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THE INTERVENTION APPROACH: THE GUATEMALA STUDY

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The staff of the Human Development Division of the Institute of Nutrition of Central America and Panama (INCAP) has been engaged during the past several years in a field program to study the effects of malnutrition on physical growth and mental development. The goal of this long-term prospective study is an integrative analysis of the nutritional and socioculutural factors affecting mental and physical development, i.e., to determine the effects of malnutrition, per se, as they interact with other variables that affect early child development. To achieve this, the project is concerned with the study of physiological and social characteristics of all preschool children and their families in four villages while providing two of the villages with nutritional supplements and all of the villages with medical care and social stimulation.

Extensive research on animals by other investigators has shown that various degrees of malnutrition disrupt physical and mental maturation, including bone growth (1), biochemical maturation (2), development of the central nervous system (3,4), and behavior and learning ability (5,6). Studies of the mental development of children in Mexico (7,8), in Africa (9), and in other parts of the world (10-12) suggest that protein-calorie malnutrition may be accompanied by altered behavioral and personality patterns.

The human studies have been primarily concerned with the effects of severe malnutrition on mental development. Little information exists on the effects of long-term mild or moderate forms of protein-calorie

malnutrition which affect even larger proportions of the children in developing countries. In interpreting human studies, it must be recognized that populations with malnutrition also may suffer a variety of sociocultural and educational deprivations which in turn affect mental development. Under these conditions, the roles of nutritional, as opposed to sociocultural, factors in mental development may be extremely difficult to separate and assess.

The ideal investigation for testing the hypothesis that malnutrition lowers mental ability would cover the entire adaptive cycle from conception to adulthood. However, for practical administrative, technical, and professional reasons, our project has been limited to the study of all pregnant women, infants, and preschool children to age seven years in each of the four villages. While serious and possibly irreversible physical damage caused by postnatal undernutrition may be clearly apparent during the first five years of life, impaired mental development may not be demonstrable until a later age. Therefore, an especially important period to study for evidence of the physical effects of malnutrition is that from birth to five years, whereas the possible effects of malnutrition on mental development are most readily demonstrable from four years on. Consequently, any study intended to demonstrate the significance of malnutrition as a factor in impaired mental development must extend over sufficient time to permit quantitative evaluation of both the malnutrition incurred and the level of intellectual ability achieved. A project restricted to seven years limits the possibility of determining fully the effects of early protein-calorie malnutrition on adolescent or adult mental development. However, the results of this study may indicate the need for a longer period of continuous investigation or for a return for reevaluation of the children at one or more later dates.

IDENTIFICATION OF THE VARIABLES

Our first task was to identify the most relevant and important variables involved in the hypothesis that protein-calorie malnutrition affects mental and physical development. Three independent variables were defined: state of nutrition, sociocultural environment, and medical care. Mental development was identified as the dependent variable. A major difficulty arose in developing methods applicable under field conditions. Available methods used under modern clinical and laboratory conditions often could not be used in field situations. To test the validity and reliability of the instruments we developed during the three years of preliminary studies, all methodology was tested under conditions similar to those anticipated for the definitive study.

State of Nutrition

The state of nutrition of a population is commonly defined by food production, distribution, availability, consumption, and utilization of

nutrients. For the individual, the state of nutrition is assessed by medical and dietary history, clinical examination that includes anthropometric measurements, radiographic studies of bone development, and biochemical and hematologic tests.

After extensive testing we developed methods adequate to define malnutrition within the populations to be studied. These methods included seven anthropometric measurements, hand-wrist radiographs, determination of morbidity patterns, and dietary surveys. All methods were found applicable to preschool age children and were incorporated into our standard operating procedure.

Sociocultural Environment

During the preliminary study we found it necessary to collect information about a wide range of variables to determine those most likely to affect mental development. Information was obtained concerning family composition, community relationships, social status, migration, patterns of communication with the wider world, and factors concerning economic status. Social class scales were developed to determine the status of all families in a village, and family expectation scales were designed for specific age groups.

Medical Care

A thorough knowledge of morbidity in our area of rural Guatemala was necessary to provide adequate medical care. Our assessment of morbidity included study of the specific diseases and injuries most often encountered, the frequency, duration, and severity of disability, and their potential for causing death.

Mental Development

A number of problems were encountered in developing tests of mental development. First of all, the existent tests for intellective function had been developed under the conditions found in the more developed countries; these were not directly applicable to the conditions found in the Guatemalan highlands. There also was a problem of which approach to testing should be taken: developmental scales, standard IQ tests, or tests of specific intellective functions (13). A major difficulty was the lack of a continuous procedure applicable at each successive age.

Two sets of tests were developed to measure mental performance: the Composite Infant Scale which utilizes a selection of items from the Bayley, Cattel, Gesell and Merrill-Palmer Scales to measure development at 6, 15, and 24 months of age; and the Preschool Battery, which explores four areas of intellectual development--perception, learning, memory, and language--and other behavioral characteristics such as attention, motivation, persistence, resistance to distraction and ability to inhibit impulsive motor responses. Four criteria determined the choice

of tests in the Preschool Battery for children aged three to seven years: 1. they should be culturally appropriate; 2. they should require simple, and when possible, non-verbal responses; 3. they should be game-like to promote interest and participation; and 4. they should measure cognitive processes.

Taken together the Composite Infant Scale and the test of the Preschool Battery may be used to measure mental performance from five months through seven years. Furthermore, several of the tests have discriminated satisfactorily between well- and poorly-nourished children in rural Guatemala.

RESULTS FROM PRELIMINARY STUDIES

Preliminary data indicate that protein-calorie malnutrition has an impact on children even earlier than was thought. The results suggest a complex interrelationship between maternal health and nutritional status and fetal development, as well as between nutrition and infant or child development. Suggestions of a correlation between early malnutrition and mental development have also been obtained. Below are some of the preliminary results.

Mental Performance After Malnutrition

The primary goal of determining the effects on mental development in children who have undergone malnutrition was approached in two studies on 5- and 6-year-olds recruited from a nutritional rehabilitation day care center in rural Guatemala (14). Twenty children were selected who had recovered from second or third degree malnutrition and all were 10% or more below their expected height for chronological age; their mean age was 67.6 months. The control group consisted of 10 well-nourished children who had had no history of serious malnutrition and who showed less than a 10% deficit in height for chronological age; their mean age was 64.4 months. The control group was selected from siblings of children who had attended the rehabilitation center during the previous year. The two groups were also matched for father's occupation, parental education, living conditions, and family structure.

In the first study, the control group performed significantly better than the previously malnourished group in four of the six mental performance tests, namely memory for sentences, memory for digits, memory for incidental learning, and memory for intentional learning. The results of the other tests, matching familiar figures and haptic-visual matching (touching a planometric geometric stimulus while looking for the identical shape among four other such stimuli and pointing it out) were not significant. The four tests which yielded significant differences had in common the need for close attention and short-term recall followed by a verbal response. Based on these results, one may hypothesize that (a) malnourished children are deficient compared with well-nourished children in tasks requiring short-term recall, and (b) the

superior performance of the normal children compared with the previously malnourished children is due to motivational and attentional rather than intellectual factors.

The second study was designed to test the two hypotheses as well as to explore a variety of cognitive and behavioral characteristics in malnourished children. The criteria for selection of 11 well-nourished and 17 previously malnourished children were identical to the first study except that the testers in this study did not know which group had been malnourished. In addition to the four tests yielding significant differences in the first study, two additional tests were used to study short-term recall: memory for visual designs and variations of the Knox cube tapping test.

The results of the second study differed from the first. The four tests that originally distinguished the previously malnourished from the control children showed no group differences in the second study. However, the previously malnourished subjects did perform significantly more poorly than the well-nourished children on the two additional short-term memory tests. These results do not support our hypothesis that malnutrition is directly associated with short-term memory capacity, since four of the six measures of short-term memory tests failed to discriminate between the well-nourished and the malnourished children. Another possible interpretation of difference in the results for the two studies is that the malnourished group in the first sample was more severely malnourished than the malnourished group in the second study. Some support for this contention comes from the finding that the malnourished children in the first study had a greater deficit in height for chronological age. A final possibility is that the results of the first study may have been influenced by observer bias, i.e., the examiner's knowledge of which were the well-nourished and the malnourished children of the two groups.

The second hypothesis, which suggests that differences between well-nourished and malnourished children may be due to attention or task concentration rather than cognitive capacity or ability, offers the more reasonable explanation for these findings. The pattern of test performance differences between the two groups in the second study suggests that motivational and attentional, rather than intellectual, factors underlie these group differences. Only when the attentional demands on the children were increased, for instance in the Knox cube test, did the control group out-perform the experimental one. Again, the memory for visual design tests, which require high attention, yielded poor results in the previously malnourished childern, and the failure of these children to improve their performance from trial two to trial three suggests a loss of interest in the task. Furthermore, the matching familiar figures test is considered a sensitive measure of motivation or task involvement and requires the use of systematic visual search strategies, without being inherently interesting. The absence of group differences for the

easy items indicates that both groups were equally capable of searching and finding the correct figures. The poorer performance of the previously malnourished group for the difficult items indicates failure to employ the solution strategies that the easy items demonstrated the children possessed.

Accepting the interpretation that the differences between the well-nourished and the malnourished children are caused by motivational rather than intellectual factors, the problem remains whether these motivational differences are directly related to the health and nutritional history of the child, or whether they result from a sampling bias in which the well-nourished children benefited from advantageous childrearing conditions. Since language development tests are generally considered the most sensitive indices of social class differences and since no statistically significant differences were found between the groups, the sample appears to have been successfully controlled for family differences between the well-nourished and malnourished children.

The evidence suggests that the performance differences may be associated with the poor health and malnutrition suffered by the experimental children. Because of more frequent and prolonged illness and lower energy levels, malnourished children apparently do not develop the normal ability to maintain high attention levels during difficult cognitive tasks. These behavioral patterns appear to persist after nutritional rehabilitation, resulting in the performance diffferences seen in these studies.

Diets of Pregnant Women

Monthly quantitative dietary records were obtained for 58 women in a rural village from the time pregnancy was ascertained until childbirth (15). Marked nutritional deficiencies were noted during the three trimesters for practically all nutrients studied, except iron and thiamine. The deficiencies were more severe during the first trimester, especially for calories, protein, vitamin A, niacin, and riboflavin. Not only was the protein intake low but only 20% of the total dietary protein came from animal sources.

Biochemical Analysis of Placentas

Thirteen placentas gathered in two rural communities over an eight-month period were analyzed biochemically and compared with similarly analyzed placentas of 23 well-nourished women studied in Iowa (16). The Guatemalan placentas contained less total protein, DNA, K, Na, Mg, Fe, and Se than the placentas from the U.S. However, the Guatemalan placentas showed a significantly higher cellular concentration of zinc than the North American placentas. The total number of cells per placenta was calculated on the assumption that DNA concentration per cell is constant (17). The value for the U.S. placentas was 2.370×10^{11} and

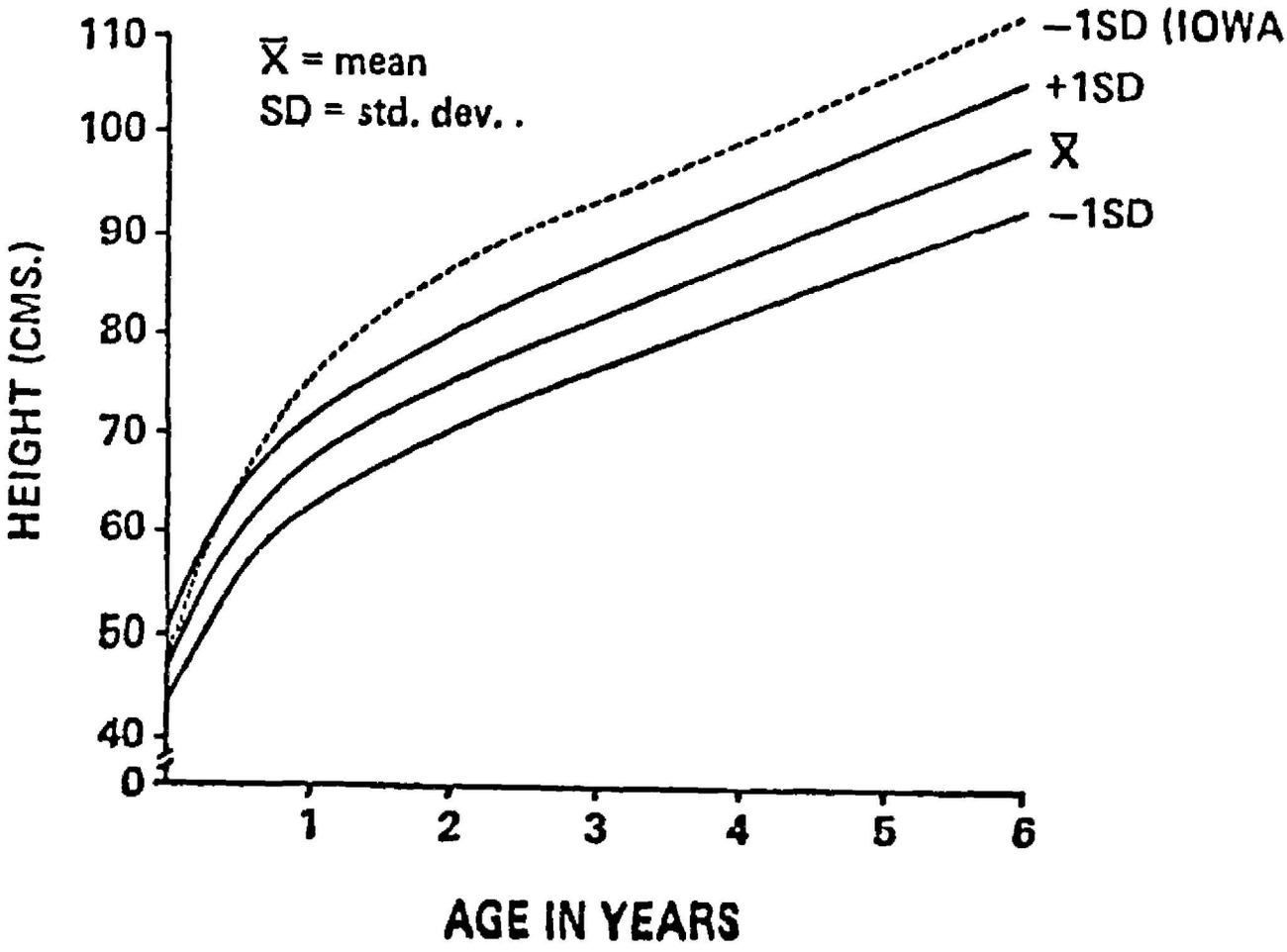
for the Guatemalan placentas 1.663×10^{11}. In view of the severe dietary deficiencies it is reasonable to postulate that the placental differences could be due in part to these nutritional deficiencies. Furthermore, the anatomical and biochemical changes could be responsible for functional alterations, which, in turn, could damage the fetus.

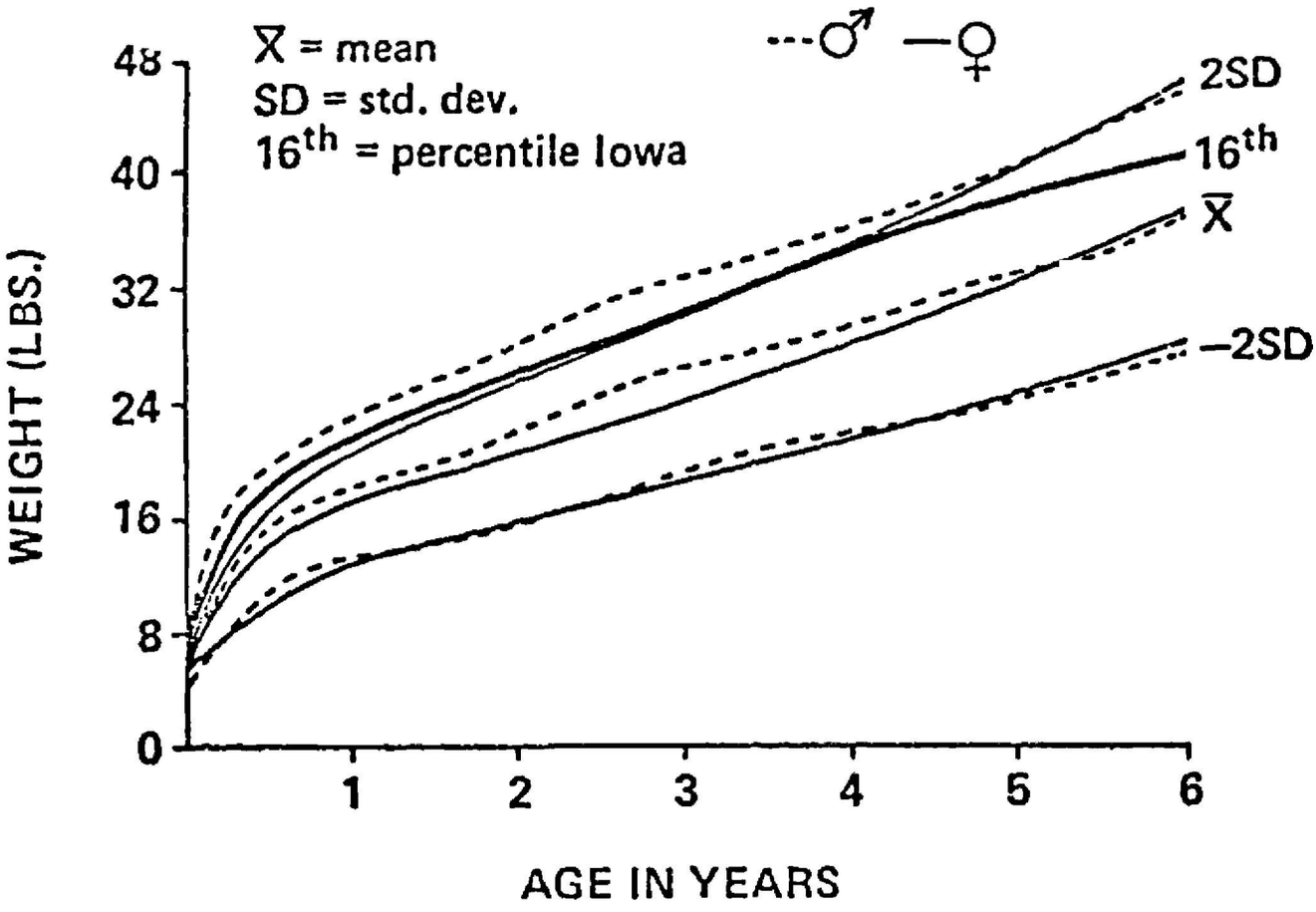
Mortality in Preschool Children

Mortality patterns among children up to seven years old in one village showed that death rates were high during the neonatal period, 64/1,000; highest during the post-neonatal period, 83/1,000; and still high during the second year of life, 52/1,000. Knowledge of specific death rates was necessary to calculate maximal possible attrition over time and thereby be assured of a sufficient number of children in each community at the end of the study.

Diet of Preschool Children

A seven-day quantitative dietary survey of preschool children in one of the villages indicated deficiences in the majority of nutrients investigated. These were most pronounced in the children under two years of age. As was found for the pregnant women, the most severe nutritional deficits were in calories, protein, vitamin A, riboflavin, niacin, and vitamin C. No estimate was made of the amount of breast milk ingested, but breast milk consumption is known to decrease markedly after the first four months of age.





Physical Growth of Preschool Children

Values for height and weight were obtained for 2,800 preschool Guatemalan children representing over 92% of the population studied in eight rural communities; these were compared with the Iowa growth curves (18). Compared with healthy urban Guatemalan infants, rural infants maintained a similar increase in length for the first three months. Beyond this age, the rural children exhibited a relative growth deficit which lasted about 30 months. Comparable growth rate was then achieved, and by 60 months their growth rate was similar to that of healthy urban Guatemalan children. However, the mean height for 6-year-old boys in this sample was two standard deviations below the Iowa standard. The weight of 6-year-old Guatemalan boys and girls was almost one standard deviation below the Iowa standard (Tables 1,2).

Bone development was studied by the Greulich and Pyle method (19) in a sample of 80 Guatemalan preschool children representing almost 100% of their age group in one village. There were marked negative deviations from the standard after 20 months of age, which were accentuated throughout the preschool age period. Also, the second metacarpal cortical thickness of a sample of Guatemalan children was significantly less than the standard of the Fels Research Institute for a North American population (20).

Socioeconomic Factors and Biological Variables

A social class scale was developed which would permit relating

socioeconomic status to biological variables for families with preschool children living in apparently homogeneous villages. Using an arbitrary social class scoring system, three groups of families were compared for adequacy of protein and calorie intake, cortical thickness of bone and the incidence and duration of diarrheal diseases. The differences observed between Group 1, the lowest, and Group 3, the highest were significant at the 0.01 level for these biological variables. Thus, even in small, isolated communities, families can be separated according to socioeconomic status which in turn is related to specific and quantifiable biological variables.

Staff Training and Restandardization

By continuous instruction and standardization of all procedures 70 people were trained during the preliminary study to obtain and interpret data with high reliability. The instruments to assess the independent and dependent variables were validated, and reliability coefficients were obtained by numerous pretesting sessions in villages that were then excluded from the definitive study. Since even under ideal conditions, changes in personnel occur in long-term studies, training of additional staff and restandardization of techniques were established as ongoing procedures.

SELECTION OF COMMUNITIES FOR THE DEFINITIVE STUDY

Criteria for Selection

For methodological and practical reasons a number of criteria were selected for matching villages for the definitive study:

1. Number of inhabitants: 500-1,000.
2. Birth rate: 35-45/1,000 annually.
3. Death rate: 14-18/1,000 annually.
4. Age distribution:

Birth-15 years	35-50%
(Birth-six years)	25-30%
16-45 years	40-45%
55 years and over	5-10%

5. Family composition: Average of five members per nuclear family.
6. Population mobility: 80% or more born in the area 2% annual migration
7. Social isolation: distance to Guatemala City 50-150 km; the nearest community included in the study should be 10 km or more and under the jurisdiction of a different municipality
8. Compact nuclear settlement: 80% of homes within a radius of one km from the research center

9. Housing and community services: 60% similarity among communities
10. Ethnocultural characteristics: 100% Ladino culture, a blend of Indian and Spanish physical characteristics; use of the Spanish language; dress and customs more European than those of Guatemalan Indians
11. Socioeconomic level: average annual income per family, $\$200 \pm \$50$
12. Sociocultural homogeneity: must exist between families and communities
13. Educational level: 80% literacy among population eight years old and over
14. Basic foods: corn and beans
15. Prevalent diseases: Malnutrition, gastrointestinal and respiratory disorders
16. Proneness to immediate change: very limited.

Several other characteristics such as accessibility, predominant economy, land tenancy, and the possibility of change in the area were also considered.

Based on information from the most recent population census (1964) a list of 300 Ladino villages of 300-1,000 inhabitants was compiled. Many of these were eliminated because of their distance from Guatemala City, number of inhabitants, and dissimilarity of ethnocultural characteristics. Lack of roads and dispersed settlement patterns also eliminated substantial numbers of communities. Visits were made to 179 villages, from which 45 were selected for further screening. Only 10 of these 45 villages fulfilled the selection requirements.

Matching the Villages

A family census was conducted in each of the 10 villages to provide information about housing, family size and composition, general sanitary facilities, religion, and means of transportation. In addition to this information, data were gathered on other sociocultural aspects of families with preschool children. A family socioquestionnaire was used to obtain information on morbidity, economic status, household conditions, annual production and consumption of crops, food habits, education, migration patterns, extent of family identification with Ladino culture, social relations with others in the community, intra-family social interactions, type of clothing worn by each member of the family, grooming, and use of free time.

Seven-day dietary surveys were taken by staff assigned to each community to define the food habits and intake among families, especially the preschool children under study.

Prevalence of tuberculosis was recognized as a decisive factor in the final selection of the communities. The presence of this disease could have made the already difficult task of determining the effects of mal-

nutrition on mental development more complicated because of its potentially adverse influence on growth and development. Since the prevalence of tuberculosis among these isolated communities was unknown, a complete photofluoroscopic and skin test survey was carried out. Persons suspected of having tuberculosis were reexamined and a chest X-ray was obtained to make a definitive diagnosis. These studies revealed a relatively low prevalence of tuberculosis, which did not materially affect the matching of the villages.

Anthropometric measurements including height, weight, and left arm circumference and tricipital skinfold thickness were made on all children from five through seven years of age. Psychological tests that focused on incidental learning, intentional learning, memory for digits, picture naming, picture recognition, and memory for sentences were administered to all 5- and 6-year-old children in the four villages.

While the study villages matched well on all of the above characteristics, there were unexplained differences among the total set of villages in both physical and psychological development. Use of the matching criteria, however, permitted the selection of two pairs of villages for the definitive study.

THE DEFINITIVE STUDY

The final design provides for an intensive long-term prospective study of similar groups of children living in two matched pairs of villages in rural Guatemala. All villages receive medical care not previously available to them. All homes are visited regularly by interviewers to obtain epidemiologic, sociologic, and dietary information. Thus, medical treatment and social contacts in both the experimental and the control pairs of villages are designed to be comparable. In one village in each pair a systematic effort is made to provide a nutritional supplement to all pregnant women and to children under seven years of age. A popular low calorie fruit flavored drink is provided in the other (control) village of each pair. The fruit drink was selected as one which the villagers recognized as having no intrinsic food value while at the same time having an intrinsic reward value in the dry climates of the villages.

Approximately 20% of the total population of these villages, which varies from 500 to 850 inhabitants, are children aged six years or younger. The rural economy yields an income of less than the equivalent of $200 a year for each family. The diet is composed largely of beans and tortillas. Projections indicate that about 60 births per year will occur in each village thus providing a new study cohort annually. Mortality in preschool children as well as losses by migration and other causes are expected to reduce each cohort by a maximum of 20 children during the six years of the study. Therefore, the net annual addition will be only about 40 children for each of the two experimental and the two control villages.

The nutritional status of the mothers and children is assessed by a wide variety of direct and indirect measures, including the assessment of bone development, and information concerning frequency and duration of illness. The clinical examinations are based on the Manual for Nutrition Surveys (21), the 1966 INCAP-US Office of International Research Survey methods (22), and World Health Organization reports (23,24); the neurological examinations are based on the methods of Paine (25), Andre-Thomas and colleagues (26), and Gesell and Amatruda (27); and the anthropometric studies are done according to methods described by Falkner (28) and Jackson and Kelly (29). Evidence of morbidity is obtained through house visits every 15 days (30). Seven-day dietary surveys are done in accordance with methods in use at INCAP (15,22). The roentgenograms are scored by the methods of Greulich and Pyle (19) and Tanner, Whitehouse and Healy (31). Cortical thickness (32) and number of ossification centers (33) are also assessed. Hemoglobin, hematocrit, total serum proteins, albumin, globulin, the albumin/globulin ratio, serum vitamin A and carotene, urinary excretion of creatine, urea, and the urea/creatinine ratio are determined (34-39).

It is too early to draw any firm conclusions from the longitudinal study, particularly in the neurological and behavioral areas. It is of interest, however, that data have been obtained relating maternal nutritional status during pregnancy and birth weight (40,41). In the months preceding initiation of the longitudinal study it was found that the average infant birth weight for 51 infants was 2.99±0.48 kg (Ave.±s.e.). By controlling for parity of the mother, maternal height (as an index of long-term undernutrition), and sex of the infant, a difference of 305 gm was found between birth weights of infants born to mothers who consumed less than 1,800 calories vs. more than 2,200 calories daily ($P<0.05$). One hundred and thirteen pregnant women have also been followed to term in the four study villages. Eighty-five percent of the mothers who consumed supplement on more than 60 days during pregnancy had babies with birth weights equal or greater than 3.0 kg; in comparison, only 50% of the mothers who came to the center less frequently had babies with comparable birth weights. Put in other terms, those mothers who consumed more than 30 liters of supplement during pregnancy gave birth to infants with weights similar to the standards for industralized countries; almost none of those in the control villages did. These observations suggest that the nutritional supplement is having an effect such that differences in behavioral development due to malnutrition may be detected, if such differences exist.

CONCLUSION

Evidence from studies with experimental animals suggests that both moderate and severe protein-calorie malnutrition, at critical periods, can produce adverse changes in physical growth and mental development. Evidence from human studies also suggests that severe, long lasting protein-calorie malnutrition in young children may be fol-

lowed by modifications in behavior and mental performance. However, the exact mechanism by which the effects of protein-calorie malnutrition are mediated remains to be determined. The present intervention study, under more carefully controlled conditions than have heretofore been possible, should provide a great deal of information on the interaction between malnutrition, behavioral development, and the social factors in an underdeveloped community.

NOTE: Dr. Canosa and Dr. Salomon were with the Growth and Development Unit, Institute of Nutrition of Central America and Panama, Guatemala at the time of the Conference. Dr. Canosa is currently with the Instituto Nacional de Prevision, Clinica Infantil, Valencia, Spain. Dr. Salomon is now with the Universidad de Brasilia, Brasilia, D.F., Brazil. Dr. Klein is with the Division of Human Development at the Institute of Nutrition of Central America and Panama. This study was supported by the Pan American Health Organization, World Health Organization (PAHO-WHO), under Contract PH43-65-640, National Institute of Child Health and Human Development, National Institutes of Health (NICHD-NIH), to the Institute of Nutrition of Central America and Panama (INCAP).

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