 subjects between the ages of 16 and 64 years and who were not pregnant at that time. It was therefore unnecessary to start with the taking of a census since the information obtained in the above study was available. Of the first 60 subjects, 40 were randomly selected by means of a systematic selection, accepting every 10th person and if that person was unwilling or unavailable, then the 20th or 30th was taken. Because of the necessity of subjects to work all day in the fields at certain seasons, it was impossible to do this for the whole number and 20 of the 60 were volunteers from the same area, age group etc. The first 60 subjects studied were from homes where the income was satisfactory, "good income group". This was done deliberately since these people, for cultural reasons, would not have agreed to admission and study in a ward with people of the labouring og "poor income group". While it is impossible to discover the income in cash of these villagers, it is easy to allocate them to the above two income groups by means of certain criteria. (Table 1.) Thirty-eight of the "poor income group" were studied in the second series giving a total of 98. Sixteen of the second series were randomly selected and 22, for reasons already stated, were volunteers.

Age and Sex distribution. The village pattern for age and sex, although not deliberately planned, was reasonably well reproduced in the subjects studied. (Tables 11 and 111.)

Environmental Santitation. Very few homes in this village have toilets of latrines, the surrounding fields being the place where defaecation takes place. Children pass stool near the homes and therefore the surroundings are extremely unhygienic. This is worse in the poor quarter of the village but the courtyards of the "good income group" are contaminated by the buffaloes since they share

the courtyard with the people.

Water Supply. In the homes of the "good income group" water is obtained by means of a hand pump attached to a pipe which is sunk approximately 30 feet into the ground within the courtyard. The "poor income group" use water from open wells. Water was tested for E. Coli content during the dry weather and again after the rains. The count from the pumps was satisfactory during the dry weather but after the rains the water was heavily contaminated with E. Coli. During both seasons the water from the open wells was heavily contaminated with E. Coli.

Hospitalisation, Feeding and Sampling Procedures:

A ward with accommodation for 6 beds and attached bathroom, in the Rural Health Centre Hospital attached to the test village of Lalton Kalan, was used and patients were admitted in batches of 4-6 for 7 days in the case of the first 60. History, physical examination and urinalysis were dealt within the hospital, and from the time of admission the subjects began to eat a diet calculated by the C.M.C. dietician to contain 78 gms. of protein, 80 gms. of fat, and total calorie value of 2,600 - 2,800. She took a dietary history from each subject, asking what was eaten, as exactly as possible, during the preceding 3 days and it was found that, while the total calorie value and fat given to the test subjects was rather less than they ate at home in the case of males, these values were rather more for women. With regard to protein intake at home, there was a wider variation, the women and poorer class eating less protein than that contained in the test diet, while the males and richer people normally ate more. The content of the diet fed was almost identical to that eaten in the village and the protein was of vegetable origin since few villagers eat meat even if they are not strict vegetarians. Eggs were however included. The dietician sent the raw materials to the village hospital cook

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who prepared it in a special kitchen and the medical interns, doctors, posted at the centre supervised the serving so that each received an identical amount and then they persuaded the subjects to consume it all. In the first series, as was suggested at Shiraz, periodic checks of diet were done, and this confirmed that the diet did indeed contain approximately 78 gms. of protein. Fat and calories were not measured. On the first day all subjects were brought to Christian Medical College Hospital for chest x-ray since it seemed necessary to exclude pulmonary tuberculosis and on the following day all the blood estimations were done with the subjects in a fasting state. The nursing staff of the hospital acted as supervisors but a special metabolic nurse was employed for night duty since the change over of containers was important. Having eaten the diet for three days it was considered that all sampling would reflect the test diet and therefore markers were not used. At 5 a.m. on day 3 all were asked to use the common bathroom and latrine for the last time and thereafter they had to use the metabolic bathroom and were never out of the sight of the supervisor. This bathroom contained 6 locked commodes, all the usual outlets having been sealed with cement. When any subject wished to pass stool or urine he had to ask the nurse to open his commode in which were a stool tin and jerry can for urine. All were asked to pass urine before defecating and to bath in a tin tub, passing urine before bathing. At the same time each morning all were asked to empty bladder and bowel, full receptacles were removed and sent immediately to the C.M.C. laboratory each day and a new set of containers substituted. In this way we believe we ensured an accurate collection of stool and urine. On a convenient day, D-Xylose absorption was estimated using a 25 gm. dose and the following day the absorption of Vitamin B_{12} by means of the Schilling test was performed. Since Intrinsic Factor could not be obtained easily, this was done by using Cobalt 58 B_{12} alone but if the percentage excreted was abnormally low the test was repeated on a few subjects.

At Shiraz it was stressed that any extra procedure which would jeopardise the acceptability of the study would be abandoned, and this was the case with jejunal biopsy. We tried everything including monetary incentives but only a very few subjects agreed to go to the main hospital and some even ran away from there before the test was performed. In all, biopsies were obtained in 4 subjects. At the end of a four day collection of stool and urine which were sent in and estimated separately daily, the subjects were discharged. Review at Shiraz of the results of these 60 subjects showed that the figures for stool and urine nitrogen were extremely low and it was obvious that, while unexplained low nitrogen excretion in Indians has been previously reported (Patwardan 1952) these figures were much too low and it was decided to ask Dr. John Reinhold to look into the matter at Ludhiana before proceeding further and, in any case, the decision to perform calorimetry on the diet and faeces of the remaining subjects, meant inevitable delay until the Bomb Calorimeter could be delivered to India. Part of the above study was done in the summer, temperatures in that particular year being unusually low, and part in the winter, the Punjab winter being very cold. Eleven of the above were re-studied in July, 1972 using the revised method for nitrogen estimation, longer collection of samples and calorimetry. So instead of two series consisting of 60 with unreliable nitrogen figures and 38 with reliable figures, we have two series of 49 each.

The reasons for the discrepancy in nitrogen estimations have already been documented by Dr. Reinhold who expressed his belief that the figures for the second series were reliable, and therefore details of reasons for this discrepancy will not be included in this report.

Following the arrival of the Bomb Calorimeter and the visit of Dr. Reinhold we commenced the study again with the following modifications:-

1. Subjects were kept in the ward for 9 days so that, having been stabilised for 3 full days on the test diet, they could provide 6 daily collections of stool and urine for estimations.

2. The subjects without exception, from the poor or Harijan quarter of the village were adamant that no venipuncture would be done. This meant curtailment of some tests of nutrition but most were possible by means of finger prick, which, although more painful is more acceptable and dispelled the widespread fear that we were selling the blood to an unfriendly nation!

3. Daily estimation of nitrogen and calories were carried out on the diet and the result expressed as a daily average for the 6 days stay.

4. Calorimetry was performed daily, not only on food but on stool.

This procedure was repeated in 11 subjects from the first series of 60 who agreed to be re-studied after the monsoon had begun and therefore the field work less exacting. All subjects in the second plus repeat series (39) were weighed daily.

METHODS

Blood. The following blood estimations were performed:

1. Haimoglobin and haematocrit by means of standart techniques.
2. Serum folate using L. Casei. ("aseptic dilution" method.) (3)
3. Serum iron. (Ramsay)
4. Blood urea nitrogen.
5. Total and differential proteins both by Biuret method and electrophoresis.
6. Serum Cholesterol.
7. Serum carotene. (4)
8. Fasting blood sugar.

Stool. The stools were examined by means of the ether concentration method for ova and parasites and by the guiac method for occult blood.

Tests of absorption.

The concentration of xylose in the serum at 2 hours and in the urine at 5 hours after an oral dose of 25 gms. was determined by the method of Roe and Rice. (5)

The absorption of Vitamin B_{12} was determined by the Schilling (6) test using an oral dose of 0.5mg. 57Co. B_{12}.

Faecal fat values were determindd by the method of van de damer, (7) daily for 4 days in the first group of 60 subjects, and for 6 days in the second group of 38 subjects, the resulds being expressed as a daily average excretion. It was considered that an excretion exceeding 6gms. daily was abnormal. Since dietary fat was not estimated, actual fat balances were not possible. (While blood tests were not repeated on the 11 subjects who were re-studied using the corrected method of nitrogen estimation and calorimetry, faecal fat and xylose tests were repeated on these 11 subjects.)

Nitrogen concentrations were determined daily on stool and urine for 4 days in the first 60 subjects but these results are considered to be

unreliable (see Dr. Reinhold's report). In the second group pf 38 subjects and in 11 of the first group who were re-studied, the technique advised by Dr. Reinhold was employed and estimations were performed on food, stool and urine for 6 consecutive days, the results being expressed as a daily average, and the nitrogen balances of these 49 subjects calculated. It was considered that a daily faecal nitrogen excretion exceeding 2.5gms. was abnormal.

Morphology. Small intestinal biopsies were obtained in only 4 subjects of the first series and in none of the second series, using the Crosby Capsule after an overnight fast. Three more biopsies were obtained from villagers who agreed to come in later for study but they subsequently refused to be studied.

RESULTS.

Dietary Intake at Home. (Table IV)

Protein intake at home was evaluated at more than 50gms. daily in all of the 29 males in the good income group, but 10 of the 31 females in this group ate less than 50 gms. daily; none ate less than 40gms. The subject in the poor income group ate much less protein daily, this was especially true for the females. Seventeen of the 21 females in this group ate less than 50gms., 11 eating less than 40gms. and 3 eating less than 30gms.

Nutritional Evaluation. (Table V)

Haemoglobin. Twenty-eight subjects had haemoglobin levels less than 12gms. per 100 ml. There was a higher incidence of anaemia in the poor income group, particularly amongst the females, most of whom were in the childbearing age group. All except one of the anaemic subjects had hookworm infestation of the stool.

Serum Folate. This was evaluated in 69 instances and 24 had concentrations less than 5mg./ml. Fifteen of these subjects had steaterrhoea. Nine had hookworm infestation.

Serum Iron. This was estimated in the 60 subjects in the good income group and subnormal values were found in 12.

Serum Albumen. This was estimated in all and found to be less than 3.5gms./100ml. in 54 subjects; there was no correlation between low values and low dietary intake of protein.

Serum Carotene. This was estimated in the good income group only and found to be less than 70mg./100ml. in 3.

Serum Cholesterol. Fourteen of the 60 good income subjects in whom it was estimated had values less than 150mg./100ml.

Nitrogen Balance. Reliable results were obtained in 49 subjects (see reports of Dr. Anderson and Dr. Reinhold). These subjects were from the poor income

group with the exception of 11 from the first group who were re-studied. It was a matter of some note that only one of these subjects was in negative nitrogen balance, the remainder having large positive balances (maxium 7.6g.) with a mean positive balance of 4.0g. of nitrogen daily. (S.D. 2.0.) Faecal excretion of nitrogen was within normal limits in all but two subjects who excreted 2.65 and 2.63gms. daily respectively.

Calorimetry. This was done in the 38 subjects of the poor income group. Six had a faecal calorie loss exceeding 10% of intake. Only one of these had steatorrhoea.

Intestinal Evaluation. None of the subjects studied gave a history of diarrhoea during the proceeding year, the stool habit of most was 2 daily. Almost all gave a history of gaseous distension of the abdomen from time to time after meals.

Intestinal Parasites. Parasites were found in the stools of 44 of the 98 subjects. Thirty-four had hookworm but E. Histolytica, Giardia Llamblia and Iod. Butschlii were also found. There was no correlation between the presence of parasites and impaired intestinal function.

Intestinal Function.

Nitrogen Absorption. There was no significant malabsorption of nitrogen, only two of the 49 subjects excreting more than 2.5gms. daily. (2.63 and 2.67g.)

Xylose Absorption. Urinary excretion of D-Xylose was less than 5gms. 5 hours after the oral dose of 25gms. in 64 instances. Dr. Anderson has calculated that there is a statistically significant negative correlation between D-Xylose values and feacal fat loss, i.e. large fat losses occur with low urine D-Xylose values.

Fat Absorption. (quoted from Dr. Anderson's report) "Statistical treatment of stool fats for all 98 subjects (both male and female) indicates no statistically significant malabsorption of fat. The arithmetical mean for males and

females is 6.12gm./24 hours. With a standard error of ± 0.2149gms./24 hours. This is not statistically greater then 6.0gms./24 hours.

2. Statistical treatment of female stools alone indicates there is no significant malabsorption of fat. The arithmetical mean is below 6.0gms./24 hours. (5.69 ± 0.270)

3. Statistical treatment of males alone shows a statistically significant malabsorption of fat with a mean loss of 6.60gms./24 hours. This is just significantly higher than the 6.0gms./24 hours.

4. There is no statistically significant correlation between the age of males and females and fat malabsorption."

Vitamin B_{12} Absorption. This was abnormal in only one subject who had steatorrhoea.

Morphology.

Unfortunately the number of biopsies obtained is too small to allow any valid conclusions, but in these obtained the changes were minimal and confined to an increase in inflammatory cells, the normal villous architecture being preserved.

DISCUSSION.

While few of the village subjects were obese, their nutritional status was satisfactory and anaemia was not found in any except males with hookworm and parous women with or without hookworm.

The rather high incidence of lowered serum folate concentrations is unexplained since this did not correlate with abnormality of intestinal function. Low values are not uncommon in apparently healthy Punjabis and are invariably found in patients with chronic blood loss (personal observation) suggesting that the increased turnover secondary to blood loss quickly depletes the folate stores in this population. The high incidence of low serum albumen values is unexplained and is not related to low protein intake or, apparently, abnormality of absorption. A low serum albumen and high Y globulin is not infrequently found in our laboratory in the local population.

The unexpected high positive nitrogen balances in 48 of the 49 subjects is interesting. All had normal blood urea nitrogen values and none gained weight during the period of study. This may indicate a hitherto unsuspected source of nutrient loss and, since it appears unlikely that the subjects were retaining the nitrogen, it is possible that a proportion was rendered unavailable for absorption, having been converted into ammonia, possibly by the action of abnormal microflora and aggravated by a diet consisting largely of vegetable protein. Patwardhan remarked upon the low urinary nitrogen values of Indians when compared with Americans but no explanation was suggested. Deesthole et/ al. commented upon the effect of intestinal flora on nitrogen metabolism in Indians and Levitt and Bond reported an increased rate of passage of nitrogen in faeces after a bean meal.

While our study was in progress four western subjects were studied. They

ate their normal western diet and their nitrogen balance was perfectly normal for western subjects. The study was repeated on one subject who ate Hindustain vegetarian diet one week prior to and during the test. Again the daily balances were normal for a western subject. While this finding is obviously not due to malabsorption it does suggest an abnormal intestinal situation which may be the cause of significant protein wastage.

TABLE I

SOCIO-ECONOMIC STATUS OF SUBJECTS

INCOME GROUP	CRITERIA	NUMBER STUDIED
"GOOD"	BRICK HOUSE.	60
	LAND.	
	AT LEAST ONE BUFFALO.	
	MACHINERY.	
	SELF - EMPLOYED.	
"POOR"	MUD HOUSE.	38
	NO LAND.	
	NO CATTLE.	
	NO MACHINERY.	
	LABOURER.	

TABLE II

AGE-SEX DISTRIBUTION OF SUBJECTS

		TOTAL	STUDIED
MALES.		778 (54%)	46 (47%)
FEMALES.		662 (46%)	52 (53%)
TOTAL.		1,440 (100%)	98 (100%)
AGES.			
	18 - 24	533 (37%)	25 (26%)
	25 - 34	304 (22%)	27 (28%)
	35 - 44	243 (16%)	16 (16%)
	45 - 54	208 (15%)	17 (17%)
	55 - 64	152 (10%)	13 (13%)
TOTAL.		1,440 (100%)	98 (100%)

TABLE III

PERCENTAGE OF POPULATION STUDIED

	TOTAL	STUDIED	% OF VILLAGE STUDIED
MALES.	778	46	3.2%
FEMALES	662	52	3.8%
TOTAL	1,440	98	7.0%

TABLE IV

DIETARY INTAKE AT HOME

"GOOD INCOME GROUP"

	MALES		FEMALES	
	MEAN	S.D.	MEAN	S.D.
PROTEIN	83.8 g.	± 18.4	57.0 g.	± 11.2
FAT	101.4 g.	± 24.4	65.3 g.	± 11.1
CALORIES	2645		1956	

"POOR INCOME GROUP"

	MALES		FEMALES	
	MEAN	S.D.	MEAN	S.D.
PROTEIN	56.3 g.	± 13.8	38.9	± 8.0
FAT	76.6 g.	± 19.3	57.2 g.	± 8.2
CALORIES	2197		1636	

TABLE V

NUTRITIONAL EVALUATION

	NORMAL RANGE	SUBJECTS STUDIED		MEAN	S.D.	SUBNORMAL
HAEMOGLOBIN						
MALE.	14 - 16 g./100ml.	GOOD INCOME.	(29)	14.7	2.3	8
		POOR INCOME	(17)	11.8	2.4	13
FEMALE.	12 - 16 g./100ml.	GOOD INCOME	(31)	13.2	2.1	20
		POOR INCOME	(21)	10.8	2.7	21
SERUM IRON						
(GOOD INCOME ONLY)	70 - 160mg./100ml.	MALES		100	37	5
		FEMALES		76	22	7
SERUM FOLATE						
(GOOD INCOME ONLY)	5 - 16mg./ml.	MALES		6.8	3.6	13
		FEMALES		6.1	2.9	11
SERUM ALBUMIN						
(WHOLE SERIES)	3.5 - 5.0g./100ml	MALES		3.4	0.5	22
		FEMALES		3.2	0.6	32
SERUM CAROTENE						
(GOOD INCOME ONLY)	70 - 300mg./100ml.	MALES		166	64	2
		FEMALES		189	56	1
SERUM CHOLESTEROL						
(GOOD INCOME ONLY)	150 - 250mg./100ml.	MALES		163	23	5
		FEMALES		167	37	9.

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FINAL REPORT

A. Title. Measurements of Nutrient Loss from the Intestinal Tract Due to Malabsorption

B. Project No. HSM-110-69-425

C. Institution. University of Rochester School of Medicine

D. Principal Investigator. Frederick A. Klipstein, M. D.

E. Summary of Findings

Dietary intake, nutritional status and intestinal structure and function were evaluated in 96 representative adult residents of a rural barrio in Puerto Rico. Protein intake exceeded 0.7 g per kg body weight per day in 86. Deficiencies noted included anemia in 4, and reduced serum concentrations of iron in 10, folate in 6, vitamin B_{12} in 1, albumin in 4, carotene in 6, and cholesterol in 8. Twelve of 50 subjects receiving 12-14 g nitrogen per day and 19 of 46 receiving 8-10 g per day were in negative nitrogen balance; this was solely attributable to excessive fecal loss in only one. Impaired intestinal function clearly resulted in a nutritional deficiency in 2 subjects and possibly contributed in 6 others. Thirty-two persons had a single and 11 multiple abnormal determinations of intestinal function. These included xylose in 24, folic acid in 1, fat in 4, nitrogen in 1, and vitamin B_{12} in 28. Intestinal morphology was normal in 17 and showed mild changes in 48, moderate changes in 24 and severe abnormalities in 4. No relationship was evident between the presence of intestinal abnormalities and protein intake or sanitary facilities at home or parasitic infestation. The presence of certain intestinal abnormalities detected in persons investigated during the winter was double that observed in persons studied during the summer.

F. Comments. The findings of this investigation indicate that intestinal function is abnormal in one-half and multiple absortive abnormalities coupled with morphologic abnormalities are present in 6 per cent of the rural population of Puerto Rico. Projecting the incidence of 6 per cent of severe abnormalities found present in this sampling to the total population of 2.8 million persons on this island, results in a figure of 168,000 Puerto Rican who may have similar intestinal changes. Compromised intestinal function resulted in nutritional deficiencies in only a small proportion of subjects evaluated. This is probably attributable to the fact that these individuals were receiving a good dietary intake so that mildly impaired intestinal absorption was insufficient to cause a net nutritional deficit. In contrast, similar investigations conducted by us elsewhere in the West Indies (Haiti and the Dominican Republic) have shown that compromised intestinal absorption of this severity when combined with marginal or poor dietary intake commonly is associated with the presence of nutritional deficiencies.

Frederick A. Klipstein

Principal Investigator
January 20, 1972

INVESTIGATIONS CONCERNING THE PREVALENCE OF NUTRITIONAL DEFICIENCIES AND INTESTINAL MALABSORPTION AMONG RURAL POPULATIONS OF THE WEST INDIES. II. BARRIO NUEVO, PUERTO RICO

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SUMMARY

Dietary intake, nutritional status and intestinal structure and function were evaluated in 96 representative adult residents of a rural barrio in Puerto Rico. Protein intake exceeded 0.7 g per kg body weight per day in 86. Deficiencies noted included anemia in 4, and reduced serum concentrations of iron in 10, folate in 6, vitamin B_{12} in 1, albumin in 4, carotene in 6, and cholesterol in 8. Twelve of 50 subjects receiving 12-14 g nitrogen per day and 19 of 46 receiving 8-10 g per day were in negative nitrogen balance; this was solely attributable to excessive fecal loss in only one. Impaired intestinal function clearly resulted in a nutritional deficiency in 2 subjects and possibly contributed in 6 others. Thirty-two persons had a single and 11 multiple abnormal determinations of intestinal function. These included xylose in 24, folic acid in 1, fat in 4, nitrogen in 1, and vitamin B_{12} in 28. Intestinal morphology was normal in 17 and showed mild changes in 48, moderate changes in 24 and severe abnormalities in 4. No relationship was evident between the presence of intestinal abnormalities and protein intake or sanitary facilities at home or parasitic infestation. The presence of certain intestinal abnormalities detected in persons investigated during the winter was double that observed in persons studied during the summer.

During the past decade it has become evident that a sizable proportion of the asymptomatic population of tropical areas has changes in intestinal structure and function which differ from that found present in normal residents of temperate climates. Such has been found to be the case in Southeast Asia[1,2], the Indian subcontinent[3-5], Africa[6-8], and the West Indies[9-11]. The pathogenesis of these changes and their relationship to the usually more severe abnormalities found present in persons with clinically recognizable tropical sprue are unknown.

The effect of mildly compromised intestinal function on the nutritional status of the affected population is uncertain. In a few reported cases, reduced absorptive capacity apparently has been of sufficient severity to result in deficiency of some nutrients either in persons who were totally asymptomatic[9,12,13] or in those who were not suspected of having malabsorption on clinical grounds since they had no gastrointestinal symptoms[14,15]. Since nutritional status represents the net result of many factors including dietary intake and absorption, it is possible that the effect of mildly impaired intestinal function on nutritional status may be related to the adequacy of dietary intake in such persons. This aspect has received scant attention in the past and, for this reason, the present study represents one of three investigations concerned with the relationship

of dietary intake, nutritional status and intestinal function among rural populations of three different regions in the West Indies who over-all have a good (Puerto Rico[16]), marginal (the Dominican Republic[17]), or poor (Haiti[18]) dietary intake. The present report describes the results obtained in Puerto Rico.

MATERIALS AND METHODS

Subject Material. Barrio Nuevo is a community of 201 houses scattered over a 2-mile area principally at an altitude of 3,200 feet, located 26 miles southwest of San Juan. The factors responsible for choosing this barrio and the method of selection of individual study cases have been described previously[19]. Of the 437 adults resident in the barrio, 14 were rejected because of chronic illness or recent intake of vitamins or antibiotics and 17 declined to participate in the study because of personal reasons, making an acceptance rate of 96%. Subjects were selected to match the age-sex distribution of the population; this method of sampling also resulted in study groups which were representative for income and sanitation (Table 1). Dietary intake at home was determined during daily visits over a 5-day period by the dietician and was calculated according to standard tables for foodstuffs used in Puerto Rico. The constituents of this diet have been described in detail[20].

Methods. Study cases were hospitalized for 6 days at the Clinical Research Center. During this time, dietary intake was weighed and calculated using standard tables. Fat intake was maintained at 80 g per day. The first 50 subjects studied (Cases 1-50) were placed on a diet containing 12-16 g of nitrogen daily. Thirty-five subjects had an intake of more than 12 g per day. Intake on this diet (average, 12.4 g) exceeded that taken by the subjects at home (average, 8.0 g) in 45 of the 50 cases (Fig. 1). The next 46 subjects studied (Cases 51-96) were placed on a diet containing 7-10 g of nitrogen daily. Intake on this diet (average, 9.6 g) approximated more closely that (average, 8.5 g) taken by this group at home (Fig. 2).

Serum and whole blood folate levels were assayed using *Lactobacillus casei*[21,22]; serum vitamin B_{12} concentrations were assayed using *Lactobacillus leishmanii*[23]; serum iron and carotene values were determined according to published methods[24,25], and serum albumin concentrations were determined by protein electrophoresis. Fat and nitrogen balances were determined on pooled 4-day stool and urine collections. Fecal fat values were determined by the method of Van de Kamer[26] Nitrogen concentrations were determined by the micro Kjedahl technique; normal subjects are found to absorb more than 85%

of dietary nitrogen and to excrete less than 2.5 g per day of nitrogen in the feces[27]. The concentration of xylose in the serum at 1 and 2 hr and urine for 5 hr following a 25 g oral dose was determined by the method of Roe and Rice[28]. The absorption of vitamin B_{12} was determined by a Schilling test[29] using an oral dose of 0.5 μg $^{57}CoB_{12}$. Folic acid absorption was determined by assay with Streptococcus faecalis of serum levels at 1 or 2 hr after an oral test dose of 40 μg per kg body weight[30]. Small intestinal biopsy samples were obtained after an overnight fast using a Crosby capsule; written, informed consent for the biopsy procedure was obtained from all subjects. Methods used in processing and criteria for the interpretation of the specimens have already been described[31]. Those biopsies which showed moderate, 2+, or severe, 3+, changes are referred to as abnormal.

RESULTS

Nutritional Status

Dietary Intake at Home (Table 2). Protein intake exceeded 50 g per day in 72 subjects, ranged between 40 and 50 g in 20, and was less than 40 g in 4. Values were less than 0.7 g per kg body weight in 10.

Nutritional Evaluation (Table 3). Four subjects had a subnormal hemoglobin concentration (9-12 g per 100 ml); 3 had low serum iron, and 1 a low serum folate concentration. None had evidence of occult blood loss. Serum iron concentrations were subnormal in 7 multiparous females and 3 males, 2 of whom were infested with hookworm. Serum folate concentrations were between 4-5 ng per ml in 4 subjects and between 3-4 ng per ml in 2, one of whom had a reduced whole blood folate value (75 ng per ml) and was anemic. The absorption of a pharmacologic dose of crystalline folic acid was normal in all 6 of these cases. The serum vitamin B_{12} concentration was subnormal (75 pg per ml) in one non-anemic individual who had a reduced absorption of this vitamin.

Serum albumin determinations ranged between 3.0-3.5 g per 100 ml in 3 and were lower in one subject; dietary intake of protein exceeded 50 g per day in all[4]. Serum carotene concentrations were between 40-60 µg per 100 ml in 6 persons. Two had normal intestinal function but one had a markedly reduced dietary intake; two or more determinations of intestinal function were abnormal in the other 4 subjects. Serum cholesterol concentrations ranged between 120-150 mg per 100 in 8 cases. Dietary intake was adequate in all. Three had a single abnormality of intestinal function, and a fourth (Case 76) had severe malabsorption.

Nitrogen Balance. Thirty-eight (76%) of the 50 subjects receiving 12-14 g of nitrogen per day were in positive balance (Fig. 1), even though nitrogen absorption was slightly reduced in 5. Twelve persons (24%) were in negative balance. In one (Case 24), this was due to a low intake of 5.1 g, resulting in reduced absorption of 77% despite a normal fecal excretion. In 10, it was secondary to urine losses which exceeded intake in 3 and approximated intake by 1 g in 7. Four subjects also had reduced absorption (75-84%), and 3 had increased fecal excretion of nitrogen.

Twenty-seven (59%) of the 46 cases receiving 8-10 g of nitrogen per day were in positive balance (Fig. 2). Seven had reduced absorption of nitrogen (<80% in two), but all had a normal fecal excretion. Nineteen (41%) subjects were in negative balance. Urinary excretion exceeded nitrogen intake in 6 and was within 1 g of intake in 12. Nitrogen absorption was reduced (62-84%) in 8, but fecal loss exceeded 2.5 g per day in only 2. Negative balance was the result of excessive loss (3.8 g per day) in one subject (Case 82).

Intestinal Evaluation

Symptoms. Fourteen subjects complained of intermittent episodes of diarrhea, not associated with anorexia or weight loss, within the 6-month period preceding their investigation. Nine had no parasites, 2 had *Necator americanus*, and 3 had

Trichuris trichiura. Intestinal function was normal in 5; 5 had malabsorption of vitamin B_{12} only, and 4 (Cases 3, 35, 79, 82) had reduced absorption of both xylose and vitamin B_{12} with morphologic abnormalities in 3 and elevated fecal fat values in 2.

Complaints of epigastric distress led to radiologic examination of the upper gastrointestinal tract in 16 subjects, 2 of whom were found to have a hiatus hernia and one an active duodenal ulcer. Gall bladder series were normal in 7 women. Barium enema and sigmoidoscopy were normal in 3 persons examined who complained of long-standing diarrhea and were found to have normal intestinal function.

Function

Determinations of intestinal function (Table 4) were normal in 53 subjects (with the exception that fecal nitrogen excretion was in the borderline elevated range of 2.5-2.7 g per 24 hr in 4). Ten of these subjects had a normal jejunal morphology, 34 had mild 1+ changes, and 9 had 2+ or 3+ abnormalities of villous structure. Thirty-two cases had an isolated abnormality of one test substance (xylose in 14, vitamin B_{12} in 18); 11 had two or more abnormal determinations of intestinal function (Table 5).

Xylose Absorption. Urinary excretion was less than 5 g per 5 hr in 24 subjects, among whom it was less than 4 g in 11. Peak serum concentrations were subnormal (24-30 mg per 100 ml) in 4. A reduced xylose value was the only defect present in 14 subjects, 5 of whom had abnormalities of jejunal morphology. Among the 10 other subjects with low xylose values, 9 had malabsorption of vitamin B_{12}; 4 had elevated values of fecal fat excretion; one had an elevated (3.8 g per 24 hr) excretion of fecal nitrogen, and 6 had abnormal intestinal morphology (Table 5).

Folic Acid Absorption. One subject (Case 1) had a subnormal peak serum concentration of 30 ng per ml following the oral test dose; vitamin B_{12} absorption was also reduced in this subject.

Fat Absorption. Fecal fat values were in the borderline elevated range of 5-6 gm per 24 hr in 4 subjects, all of whom had subnormal absorption of xylose, and 3 of whom also had reduced absorption of vitamin B_{12} and abnormal jejunal morphology.

Nitrogen Absorption. Absorption in the 50 subjects who received a diet containing 12-16 g of nitrogen daily was normal (>85%) in 39, borderline (80-85%) in 6, and subnormal (67-78%)

in 5, 3 of whom had an increased fecal excretion (Fig. 1). Twenty-six of the 46 subjects receiving 7-10 g of nitrogen daily had normal, 11 had borderline, and 9 had subnormal (62-79%) absorption (Fig. 2). Fecal excretion was increased in 3 persons who had subnormal absorption.

Of the 6 subjects with increased fecal excretion, values were borderline (2.5-2.7 g) in 5; in 4, other tests of intestinal function were normal, and in one xylose absorption was reduced. One (Case 82) excreted 3.8 g per day and had generalized malabsorption (Table 5).

Vitamin B_{12}. The 24 hr urinary excretion of $^{57}CoB_{12}$ was less than 8% in 28 subjects, among whom it was less than 6% in 14 and less than 4% in 6. Forty-eight hr urinary excretion values were less than 12% in all 28 of these cases and in 2 others as well; the slightly reduced (10% and 11%) values in the latter 2 were not construed as indicative of vitamin B_{12} malabsorption. Reduced absorption of vitamin B_{12} was the only functional defect present in 18 subjects, 8 of whom had morphologic abnormalities. Malabsorption of vitamin B_{12} was associated with subnormal xylose values in 9 cases, increased fecal fat excretion in 3, reduced absorption of folic acid in one, and abnormal jejunal morphology in 6.

Structure

Jejunal biopsies were obtained in 93 subjects. Intestinal structure was normal, resembling that seen in normal North Americans, in 17. Mild, 1+ changes were present in 48 specimens. In 18 of these, villous structure was normal and changes were limited to increased infiltration of the lamina propria; in the other 30, there was mild blunting of villous architecture. Moderate, 2+ changes were present in 24 specimens; villous architecture was abnormal, and increased infiltration of the lamina propria was present in all. Four subjects had severe, 3+ changes. Details concerning immunoglobulin concentrations and lipid distribution in these biopsy specimens have been described separately[32].

In general, the prevalence of functional abnormalities increased with progressively severe changes of morphology, with the notable exception that 2 of the 4 subjects with severe, 3+ changes had normal function (Table 6).

Factors Related to the Presence of Intestinal Abnormalities

Dwelling. No consistent pattern of intestinal abnormalities was evident among the 38 persons investigated who shared housing with one or more other subjects in the study. Two persons from the same dwelling were studied in 16 instances. In 4 houses, both persons tested had normal intestinal structure and function; in 8, one subject was normal and the other

had an abnormality of function and/or structure; in 4, both subjects had abnormalities. Three subjects from the same dwelling were investigated in two instances; in both, 2 of the 3 subjects had intestinal abnormalities.

Location in Barrio. Abnormal determinations of intestinal function were detected in persons living in 6 (30%) of the 20 houses on the road leading up the mountain to the barrio, in 14 (70%) of the 20 houses in the center of the barrio, and in 19 (52%) of the 36 outlying houses (Fig. 3). Abnormalities were present in 6 (26%) of 23 persons living on the road to the barrio, in 15 (60%) of 25 persons living in the center of the barrio, and in 23 (47%) of 48 persons examined in the outlying areas.

Sanitation. Nineteen (59%) of the 32 subjects who had use of a toilet had abnormalities of intestinal function and/or structure, while 33 (52%) of the persons who used a latrine had an intestinal abnormality.

Protein Intake. Nine (41%) of the 24 subjects who had a dietary intake at home of less than 50 g of protein per day had an intestinal abnormality, as did 43 (58%) of the 74 persons who had a larger intake of protein.

Parasitic Infestation. Forty-six subjects had a parasitic infestation, with one type in 37 and with two different parasites in 9. Twenty-two were infested with hookworm (*Necator americanus*), 12 with *Trichuris trichiura*, 9 with *Entamoeba coli*, 3 with *Schistosoma mansoni*, 2 with *Entamoeba histolytica* (cysts), 2 with *Giardia lamblia*, and one with *Strongyloides stercoralis*. Intestinal abnormalities are recognized to occur secondary to infestation with *Giardia* or *Strongyloides*[33] and were present in one of the two persons with *Giardia* but not in the subject with *Strongyloides*. Of the 46 persons who had a parasitic infestation, 23 (50%) had an intestinal abnormality.

Season. An average of 12 subjects was studied monthly over the 8-month period of the investigation which extended between July 1970 and February 1971 (Fig. 4). The percentage of subjects who had an abnormality of xylose or vitamin B_{12} absorption or of intestinal morphology, either singly or in combination, increased from the 33-46% range present in July through September to 61% in October, and thereafter rose to 78% in January. The prevalence of subnormal absorption of vitamin B_{12} (but not that of xylose) and of abnormal intestinal morphology rose strikingly in January. Further, of the 6 cases found in the study to have combined malabsorption of both xylose and vitamin B_{12} and morphologic abnormalities, 4 were seen during the month of January.

Repeat Investigations

Tests of intestinal function were repeated in 15 individuals:

a) Six persons who initially were found to have subnormal xylose values were treated with folic acid, 5 mg daily, for from 3 to 4 months. When retested in February 1971, xylose determinations had returned to normal in 3 and remained low in 3 (Fig. 5). The absorption of vitamin B_{12} remained subnormal in 2 and had become subnormal in the other 3 tested. In 2, mild 1+ changes of jejunal morphology were unchanged; in one, 2+ abnormalities had reverted to 1+ (xylose excretion returned from 2.0 to 5.9 g in this subject); and in the fourth case biopsied, who had persistent malabsorption of xylose and vitamin B_{12}, 2+ abnormalities were unchanged. One subject (Case 25) was continued on folic acid and re-evaluated nine months later; absorption had returned to normal (Table 7).

b) Eight subjects found to have normal intestinal function and morphology during their initial examination were selected at random for re-evaluation in connection with other investigations conducted during September and October, 1971, six months after the completion of the study (Fig. 6). Xylose determinations remained within the normal range in 5 and had become subnormal in 3. Vitamin B_{12} absorption continued to be normal in all

except one subject (Case 12) who complained of intermittent diarrhea for two months. This individual was not anemic but had megaloblastic changes in the bone marrow, reduced serum concentrations of folate (4.5 ng per ml) and vitamin B_{12} (10 pg per ml), and was found to have severe generalized malabsorption (Table 7). These abnormalities were reversed to normal by 10 weeks of treatment with vitamin B_{12}, folic acid, and sulfasuxidine.

DISCUSSION

Recent surveys conducted among both the rural and poor urban populations of Puerto Rico have shown that deficiency states occur in children but are relatively uncommon among the adult population[16,34,35]. Such were our findings in the present investigation. Anemia was rare. Iron deficiency was confined to multiparous females or, less frequently, males infested with hookworm. The significance and pathogenesis of the mild hypoalbuminemia found present in 4 persons are obscure; reduced values were not related to dietary intake, negative nitrogen balance, or the presence of abnormal intestinal or hepatic function. Depressed serum concentrations of carotene and cholesterol, present in 6 and 8 cases, respectively, could be related to either suboptimal dietary intake or to intestinal malabsorption in most instances.

The most striking finding concerning changes in structure and function of the small intestine was the patchy nature of the functional and morphologic abnormalities noted. Forty-three of the 96 subjects examined had one or more abnormalities of function. The majority (32) had a single defect of absorption, most commonly of vitamin B_{12}. Among the 11 persons who had multiple abnormalities of intestinal function, 6 had definite morphologic abnormalities and 3 had mild steatorrhea. It is difficult to compare these results with those reported previously among randomly selected adults studied in the tropics since, in most instances, assessment of intestinal function was restricted to determinations of xylose absorption. Reduced absorption of this substance, present in 24% of persons in the current study, has been detected in from 20 to 60% of groups tested in most tropical areas[36]. Vitamin B_{12} absorption was subnormal in 15 (65%) of 23 asymptomatic Haitians examined[37]; in one (3%) of 35 persons tested in Thailand[2], and in 6 (20%) of 30 native residents evaluated in East Pakistan[4]. Steatorrhea was detected in 2 (6%) of 35 subjects examined in Thailand[2]; in 1 (7%) of 14 persons tested in East Pakistan[4], and in 4 (10%) of 39 poorly nourished peasants evaluated in Egypt[8].

Previous evaluation of intestinal function among asymptomatic residents of Puerto Rico has been confined to two investigations. Angel et al found reduced urinary excretion of xylose, using a 5 g test dose, in 24% of 53 residents of a rural village[38]. Robbins et al examined two groups who were specifically selected for study on the basis of the previous finding of either a subnormal or elevated serum cholesterol concentration[9]. Fifty-six per cent of the 41 subjects in the group with low values and 15% of the 61 persons in the group with elevated concentrations had a reduced absorption of xylose. Fat absorption was less than 90% in 5 of 15 persons with low xylose values tested, and 12 of 17 examined had morphologic abnormalities.

In only two instances in the present study could deficiencies of a nutrient be clearly attributed to impaired intestinal function. In one, deficiency of vitamin B_{12} was shown to be secondary to malabsorption of this vitamin. In the other, subnormal values for both serum carotene and cholesterol were present in a subject who had generalized malabsorption. In 6 others, there was a suggestive relationship. Three subjects with reduced serum carotene levels and 3 with subnormal serum cholesterol concentrations had one or more abnormal determinations of intestinal function. Excessive fecal loss of nitrogen present in one subject who also had multiple other abnormalities

of function and morphology resulted in a negative nitrogen balance, but this was not associated with hypoalbuminemia. These observations indicate that the mildly compromised intestinal function frequently detected among Puerto Ricans living in rural areas was not of sufficient severity to result in the development of nutritional deficiencies in the presence of a dietary intake that was adequate in nearly all instances.

Several observations in the present study indicate that the occurrence of intestinal abnormalities among the rural population is not in a static state. 1) The incidence of malabsorption of vitamin B_{12} (but not of xylose) and of morphologic abnormalities found present during the months of January and February was double that noted during the summer months. 2) Two-thirds of the subjects found during the entire study to have both malabsorption of two substances and morphologic abnormalities were seen during the month of January. 3) Previously normal absorption of vitamin B_{12} became subnormal in all 3 subjects who were re-examined during the month of February; in contrast, absorption became subnormal in only one of 7 other subjects who were re-evaluated during the summer. 4) One subject was found to have developed overt tropical sprue one year after initial evaluation showed him to have normal intestinal function.

Several of these observations suggest that there is a seasonal variation in the incidence of intestinal abnormalities among the rural population in Puerto Rico. Variations in the absorption of xylose in individual asymptomatic Pakistanis[4] and of other substances in Indians[39] studied over a prolonged period of time have been reported but, to our knowledge, seasonal variations have not been described previously among such groups investigated in the tropics. This may possibly be attributed to the facts that other investigations have involved smaller groups of subjects which were presumably studied over a shorter period of time, and that in most instances evaluation of intestinal function was confined to xylose determinations.

The factors responsible for this seasonal variation are unknown, but it is of interest that the period during which peak abnormalities occurred heralds the "sprue season." It has long been recognized that there is a seasonal variation in the incidence of tropical sprue in Puerto Rico: the majority of patients present between the months of February and July with a peak in May and June. It is likely that most patients seen during this period have had the disease for at least six months, since most have experienced gastrointestinal symptoms (when present) for from 3 to 6 months and over 90% have

reached the advanced stage of the disease with a florid megaloblastic anemia. Two events occur during the autumn or early winter that are out of the ordinary for this island. The rainy season usually extends from mid-summer to October or November. The peak incidence of tropical sprue among British expatriates in India[40] and Burma[41] during World War II was identical to that seen in Puerto Rico, and followed the rainy season in these countries. The second event is the month-long period during the Christmas season which is associated with a markedly increased intake of both alcoholic beverages and foodstuffs rich in unsaturated fats, such as pork drippings. Intestinal sensitivity to substances produced by the oxidative rancidity of certain unsaturated fats used in cooking has been suggested as a possible factor in the pathogenesis of tropical sprue[42].

Other factors such as sanitary facilities, dietary intake and parasitism did not appear to be relevant to the occurrence of intestinal abnormalities. "Sprue houses" have been described in India[43], and an increased prevalence of reduced serum folate levels detected among asymptomatic relatives of persons with overt tropical sprue in Puerto Rico[44]. No clustering of intestinal abnormalities within a single household was evident in the present study, although there did appear to be a higher prevalence of abnormalities in certain

areas of the barrio.

The pathogenesis of the intestinal changes found present among some asymptomatic persons in the tropics is unknown. Also uncertain is whether they have any relationship to the qualitatively similar[45] but quantitatively more severe functional and structural changes found present in the disorder recognized as tropical sprue. During the past several years it has become increasingly apparent that the rigid criteria previously employed to define tropical sprue are not applicable to all cases, and it has been questioned whether this disorder represents a syndrome rather than a single disease entity[46]. In India and Haiti it has often proved difficult to delineate a clear line of demarcation between persons with tropical sprue and those with asymptomatic malabsorption[37,47]. It is noteworthy that had any of the 6 subjects in the present survey found to have impaired absorption of multiple substances and morphologic changes presented to us for medical care because of symptoms (3 had diarrhea), we would have considered them early cases of tropical sprue. Such was the case in one individual who, when randomly selected for restudy, was found to have early megaloblastic changes in the bone marrow and severe intestinal abnormalities which were correctable by

treatment with folic acid and sulfa. In some instances, the abnormalities detected in the Puerto Rican population may represent residual changes of initially unrecognized and partially treated tropical sprue. Treatment with folic acid or vitamin B_{12} only rarely reverses intestinal abnormalities to normal in chronic tropical sprue[48-50]. In Puerto Rico, unlike in India or Haiti, these vitamins are both easily available and commonly used for a variety of ailments. Our experience at this clinic and that of other investigators in Puerto Rico[51] leads us to believe that there is a sizable reservoir of such individuals among the population. In most persons, however, the etiology of the intestinal changes remains unknown.

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TABLE 3

NUTRITIONAL EVALUATION

	Normal	Range	Subjects Studied Mean	SD	Subnormal
Hemoglobin male	14-16	g/100ml	15.2	1.4	2
female	12-16	g/100ml	13.9	1.7	2
Serum iron	70-160	µg/100ml	107	33	10
TIBC saturation	25-50	%	33	9	13
Serum folate	5-18	ng/ml	9.5	4.2	6
Whole blood folate	100-350	ng/ml	187	65.6	1
Serum vitamin B_{12}	150-900	pg/ml	410	206	1
Serum albumin	3.5-5.0	g/100ml	3.8	0.4	4
Serum carotene	70-300	µg/100ml	128	46	6
Serum cholesterol	150-250	mg/100ml	211	56	8

TABLE 4

INTESTINAL FUNCTION

	Normal		Subjects Studied			
			Range	Mean	SD	Subnormal
Xylose Absorption						
Urine excretion	5-9	g/5hr	2.0-10.7	6.0	1.9	24
Serum peak*	>30	mg/100ml	24-71	43.4	12.0	4
Folic acid absorption*	>40	ng/ml	30-120	77.0	27.0	1
Fecal fat	<5.0	g/day	0.6-6.0	2.8	1.2	4
Fecal nitrogen	<2.5	g/day	0.4-4.3	1.6	0.6	6
$^{57}Co B_{12}$ absorption	>8.0	%/24hr	2.7-22	10.9	5.2	28
" "	>12	%/48hr	3.3-30	14.6	6.6	30

* Peak serum concentration at 1 or 2 hr after the oral test dose

TABLE 5

INVESTIGATIONS IN SUBJECTS WITH TWO OR MORE ABNORMALITIES OF INTESTINAL FUNCTION

Case No.	Xylose Urine	Xylose Serum*	Folic Acid*	Fecal Fat	Fecal Nitrogen	$^{57}CoB_{12}$	Jejunal Morphology
	g/5hr	mg/100ml	ng/ml	g/24hr		%/24hr	
Nl.	>5	>30	>40	<5	<2.5	<8	0
1	5.1	64	30	1.5	0.6	5.2	1+
3	3.7	32	80	1.5	1.4	7.1	2+
17	3.2	32	51	6.0	1.4	11.0	1+
25	3.2	41	90	2.9	2.4	5.5	1+
44	3.7	48	87	4.4	1.6	5.0	0
45	4.6	48	82	1.9	1.0	7.3	1+
73	4.7	54	65	1.8	1.2	6.0	2+
76	4.4	30	51	5.6	1.9	3.1	3+
79	2.1	36	63	5.2	0.6	3.4	2+
81	4.1	30	75	1.9	1.0	6.0	2+
82	2.1	44	49	5.0	3.8	5.2	2+

* Peak serum concentration at 1 or 2 hr after the oral test dose

TABLE 6

RELATIONSHIP OF INTESTINAL MORPHOLOGY

TO FUNCTIONAL ABNORMALITIES

Morphology Severity	No.	Functional Abnormality			
		None	Xylose	Fat	$^{57}CoB_{12}$
			<5 g	5-6 g	<8%
0	17	13(76%)	2(12%)	0	2(12%)
1+	48	24(50%)	11(30%)	1(2%)	12(25%)
2+	24	5(21%)	9(37%)	2(8%)	13(54%)
3+	4	2(50%)	2(50%)	1(25%)	1(25%)

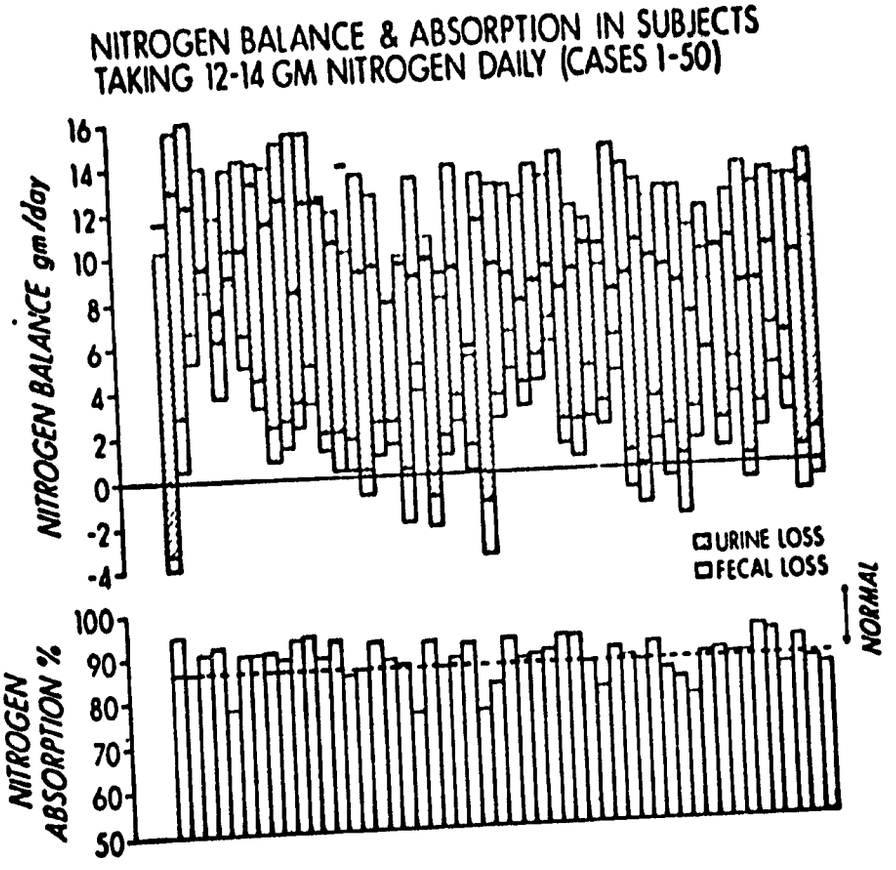

Fig. 1. Nitrogen balance and absorption in subjects receiving 12-14 g nitrogen daily (Cases 1-51). Single, heavy horizontal line indicates intake at home prior to hospitalization. Top of vertical bar indicates average intake and bottom of vertical bar indicates average balance during the study period.

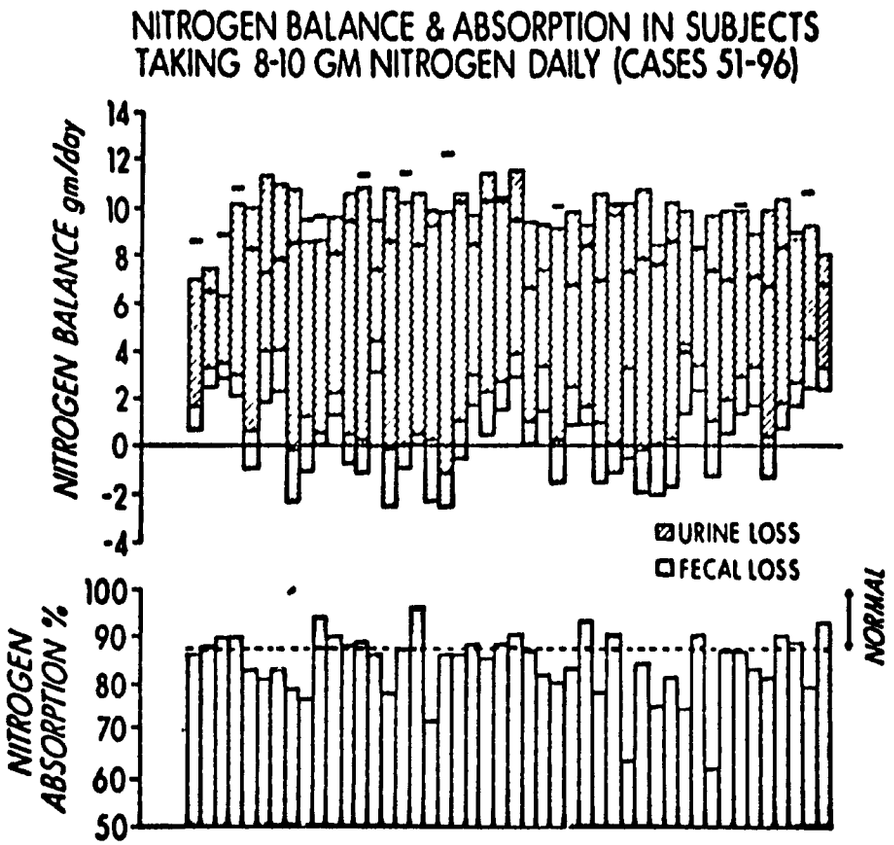

Fig. 2 Nitrogen balance and absorption in subjects receiving 8-10 g nitrogen daily (Cases 51-96). Single, heavy horizontal line indicates intake at home prior to hospitalization. Top of vertical bar indicates average intake and bottom of vertical bar indicates average balance during the study period.

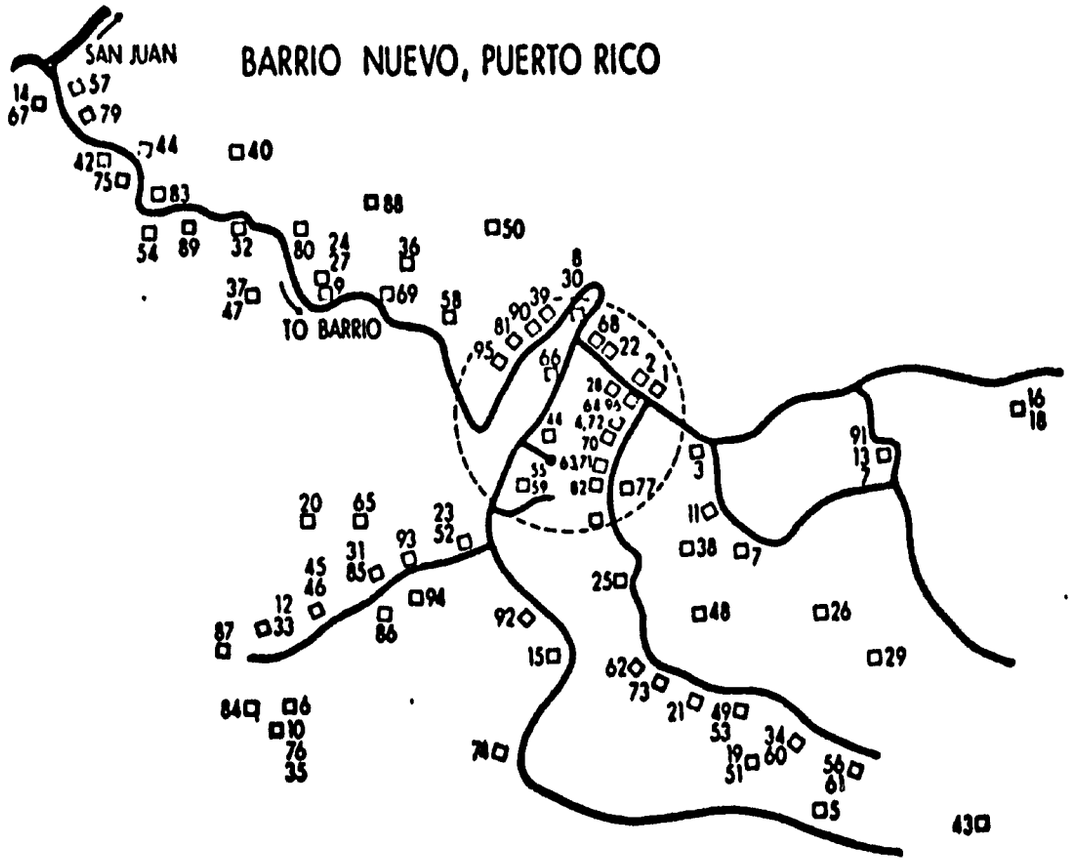

Fig. 3. Location of houses studied in the barrio. Houses within the circled area are referred to as in the center of the barrio. Cross hatching indicates presence of abnormalities of intestinal function among one or more inhabitants. Numbers refer to cases residing there.

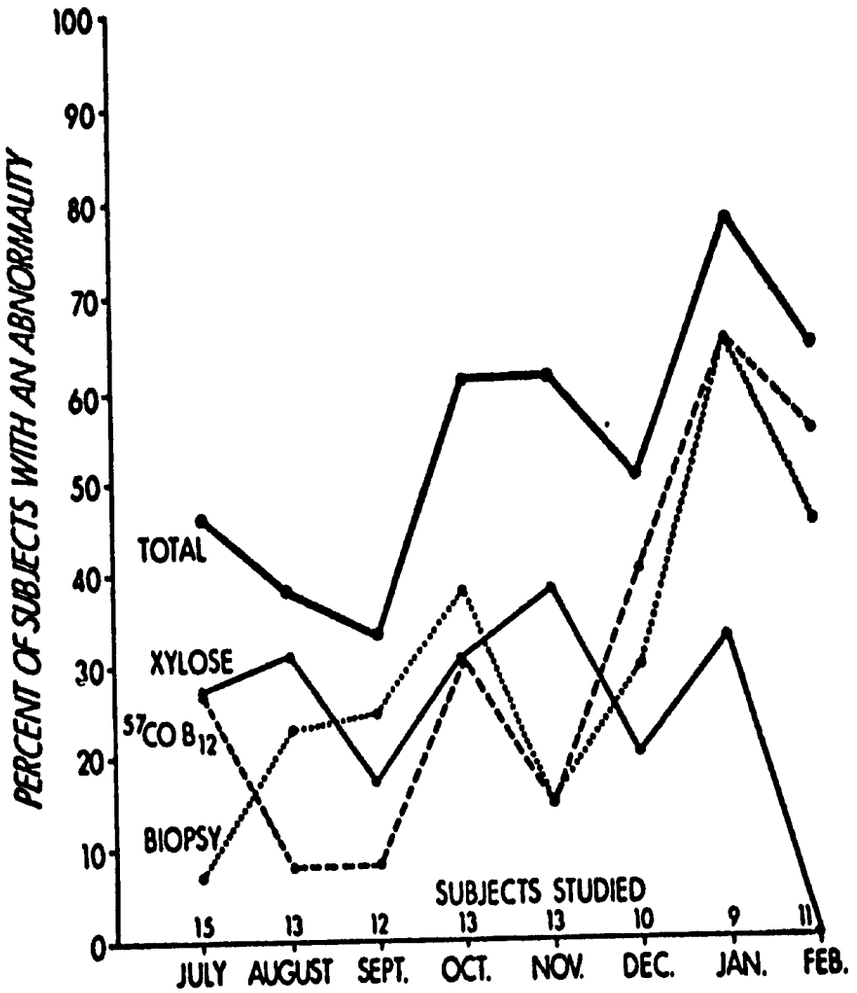

Fig. 4. Incidence of intestinal abnormalities in relation to time of investigation. Total refers to individuals who had an abnormality of morphology or xylose or vitamin B_{12} absorption, either singly or in conbination.

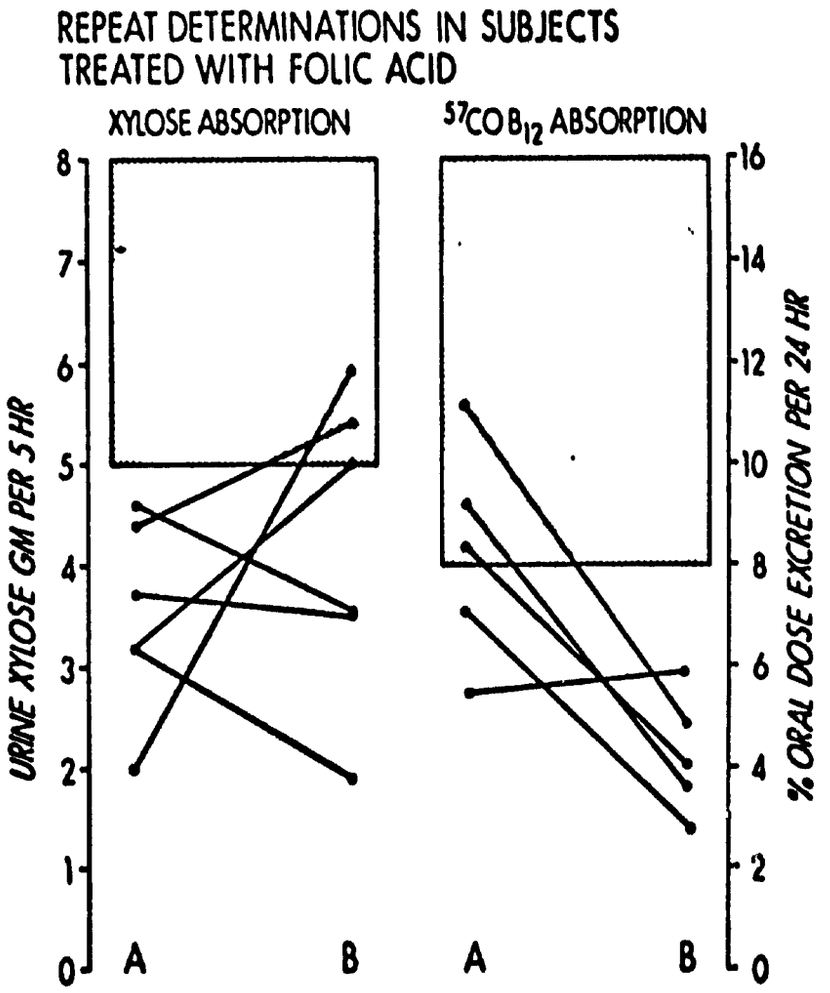

Fig. 5. Repeat observations in subjects treated with folic acid. A. Pretreatment. B. Observations conducted in February after 3 to 4 months' treatment. Hatched areas indicate the range of normal values.

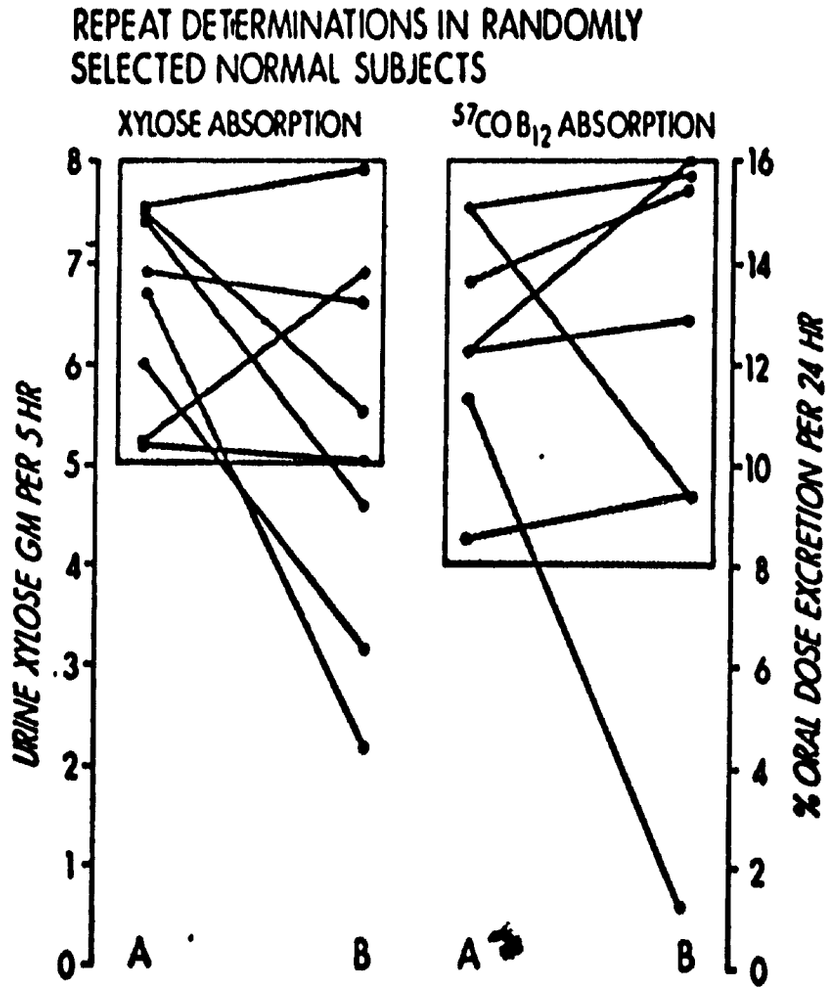

Fig. 6. Repeat determinations in randomly selected subjects who initially had normal determinations of intestinal function and morphology. A. Initial observations. B. Repeat observations 6 to 12 month later in October. Hatched areas indicate the range of normal values.

AVISO IMPORTANTE

COMUNIDAD DEL BARRIO NUEVO

El Hospital (Distrito) Universitario del Centro Médico y la Escuela de Medicina de la Universidad de Puerto Rico van a llevar a cabo estudios especiales para determinar el estado de nutrición y las funciones intestinales en personas adultas en varias comunidades de la isla. Estos estudios consisten en lo siguiente: (1) determinación de hemoglobina y glóbulos blancos (2) análisis de orina y excreta (3) pruebas de función del riñón (4) nivel de azúcar en la sangre, (5) radiografía de pecho, (6) electrocardiograma, (7) nivel de vitaminas en sangre, (8) colesterol en sangre, (9) pruebas de función y absorción del intestino y (10) estudios de la dieta y análisis de los alimentos de cada persona. Además, se expedirán certificados de salud a las personas que lo soliciten. Si se encontrara alguna anormalidad en los resultados de las pruebas y análisis, las facilidades del Centro Médico de Puerto Rico estarán disponibles para el tratamiento de la enfermedad que estuviera padeciendo la persona.

Se incluirán en este estudio todas las personas entre la edad de 20 a 65 años. Todos aquéllos que estén interesados en recibir este examen serán hospitalizados por un período de 3 a 6 días en un nuevo hospital del Centro Médico, habilitado con aire acondicionado y con todas las facilidades médicas. Se facilitará transportación de ida y vuelta a todos los pacientes. El examen completo y transportación serán completamente GRATIS y se les notificará a todas las personas sobre los resultados obtenidos.

ESTAMOS CONSCIENTES DE QUE ALGUNAS DE LAS PERSONAS QUE SE INCLUIRAN EN EL ESTUDIO TRABAJAN Y POR ESO COMPENSAREMOS A TODOS LOS QUE SEAN INCLUIDOS EN EL ESTUDIO CON ALREDEDOR DE $40.

Su comunidad ha sido escogida preliminarmente para ser estudiada y esperamos toda la cooperación posible, ya que los resultados a obtenerse van dirigidos a mejorar su salud. Próximamente los visitaremos para comunicarnos personalmente con ustedes y contestar las preguntas que pudieran tener.

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Departamento de Medicina
Hospital (Distrito) Universitario
Centro Médico
Escuela de Medicina de Puerto Rico

EHimrl

May, 1970

ENCUESTA DE BARRIO NUEVO - 1970

1- Nombre _____
Lugar y fecha de nacimiento:
_____

3- Dirección Actual
Calle y número _____
Pueblo _____
Tiempo residiendo _____

4- Dirección Anterior
Calle y número _____
Pueblo _____

5- Es dueño de la casa _____

6- Construcción de la vivienda
Materiales _____
Abastecimiento de Agua _____
Servicio Sanitario _____

7- Trabajo actual _____

8- Sitio _____

9- Nombre del Patrono _____
_____

10- Salario $ _____

11- Veterano _____ Pensión $ _____

12- Seguro Social $ _____ Retiro$ _____

12- Posee ganado, animales
_____
_____
_____

13- Trabaja la esposa _____

14- Patrono _____

15- Salario $ _____

16- Otros miembros de la familia trabajando:

Nombre	Sitio	Salario
		$

17-¿Ha estado enfermo durante los últimos cinco años? _____ ¿De qué?
_____
_____
_____
_____
_____
_____

18-¿Ha tomado alguna medicina durante los últimos seis meses? _____
¿Cuáles? _____
_____
_____
_____

GRUPO FAMILIAR CONVIVIENDO EN EL HOGAR ACTUALMENTE

Nombre	Parentesco	Edad	Estado Civil	Sexo	Instrucción	Ocupación

Observaciones: _____
_____

Fecha _____

PROJECT TITLE : NUTRIENT LOSS DUE TO MALABSORPTION IN APPARENTLY NORMAL VILLAGERS FROM SOUTHERN IRAN.

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C O N T E N T O U T L I N E:

I Objectives.

II Materials and Methods.

a) Fars Province.
b) Pahlavi Clinical Research Unit.
c) Subject Material.
d) Subject Evaluation.

III Nutrient Loss.

a) Direct evidence relating to absorption of nutrients.

1. Stool Fat.
2. Stool nitrogen.
3. Calorie loss in stool.
4. Vitamin B12 absorption.
5. Xylose absorption.
6. Multiple abnormalities.

b) Indirect evidence relating to absorption of nutrients.

1. Hemoglobin.
2. Mineral elements in blood :-
   i) Iron.
   ii) Calcium.
   iii) Zinc.
3. Serum Carotene.
4. Serum cholesterol.
5. Serum albumin.

IV Balance Studies.

i) Nitrogen.
ii) Mineral : Calcium, Phosphorus, and Zinc.

V Small intestinal morphology.

IV Summary of abnormalities.

VII Discussion.

VIII Conclusion.

IX Recommendations.

X References.

XI Tables.

XII Cases : 3 cases with steatorrhea.

XIII Data for the 100 subjects.

I OBJECTIVES.

The determination of malabsorption of nutrients in apparently normal villagers of Southern Iran.

II MATERIAL AND METHODS.

a) Fars Province.

This province is located in Southern Iran and covers a 52,069 square mile area (8.09% of area of Iran) at 27°3' to 31°42' longitude North and 50°37' to 55°38' latitude East.

Fars is mountainous with an average altitude of 5000 feet. The climate is variable but usually dry and hot with mild winters and an occasional snow.

The 1966 census showed the population to be 1,439,804 with a population density of 27.6 per square mile.(1) Nearly 60% of the population live in rural area and 1.5% are nomadic and migratory. The capital city, Shiraz, has a population of 270,000. The male to female ratio is 1.07 with a mean age of 21.6 years.

Only 3% of the population is technical and professional, the remainder being primarily agricultural, labor and service work. There is very little heavy industry in the Province.

Over 99% of the population is Moslem. The literacy rate has been estimated at 31.9% (urban 50.8%, rural 18.4%). Consanguinity is quite common and first cousin marriages represent 30% of the total.

All dietary habits are variable. The rural community has been estimated of having a daily caloric intake of about 1700 calories. The diet is particulary deficient in animal proteins. The total protein and fat intake are also lower than comparable city dwellers of Fars. Thus the main source of caloric intake remains to be carbohydrates, especially the village bread. The type of food may, otherwise, be described as spicy, and tea is consumed to a great extent. Waterpipe, opium and cigarette smoking are prevalent. Folk medicines, including many herbs, are commonly employed. Environmental hazards in terms of industrial air pollution is not a problem.

Medical care in Fars is variable considering the scattered and low populated rural communities with poor means of transportation. Some of the rural communities are served through a health corps system (recently graduated physicians, nurses etc.). The larger towns and cities are covered by private and welfare organization as well as an Insurance system, primarily sponsered by the government. According to the 1966 census, Fars had a total of 1306 hospital beds (1 bed for 1109 inhabitants), with 700 of them in the capital city of Shiraz. Of these over 800 are affiliated with Pahlavi University.

II 3. Pahlavi Clinical Research Unit.

This is a multidisciplinary clinical research unit whose facilities include :-

1. A ten bed metabolic service located in Nemazee Hospital of Pahlavi University.
2. Research laboratories adjacent to the metabolic service.
3. A"10,000 population" rural community in

19 villages within 40 minutes of Shiraz
(rural population research unit).

The subjects for the present study were chosen from the Rural Population Research Unit and the study was performed in the metabolic service and the research laboratories located in Nemazee Hospital.

Rural Population Research Unit: The 19 villages for this total rural population study were chosen in such a manner that the only prejudicial factor in their choice was their availability through some kind of a road in all seasons and their being within reach from Shiraz. Otherwise, they may be considered representatives of villages in Fars. They are located in the Marvdasht area with 60km. of Shiraz. The 19 villages are amongst over 50 within this area. Their total population is 10,466 (1970) with the range of 76 to 2168 people in each village. The median age of this population is 15.7.

Studies on food consumption in a limited number of individuals (13-14 year old boys) from this general area is available.[2] The total daily caloric intake was 1583 ± 342. This included daily means of 41.1 gm. protein and 18.5 gm. fat.

II C. Subject Material.

The choice of 100 apparently normal subjects was according to the following general guidlines: -

1. Subjects below the age of 15 were not considered.
2. Pregnant women were not included. Those

recently delivered were usually not included because of refusal on part of subjects.

3. Subjects with noticeable disease either on screening or on initial hospital evaluation were not included in the final 100.

Considering the above general guidelines, the 100 apparently normal villagers were chosen from the Rural Population Research Unit according to the following two systems :-

1) There being a card computerized for each person in the Rural Population Unit, 200 subjects above the age 15 were selected. Then as each of these subjects presented to the clinics located in each of the 19 villages, They were asked to come into the metabolic service FOR evaluation. Eventually, 16 of these subjects entered the study. The major reasons for the others not being evaluated include : not referring to the clinic, being involeved WITH crops, pregnant when seen, or recently delivered. Initially, it was expected that nearly 50 of the necessary subjects would be chosen in this manner, but with the medical care delivery system organized for these villages (a clinic held in each village at the most every 2 weeks), and the then voluntary submission for evaluation, only 16 subjects were finally evaluated through this system.

2) A voluntary submission for evaluation through clinic visits. It should be noted that with one physician and this rotating system of clinic visits, this rural medical care delivery system was quite popular. It is estimated that 5000villagers saw our physician in a 17 month period. Considering the readily available care and the low cost, many of the visits were not for disease

but rather minor complaints. In this manner, and considering the general guidelines, subjects were asked to be admitted for evaluation. The only resistance to this system occurred during the work seasons or in presence of very young children. Thus, the 84 other subjects were chosen through this type of a voluntary system. It should be noted that subjects with gastrointestinal symptoms were not excluded.

The age and sex distribution of these subjects are presented in Table 1.

II D. Subject Evaluation

The 100 subjects were brought to the metabolic service of the Pahlavi Clinical Research Unit for an approximate 2 week stay. Initially a complete history and physical examinations were performed. Then, the necessary blood, urine and stool tests including the xylose. Subsequently there was the 6 day nitrogen and fat balance studies. This was followed by a Schilling test and intestinal biopsies.

Nine subjects had their balance studies performed both on a measured hospital diet and their approximate village diet, including the village bread.

Total calorimetry was performed in 8 subjects while 3 other volunteers had prolonged mineral balance studies.

III. NUTRIENT LOSS.

III. A. Direct Evidence for Malabsorption of Nutrients.

Each subject who was admitted to the metabolic service was placed on a standard test diet. This test diet contained a known

quantity of nutrients, the fat being over 80 gm/d and the nitrogen being 13± 1 gm/d. A duplicate diet was analyzed each day for fat and nitrogen contents. Food not eaten by the patient at a particular meal was offered at another time in that same 24 hour period. Food not eaten in the 24 hour period was measured in terms of its fat and nitrogen contents, and these values were subtracted from that particular day's measured fat and nitrogen offerings.

The daily intake for each patient was 2000-2400 calories.

With the start of each test meal, a charcoal marker was given. From the morning after the charcoal appeared in the stool, collections were begun and continued for a 6 day period.

III A.

1. Stool Fat.

On a known fat intake (usually greater than 80 gm/d) daily stool fats were determined over 6 days.[3] These 6 day values were then averaged. Distribution of stool fats is presented in Table 2. Only 3 subjects had greater than 5 gm. fat in their stool each day. The disease diagnosis in these subjects has not been made despite extensive evaluation including a laparotomy in one case. Case histories of these 3 subjects is presented in the addendum. One of the 3 cases was from the random selection group of 16. This patient was also treated with tetracycline (250 mg. 4 times daily for 2 weeks), with no change in the degree of steatorrhea. There was no correllation between steatorrhea and parasites, including giardia, found in the stool examination. Otherwise, in 97 subjects, the amount of fat in the stool was in a range considered normal.

In 9 subjects stool fat measurements were made on both the test meal and the villagers approximate diet (fat content 30-35gm/d, including the phytate rich village bread). There was no steatorr -

hea under these circumstances simulating the village intake.

III A

2. Stool Nitrogen.

On a known nitrogen intake of about 13 gm/d, daily stool for nitrogen was determined.[4] Six days values were then averaged (Table 3). 12 subjects had more than 2 gm. and 4 greater than 2.5 gm. of nitrogen in their stools daily. There did not appear to be a correlation between this fecal nitrogen loss and parasiticism.

In the 9 subjects who were also tested on the approximate village diet (with nitrogen content usually lower than that of the comparable standard test diet); everyone's stool nitrogen excretion was greater while on the village diet as compared to the findings while on standard test diet. In 2 subjects, the stool nitrogen values while on village diets were greater than 2 gm/d; in one of these, the value being close to 3 gm. daily.

III. A

3. Caloric Loss in Stool.

While on the standard test diet, calorimetry was performed on 8 subjects.[5] The results are presented in Table 4. The caloric loss in the stool ranged between 5.8 - 9.2% of the caloric intake.

III. A

4. Vitamin B12 Absorption.

The standard Schilling test with intrinsic factor were performed in all subjects,(Table 5). Twenty of these values may be

ted in Table 8-10, and suggest an iron deficiency in nearly 15 - 30% of the subjects.

III B.

2b. Serum Calcium and Phosphorus.[9,10]

Serum Calcium and phosphorus determinations are presented in Tables 11, 12. The results show a low serum calcium in at least 13 subjects.

III B.

2c. Serum Zinc.[9]

The determination was performed in 85 subjects,(Table 13). Thirteen of these had low serum zinc values.

III B

2d. Correlation.

Correlation of minerals in blood of these villagers is presented in Table 14. There was little correlation between a low serum zinc, percent iron saturation and low serum calcium.

III B

3. Serum Carotene.[11]

The results of serum carotene determinations is presented in Table 15. Over half had values which were below normal suggestive of a low dietary intake or malabsorption.

III B

4. Serum Cholesterol.[12]

Serum Cholesterol determinations are presented in Table 16. Twenty nine or nearly ⅓ of the subjects had serum cholesterols of less than 150 mg.%

III B

5. Serum Albumin.[13]

Serum Albumin determinations by electrophoresis on cellulose acetate is reported in Table 17. In 35 subjects the serum albumin

was lower than 3.5 gm%. All these subjects had normal liver function studies including a BSP. Moreover, none of the 35 had significant proteinuria.

IV BALANCE STUDIES.

1. Nitrogen.

Nitrogen balances were available in all 100 subjects, (Table 18). They were predominantly positive, though suprisingly 43 out of the hundred had negative nitrogen balances.

In 9 subjects nitrogen balance studies and nitrogen excretion in feces were compared between the standard test diet and the village diet containing abundance of local bread,(Table 19). The major difference between the 2 diets was in the quantity of nitrogen excreted, this being higher on the village diet. In a limited study of 3 subjects an attempt was made to clarify the reason for this increased fecal nitrogen loss. Feeding of bread was compared to feeding of an equivalent amount of phytic acid (2.5 gm. daily). Otherwise, the diets were similar. The sodium phytate feeding improved the nitrogen balance (with a decrease in fecal loss). This is suggestive of the bread and its high indigestable fibre residue being responsible for the increased fecal nitrogen loss.

IV

2. Mineral.

Balance studies of calcium, zinc and phosphorus were determined in 13 of the subjects. All showed strongly positive balances of calcium and phosphorus while 11 out of 13 also showed positive zinc balances. Retention of calcium averaged 35%, of phosphorus 23%, and of zinc 26% of quantities ingested in the diet.

Three subjects had more prolonged balance studies of calcium and zinc,(Table 21/22), including a preliminary control period, (on less than 1 gm. phytate/d for 16 days), sodium phytate period (2.5 gm. phytate / d for 28 days), Tanok period (2.5 - 3gm. phytate / d for 32 days) and a final control period (less than 1gm. phytate /d for 16 days). These show a deleterious action of phytate ingestion when given in large amounts upon calcium and zinc nutrition.

V SMALL INTESTINAL MORPHOLOGY.

The peroral small intestinal suction biopsy capsule (Rubin tube) was used and the results are presented in Table 23. Nearly ⅓ of the subjects had villous abnormalities ranging from mild to severe flattening.

VI SUMMARY OF ABNORMALITIES.

These include 3 subjects with steatorrhea, and 12 with increased fecal nitrogen loss. This fecal nitrogen loss increased when subjects were placed on their approximate village diet. Two subjects had both steatorrhea and increased fecal nitrogen loss. Twenty had abnormal vitamin B12 absorption while 3 had xylose malabsorption.

Only one subject with steatorrhea had anemia, though there was evidence of iron (29 subjects), calcium(13 subjects), and zinc (21 subjects) deficiencies. Fifty-two had low serum carotene, 29 had low serum cholesterol and 35 had hypoalbuminemia.

The balance studies showed nearly half, with negative nitrogen balances; they also confirmed the deficiency of calcium and zinc.

The small intestinal morphology could be considered abnormal in nearly ⅓ of the subjects

III. DISCUSSION.

The selection of villages and the subjects has been according to a nearly random and voluntary process so that for all practical purposes one could consider these subjects representative of the population of Southern Iran. There is no similar information available on other villages in Iran and one could not presently generalize and consider our 100 subjects representative of all of Iran's rural communities.

The findings in these 100 apparently normal villagers suggests that major nutrient loss of nitrogen and fat does not lead to significant calorie loss. This is of interest since the small intestinal morphology is quite abnormal, especially in terms of villous atrophy, in almost $\frac{1}{3}$ of the subjects. Thus, there appears to be little correllation between villous atrophy and malabsorption, especially that of fat.

The diet of this rural population does appear to be deficient in calories, protein (especially animal protein) and fat. This may explain the low serum cholesterol, albumin and carotene. On the other hand the village diet is rich in a special bread - high residue and rich in terms of phytate content. This high residue appears to lead to a greater fecal nitrogen loss but more importantly phytate appears to play a major role in mineral depletion (i.e. calcium, zinc and probably iron).

The 3 subjects with steatorrhea are of interest and more studies are necessary to clarify the pathogenesis for this malabsorption. In one subject, treatment with antibiotics, (tetracycline) did not appear to make a difference in this steatorrhea.

VIII. CONCLUSION.

Despite the frequent finding of structural abnormalities of the small intestine, described as "sprue like pattern", there is little malabsorption of major nutrients in the rural population of Southern Iran. Certainly, the caloric loss does not appear to be significant. The most significant aspect of nutrient malabsorption appears to be that of minerals and this may very well be due to the high content of phytate in the village bread.

The only other factor in the village diet which may lead to nutrient loss is the high residue which was shown to be associated with increased loss of nitrogen in the feces.

IX. RECOMMENDATIONS.

Several findings require further investigation:-

1. The degree of small intestinal morphologic changes and its possible functional importance in parameters other than absorption of major nutrients.

2. The determination of reason for fecal nitrogen loss, including protein losing enteropathy.

3. The determination of etiology of steatorrhea in the few who demonstrated this abnormality.

4. The determination of etiology of vitamin B12 intrinsic factor malabsorption.

5. Reason for high xylose excretions.

6. Confirmation of mineral deficiency and their relation to phytate intake in a significant number of subjects.
7. Importance of the village diet, from the standpoint of residue, in malabsorption.

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TABLE 1.

ONE HUNDRED APPARENTLY NORMAL VILLAGERS FROM SOUTHERN IRAN.

SEX AND AGE DISTRIBUTION.

	Male	Female
15 - 20	5	7
21 - 30	15	14
31 - 40	21	12
41 - 50	11	10
51 - 60	1	4
Over 60		
T O T A L	53	47

TABLE 2.

STOOL FATS: GM / DAY AVERAGED OVER 6 DAYS.

Gm / Day	Number of Subjects.
Less than 2	56
2.1 - 3	26
3.1 - 4	14
4.1 - 5	1
5.1 - 6	2
6.1 - 7	-
7.1 - 8	1
Greater than 8	0
T O T A L	100

TABLE 3.

STOOL NITROGEN - GM / DAY AVERAGED OVER SIX DAYS.

Gm / Day	Number of Subjects.
Less than 1.5	67
1.6 - 2	21
2.1 - 2.5	8
2.6 - 3	4
3.1 - 3.5	0
Greater than 3.6	0
T O T A L	100

TABLE 4.

CALORIE LOSS IN STOOL - 8 Subjects.

Case Number	Intake Calories	Output Calories	% Of Intake
30	2,313	140	6.1
31	2,364	145	6.2
33	2,369	217	9.2
34	2,393	141	5.9
36	2,140	148	6.9
37	2,140	124	5.8
38	2,140	125	5.8
42	2,056	181	8.8

TABLE 5.

VITAMIN B12 ABSORPTION. SCHILLING TEST

WITH INTRINSIC FACTOR

Vitamin B12 % in Urine / 24 hours	Number of Subjects.
Less than 7	7
7 - 10	13
Greater than 10	80
T O T A L	100

TABLE 6.

XYLOSE ABSORPTION, PERCENT URINARY EXCRETION IN 5 HOURS AFTER A 25GM. ORAL INTAKE

% Urinary Excretion	Number of Subjects.
Less than 20	13
21 - 40	54
41 - 60	25
Greater than 60	8
T O T A L	100

TABLE 7

HEMOGLOBIN VALUES IN 100 SUBJECTS.

Gm %	Male	Female.
Less than 8	1	0
8.1 - 9	0	0
9.1 - 10	0	0
10.1 - 11	1	0
11.1 - 12	0	1
12.1 - 13	6	9
13.1 - 14	3	14
14.1 - 15	8	15
15.1 - 16	20	8
16.1 - 17	9	0
17.1 - 18	4	0
Over 18	1	0
TOTAL	53	47

TABLE 8.

SERUM IRON

NORMAL = GREATER THAN 70 micrograms %

Values microgm%	Number of Subjects.
Less than 70	14
71 - 160	71
Greater than 160	13
TOTAL	98

TABLE 9.

SERUM IRON BINDING CAPACITY

Values Microgm %	Number of Subjects.
Less than 150	0
151 - 200	4
201 - 250	5
251 - 300	11
301 - 350	26
351 - 400	24
Greater than 400	25
T O T A L	95

TABLE 10.

IRON BINDING, PERCENT SATURATION

NORMAL 25 - 50%

% Saturation	Number of Subjects.
Less than 10	3
11 - 20	13
21 - 25	13
26 - 30	15
31 - 40	23
41 - 50	14
51 - 60	4
Over 60	8
T O T A L	93

TABLE 11.

SERUM CALCIUM IN 100 SUBJECTS.

Mg %	Number of Subjects.
Less than 7	0
7.1 - 8	4
8.1 - 8.5	9
8.6 - 9.0	23
9.1 - 10.5	63
10.6 - 11.0	0
11.1 - 11.5	0
Greater than 11.6	1
T O T A L	100

TABLE 12.

FASTING SERUM PHOSPHORUS IN 100 SUBJECTS.

Mg %	Number of Cases.
Less than 2	0
2 - 2.5	3
2.6 - 3	15
3.1 - 3.5	34
3.6 - 4	30
4.1 - 4.5	16
Greater than 4.5	2
T O T A L	100

TABLE 13.

SERUM ZINC: NORMAL GREATER THAN 75 Microgram %

Microgram %	Number of Subjects.
Less than 75	13
Greater than 75	72
T O T A L	85

TABLE 14.

CORRELATION OF MINERALS IN BLOOD OF VILLAGERS - (IRON SATURATION, SERUM CALCIUM AND SERUM ZINC.)

Correlation +	Number of Subjects.
3/3	2/79
2/3	4/79
1/3	30/79
2/2	1/21
1/2	13/21
1/1	0/0

+ The nominator represents number with low serum values. The denominator represents number having had the determination; i.e. 2/3 means that, of the number who had the 3 mineral determination (denominator), 2 were found to be low.

TABLE 15.

SERUM CAROTENE: NORMAL - GREATER THAN

60 MICROGRAMS %

Values - Micrograms %	Number of Subjects.
Less than 60	52
60 - 70	8
Greater than 70	40
T O T A L	100

TABLE 16.

SERUM CHOLESTEROL. NORMAL 150 - 250 Mg.%

Values Mg.%	Number of Subjects.
Less than 100	1
101 - 120	3
121 - 140	12
141 - 150	13
151 - 250	66
Greater than 250	5
T O T A L	100

TABLE 17.

SERUM ALBUMINS.

Values Gm.%	Number of Subjects.
Less than 2	0
2.1 - 2.5	0
2.6 - 3	12
3.1 - 3.5	23
3.6 - 4	41
4.1 - 4.5	23
4.6 - 5	1
Greater than 5	0
TOTAL	100

TABLE 18.

NITROGEN BALANCE STUDIES.

Balances.	Number of Subjects.
+4.1 → +5	4
+3.1 → +4	6
+2.1 → +3	9
+1.1 → +2	15
0 → +1	23
-1 → 0	20
-2 → -1.1	15
-3 → -2.1	4
-4 → -3.1	3
-5 → -4.1	1

TABLE 19.

NITROGEN BALANCE AND FECAL NITROGEN EXCRETION

COMPARISON OF STANDARD TEST DIET AND APPROXIMATE

VILLAGE DIET.

Case Number.	Nitrogen Balance		Fecal Nitrogen.	
	Standard.	Village	Standard.	Village.
61	+6.56	+2.74	1.24	1.67
62	+1.14	+1.73	0.94	1.67
63	+2.66	+5.76	1.16	2.94
64	+1.61	+1.75	1.22	2.31
65	+1.98	+0.99	1.21	1.80
66	+3.30	+1.47	1.31	2.15
67	+0.92	-1.19	1.09	1.61
68	+2.55	+3.12	0.80	0.96
69	-1.70	-0.96	1.76	1.89

TABLE 20.

ZINC BALANCES DURING PHYTATE ADMINISTRATION.

PERIOD	CONTROL		SODIUM PHYTATE.		TANOK		CONTROL
Phytate* per day:	<1 g.		2.5 g.		2.5 to 3 g.		<1 g.
Duration, days	16		28		32		15
	Mg. calcium / 24 hours						
Rah.							
Mean	+11.6		+6.17		-2.7		+5.2
S.E.	1.50		0.69		0.53		2.43
p**		0.001		0.001		0.001	
Teh.							
Mean	+10.3		+0.6		-2.2		-
S.E.	1.27		0.57		0.89		
p		0.001		0.001			
Ranj.							
Mean	+ 7.1		+3.1		+6.5***		+10.1
S.E.	1.37		1.45		2.29		3.6
p		0.01		0.05		N.S.	

* As phytic acid.

** Significance of difference as evaluated by t test.

*** Food intake increased by 25 % over that of control period.

TABLE 21.

CALCIUM BALANCES DURING PHYTATE ADMINISTRATION.

PERIOD	CONTROL		SODIUM PHYTATE.		TANOK		CONTROL
Phytate* per day	<1 g.		2.5 g.		2.5 to 3 g.		<1 g.
Duration, days.	16		28		32		16
Mg. Calcium per 24 hours.							
Rah.							
Mean	+236		+248		-282		+19
S.E.	34		55		48		55
p**		N. S.		0.001		0.001	
Teh.							
Mean.	+471		- 88		-209		-
S.E.	101		111		98		-
p**		0.001		N,S(0.001)			
Ranj.							
Mean	+222		+119		-184***		-65***
S.E.	47		36		38		108
p**		0.01		0.001		0.05	

* As phytic acid.

** Significance of the difference as evaluated by t test.

*** Food intake increased by 25 % over control period.

TABLE 22.

PEROEAL SMALL INTESTINAL BIOPSY.

Classification	Number.
Normal	11
Nonspecific or Minor changes.	38
Hypercellularity	14
Villous atrophy (minimal to severe).	28
Insufficient.	9
T O T A L	100

XII CASES WITH STEATORRHEA.

Case 1. No. 1 R.R. No. 101415.

This 20 year old male was completely asymptomatic and was admitted for malabsorption evaluation. The findings are presented in the data summary. The etiology of his steatorrhea was not determined by evaluations listed. He was discharged to be readmitted for exploration. Meanwhile he was drafted into the Iranian Army and moved to another area. An attempt is being made to readmit and evaluate him.

Case 2. No. 22. H.S. 107304.

This 45 year old male was asymptomatic except for minimally symptomatic hemorahoids. The remainder of history and physical were unremarkable. The lab. findings are presented in the data sheet. No diagnosis was made and patient is being followed.

Case 3. No. R 10. Gh.M No. 119454.

This 40 year old male had epigastric pain of 4 months duration and distention. These did not relate to anything. There was no nausea or vomiting. He had 1-3 bulky non-bloody stools per day. His appetite was good and he did not have any weight loss.

His physical examination was unremarkable except for pallor.

His lab. data are presented on the data sheet.

Of significance was his steatorrhea and giardiasis. He received atabrine and with continuing symptoms, Tetracycline, 250 mg. 4 times daily for 2 weeks. The stool fat studies were repeated and the steatorrhea had remained unchanged.

With an abnormal 'malabsorption' pattern of small intestinal X-Ray, the patient had a peroral small intestinal biopsy which showed almost total flattening of mucosal surface with heavy lymphocytic infiltration. At surgical exploration, the serosal surface appeared normal and ther was no mesenteric adenopathy. A full thickness small intestinal biopsy showed broadened villi with lymphocytic infiltration of lamina propria.

He is presently being followed, there being no definitive diagnosis.

Part B. Impaired Dietary Mineral Utilization in Rural Iran.

Summary Consumption of large amounts of phytate in the form of unleavened wholemeal wheat bread by the rural population of Iran interferes extensively with the utilization of mineral nutrients including calcium, iron, zinc, probably magnesium and phosphorus. Preliminary experiments indicate that the phytate of village bread is more potent in its effects upon calcium and zinc metabolism than is pure sodium phytate. The prevalence of rickets and osteomalacia, retardation of growth and sexual development, and iron-deficiency anemia are direct effects of high phytate intakes.

Practical methods for overcoming the harmful effects of phytate are proposed.

High phytate consumption may be a major cause of malnutrition in developing countries.

Introduction:

The Iranian villager subsists largely upon unleavened wholemeal wheat bread made by primitive methods. The bread contains an abundance of mineral elements required for human nutrition. Calcium intakes approach 1 g. daily. Average iron intakes are more than twice the allowance recommended (18 mg. daily) by the Food and Nutrition Board of the National Research Council. Those of zinc exceed by a substantial margin the intakes of Americans consuming a nutritious diet. Magnesium and phosphorus are also present in the village diet in large amounts. Despite these seemingly safe margins, there is strong evidence that disturbances of calcium, phosphorus, iron and zinc metabolism are widespread in the rural population and probably among certain poor in the cities. Moreover these disturbances are undoubtedly of nutritional origin. They are the result of faulty nutrient utilization.

Mineral metabolism of villagers

A survey of more than 2000 school children 6 to 9 years old in Isfahan by Emami etal (1) showed that about 10% were suffering from rickets and an equal number to have bone deformities due to other causes. Children of both rural and urban

background were included. Rickets is prevalent also in the Shiraz region (2), and may lead to serious deformities (3). It responds rapidly to treatment with Vitamin D (2). Bone pain is among yhe most frequent complaints of adult villagers and is suspected to be a sign of osteomalacia.

Iranian villagers are small in stature (4). Growth retardation appears in early infancy and continues through childhood. On the other hand, children belonging to the urban middle class differ only little from the growth norms of developed countries. Puberty is delayed in village children and in some instances fails to take place. The latter results in the hypogonadal dwarfism syndrome which has been corrected by administration of zinc together with a nutritious diet (5). One estimate of the frequency of the latter is that from two to three per cent of the rural population of Fars Province is affected (6).

The incidence of iron deficiency anemia is high and it is not unusual for 30% of the populations of villages to fall below 12.5 g/100 ml in hemoglobin concentration despite an average altitude of 1700 m. Ten per cent may fall below 10 g/100 ml. The anemia develps despite iron intakes that are high and, despite the latter, responds promptly and effectively to medication with iron (7).

Abnormally low concentrations of mineral elements in blood

Zinc: 17% of the adult villagers included in the present study, 72% of older children (Marvdasht), and 80% of younger children (Kherak) in villages had plasma zinc concentrations below 75 ug/100 ml., the minimum found in well-nourished persons. Concentrations below 50 ug./100 ml. were frequent. Moreover, zinc concentrations in plasma failed to rise during zinc treatment while a village diet was consumed, unless large amounts (40 g. daily of elemental zinc) were given. The failure is attributed to poor utilization.

Calcium: Concentrations of calcium in plasma were less than 8.8 mg./100 ml. in 17% of adults and 24% of younger children. Fewer low values were found in older children who had been receiving vitamin D along with other vitamins in the course of

the study before calcium analyses were made. Low values were more frequent in blood samples collected in February to May as compared with October and November.

Iron: The frequency of low iron concentrations in serum is similar to that of low calcium in the adult group. For reasons that are not clear, anemia was less frequent in the adults studied for malabsorption than in many samples of village adults.

Phosphorus: All samples for phosphorus analysis in serum were collected with subject in fasting state. Under these conditions the minimal concentration found in normal subjects if 2.8 mg/100 ml. 9% of the adults had concentrations below this level. The mean and mode are significantly lower than in well-nourished adults. The lowered serum phosphorus concentrations may be a response to hypersecretion of parathyroid hormone stimulated by calcium depletion.

High retentions of mineral elements by villagers consuming a nutritious diet: (See preprint of paper by Reinhold, Hedayati, Lahimgarzadeh, and Nasr).

Balances of calcium, zinc, phosphorus and nitrogen were measured in 13 villagers undergoing evaluation for nutrient utilization. They received the hospital diet modified by addition of fat as described. All showed strongly positive balances of calcium and phosphorus. 11 of 13 also showed markedly positive zinc balances. On the other hand, nitrogen balances averaged close to zero. Retentions of calcium averaged 35%; of phosphorus, 23%, and of zinc, 26% of quantities ingested in the diet. Well-nourished adults retain at most 3% of the amounts in their diets. The strikingly positive retentions of the villagers are evidence of severe depletion of the mineral elements. Avidity of retention of calcium has been shown to depend upon degree of calcium depletion (8).

Effects of phytate added to diets of human volunteers

a. On calcium and zinc balances. Phytate was fed in two forms. First, as neutralized sodium phytate (pure crystalline from maize) and, secondly, as tanok, the phytate-rich village bread. 2.5 g. daily as phytic acid was fed during the sodium phytate experiment, and between 2.5 and 3 g. daily during the tanok period. An initial

control period preceded administration of phytate, lasting 16 days. Phytate was fed for 28 days and tanok for 32. A final control period of 15 days terminated the experiment in two subjects. Complete collections of urine and feces were made. Duplicate diets were prepared and analyzed together with excreta for calcium, zinc, phosphorus and nitrogen.

During the initial control period, balances of calcium and zinc were strongly positive (Tables 2 and 3). They became less positive during the sodium phytate period, but during the tanok period became negative. Losses of calcium, particularly, were large. Zinc balances were strongly negative in 2 of the 3 subjects during the tanok period, but positive in a third whose food intake was increased by 25% at this time. After discontinuation of the high phytate intakes, balances became positive or less negative.

Phosphorus balances tended to become more positive while phytate was fed. Whether this represents utilization of some of the phosphate of phytate or increased absorption of phosphorus because of decreased calcium activity remains to be established.

The deleterious action of phytate ingested in large amounts upon calcium and zinc nutrition is demonstrated by these experiments. Especially noteworthy is the potency of phytate in the form of village bread for causing losses of these nutritionally important elements.

b. <u>On concentrations of mineral elements in plasma</u>. Blood was collected at two-week intervals before, during, and after administration of phytate. Decreased concentrations of calcium and zinc in plasma and iron in serum followed sodium phytate ingestion. Calcium concentrations remained low, but those of iron and zinc returned to normal. Serum phosphorus concentrations also showed a gradual decline paralleling the decrease in plasma calcium. A stimulation of parathyroid secretion brought about by falling concentrations of calcium is suggested as the explanation. Alkaline phosphatase activity rose in two subjects after 6 weeks of phytate ingestion.

Hemoglobin concentrations which were low in one subject and at the lower limit of normal in the others rose rapidly during the initial control period. The rise was interrupted during the sodium phytate period but was resumed during the tanok period.

The fall in iron, zinc and, especially, calcium concentrations in the plasma of each of the 3 subjects is further evidence of the harmful action of phytate when consumed in amounts comparable to the daily phytate intakes of villagers.

Phytate intakes of villagers

Although several types of bread are consumed in the villages of central and southern Iran, they have in common the use of flour that includes 90% to 100% of the substance of the wheat kernel. By comparison, Western milling practice extracts only 65% for white flour and 70% to 75% for wholemeal flours. Because of the high rate of extraction, the flour used by villagers includes nearly all of the phytic acid of the wheat. Furthermore, leaven is omitted by the village breadmakers and the phytic acid escapes the destructive action of the yeasts and bacteria of the leaven. Finally, the Iranian villager depends upon bread to supply 50% to 75% of his caloric intake and must therefore consume large amounts. The combined effects of high phytate content and high bread consumption is estimated to lead to intakes of 3 to 5 g. daily. Smaller amounts have been shown to produce toxic effects in animals (9).

Phytate consumption continued at this rate for years must inevitably impair the metabolism of calcium, iron, zinc and perhaps other divalent metals by interfering with their absorption from the intestine. Although claims have been made that adaptation occurs to phytate in the diet, these are based on experiments of a few weeks or months duration in which phytate intakes were lower than those of the villagers. (10,11). Our findings indicate that if adaptation occurs it does not fully overcome the disturbing effects of high phytate intakes.

Implications for nutrition in developing countries.

The breads prepared by Iranian village housewives are typical of those that are the staple foods of bread-consuming developing countries. Made from wholemeals of

high extraction rate they are mostly unleavened. When leaven is included, the time allowed for its action is likely to be too short for effective destruction of phytate. Examination in our laboratory of chupattis from India, tortillas from Mexico, maize-wheat breads from Egypt, and tef breads from Ethiopia has shown phytate concentrations that range from 500 to 1000 mg. per 100 g. of dry bread. This is the same as that found in the village breads of Iran.

It is not surprising that disturbances of mineral metabolism such as tropical rickets, osteomalacia, and iron deficiency anemia have been causes of concern in these countries.

Approaches to corrective action

The likelihood that high intakes of phytate contribute to an important extent to the disturbances of mineral metabolism and consequent ill health in populations depending upon crudely processed grain and primitive breadmaking methods calls for efforts to eliminate excessive amounts of this harmful substance from food. However, before undertaking concrete measures for this purpose, it may be advisable to assess in a number of populations the extent and severity of phytate toxicity. Remedial efforts could then be focused upon those showing the most severe effects.

A number of practical methods are available for lowering phytate intakes. These include: (a) Refinement of grain milling so as to eliminate the phytate-rich outer seed coats of grains from flour. Wheat bran contains 2.5% phytate. (b) Introduction of leaven into breadmaking methods. Either starter or manufactured yeast is capable of lowering phytate intakes to tolerable levels---within two hours. (c) Provision of more varied diets. In addition, the establishment of maximal permissible phytate contents by governmental action would be helpful. Evidence has been obtained in the course of examination of village breads that suggests that some flours used in villages have been adulterated by addition of finely ground bran.

Vitamin D markedly improves calcium and phosphate utilization in the presence of

phytate in rats. (12, 13). Although information is lacking about its action in the presence of phytate in man, the responsiveness of rickets in childhood in Iran to Vitamin D suggests that it would be effective.

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PHYTATE AND CALCIUM BALANCES

Subject	Control	Sodium phytate	Tanok	Control
	- -	2.5 g/day	2.5-3.0 g/day**	
	16. days	28 days	32 days	16 days
	mg.Calcium per 24 hours			
Rah.				
Mean	+236	+248	-282	+ 19
S.E.	34	55	48	55
p		N.S.	0.001	0.001
Teh.Mean	+471	-88	-209	-
S.E.	101	111	98	-
p		0.001	N.S.(0.001)	
Ran.				
Mean	+222	+119	-184*	- 65 *
S.E.	47	36	38	108
p		0.01	0.001	0.05

*Intake increased by 25%

**As phytic acid

PHYTATE AND ZINC BALANCES

Subject	Control	Sodium phytate	Tanok	Control
	16 days	2.5g/day 28 days	2.5-3.0 g/day ** 32 days	16 days
	mg Zinc per 24 hours			
Rah.				
Mean	+11.6	+6.17	-2.7	+5.2
S.E.	0.50	0.69	0.53	2.43
p	0.001	0.001	0.001	
Teh. Mean	+10.3	+0.60	-2.2	-
S.E.	1.27	0.57	0.89	-
p	0.001	0.001		
Ran.				
Mean	+ 7.1	+3.1	+6.5*	+10.1*
S.E.	1.37	1.45	2.29	3.6
p	0.01	0.05	N.S.	

* Intake increased by 25%

**As phytic acid

PHOSPHORUS BALANCES DURING PHYTATE ADMINISTRATION

Subject	Control	Sodium phytate	Tanok	Control
		2.5 g./day	2.5-3.0 g/day**	
	16 days	28 days	32 days	16 days
	mg.Phosphorus per 24 hours			
Rah.				
Mean	+267	+552	+558	+121
S.E.	95	59	114	179
p	0.01	N.S.	0.01	
Teh.				
Mean	+119	+124	+315	-
S.E.	145	100	180	-
p	N.S.	N.S.		
Ran.				
Mean	-169	-345	+473*	+305*
S.E.	79	97	70	266
p	0.05	0.001	N.S.	

*Intake increased by 25 %
**As phytic acid