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SOME METHODOLOGICAL PROBLEMS IN FIELD STUDIES OF NUTRITION AND INTELLIGENCE

by

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In a world containing perhaps a billion people who are or have been malnourished (Behar, 1968), the possibility of a relationship between malnutrition and some form of impaired psychological performance has provoked a great deal of attention. The need for large scale collection of longitudinal data in order to assess the effects of malnutrition has led to a number of major field studies. All such studies of malnutrition and mental development are bedeviled because there are no generally recognized operational definitions of the two terms involved. We, at INCAP, have formed an interdisciplinary group to attack this problem. Our group consists of a child psychologist, an anthropologist, a statistician, a pediatrician, an epidemiologist, an immunologist and a biochemist. We are collecting data in four rural *ladino* Guatemalan communities, studying a total of 600 children under the age of seven, and their mothers. Moreover, during our projected seven years of longitudinal data collection, births will add another 100 children a year to our sample.

A major concern in such a project is data collection procedures. Not surprisingly, after one year we found that our ideas as to the kind of data to collect and how to do so have undergone considerable elaboration and extension. In this paper we shall discuss the current range of variables we feel important, how they might be defined, and into what general scientific context they fall. Since the data and results of our first year are being presented elsewhere, the findings we present here are offered only as examples for discussion.

In many ways, a prospective field study of the effects of malnutrition on mental development seems deceptively easy. After all, what is required is only a measure of nutrition

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which displays variation in a naturally occurring population, a measure of intelligence with the same characteristics, a clearly demonstrated statistical association (or its clearly demonstrated absence), and the absence of any obviously confounding relationship. A rigorously scientific solution based on a carefully elaborated model of the physiology of nutrition and learning would be esthetically pleasing, but as a first step, a simple statistical association would do. Unfortunately, we found that available nutritional measures of adequate precision were not practicable. While casting around for satisfactory measures we found ourselves reexamining many of the assumptions involved in developing accepted measures of nutrition. The need for rigorous definitions of objectives and testing of hypotheses was apparent. Similarly, it seems that no demonstration of an effect on psychological performance will serve without an intellectual underpinning of considerable detail.

Nutritional Status

Nutrition must be quantified if its effect is to be measured and there are many widely accepted measures for this purpose (Jelliffe, 1966; Arroyave, 1968). These can be arbitrarily classified as those which measure nutrient intake and those which measure the results of nutrient intake.

First, we will discuss measures of nutrient intake. Estimates based upon the total food consumption in an entire area are of considerable epidemiologic value, and have been shown to have high correlation with physical growth characteristics of the population as a whole in the same area. In our study, however, the differences in average consumption between villages is quite modest, even after an effort to supplement the diet in two of the villages. In view of a large natural variability in the intake among children, measurement of mean village nutrient intake does not discriminate with sufficient precision for statistical analysis. Moreover, such a technique may lead to fallacies of ecological correlation (Robinson, 1950).

Although estimates based upon total food consumption of populations are of little use in this context, dietary surveys of individuals seem to have the advantage of discriminating among various types of malnutrition and of defining the relative degree of malnutrition among children. This could be extremely useful, as malnutrition is not a single entity due to just one nutrient deficiency. For example, calorie deficiency producing marasmus may be found in some children of a village, while others will be suffering primarily from protein deficiency, kwashiorkor. Indeed, a marasmic child's diet may shift slightly and he will

develop kwashiorkor. Clear labeling is simply not possible, so the agent is called protein-calorie malnutrition. We believe, however, that just as the biochemical, physiological and clinical expression of marasmus and kwashiorkor are fundamentally different, so will their effects on mental development also be fundamentally different. They must be separated.

Unfortunately, dietary surveys of individuals have proved, so far, unequal to the task in the context of our field study. The variation among children in the same age group, in calories, is 120Kcal. (standard deviation). In vitamin A it is 213% (coefficient of variation). Thus, the normal variability of dietary intake is large within an age group. One would expect that the variability of the dietary survey method would be smaller than this population variability. Therefore, we examined the daily registry method, which combines weighing of foods with estimates of amount consumed (Flores, Menchu, Lora and Guzman, 1970), the most precise dietary survey method practicable. Here we found that the variation from survey to survey for calories was 255Kcal. (standard deviation) and for vitamin A 212% (coefficient of variation). We present these two nutrients because they represent the extremes in variability among the nutrients measured. Thus, it is clear that an instrument of such gross relative imprecision is inadequate to define the dietary intake of these nutrients, except as an estimate of mean nutrient intake per village. However, we must differentiate between the better and worse nourished groups within each village. The precision of estimates for individuals could be improved by increasing the survey frequency, but expense renders this impractical. Thus, what appeared to us the obvious solution to the problem of estimating nutritional status did not satisfy the rudimentary criteria of statistical association from one survey to the next.

Therefore, we reassessed the prerequisites of an adequate dietary survey method. We decided the method need only be precise enough to permit the reliable ranking of children and to separate the better and worse nourished groups. The reliability is measured first by how reproducible the ranking is from survey to survey. Then independent biochemical and growth estimates of nutritional status must be shown to be correctly associated with the ranking, to show that the dietary survey method is reflecting a physiologically meaningful variation in nutrition.

A three-day recall method is less precise but also less variable than the daily registry method. Validation of this three-day recall method indicates that the error of recall for calories and proteins is significantly less than the daily variation of the diet (table 1) and that this error tends to reduce the variability from survey to survey. This recall method does provide reliable rank-

TABLE 1
Comparison between the daily registry and three-day recall methods

	Standard deviations	
	Calories	Proteins (gms)
1. From survey to survey		
a. Daily registry method	255	4.25
b. Three-day recall method	132	3.53
2. Daily registry method compared to three-day recall method of the same children of the same day	154	1.62

TABLE 2
Dietary surveys
Correlations among two estimates of nutrient intake of same children performed three months apart

Nutrient	Daily registry method	Three-day recall method	Significance of difference between correlation coefficients
	N = 138	N = 46	P
	Correlation coefficients		
Calories	.263	.570	< 0.05
Protein	.419	.476	not significant

ing (table 2) in both calories and proteins. The validation of this ranking of the children for protein and calorie intake will have to be undertaken by biochemical surveys and by judging total nutritional adequacy from growth rates. However, none of the dietary methods provides a ranking of children within a village for vitamin A, iron, niacin, or riboflavin intake.

We considered omitting the dietary surveys and relying upon randomization of nutrition among our villages. In this fashion, by supplementing one group of villages and not the other, we would be assuring adequate nutrition in some children while the others suffered their usual inadequate diets. Unfortunately, children have different attendance habits at supplementation centers. Thus, this plan presents more problems than it solves in estimating nutritional adequacy because some children always attend, others never, with the majority somewhere in between. Also, dietary adequacy is probably related to family characteristics which may affect mental development independent of diet. It is, therefore, vitally important to be sure that there is an adequate representation of children partaking of the supplement from each level of home dietary adequacy to permit comparison with a similar ranking of children whose home diets are not supplemented. Therefore, dietary surveys must be included unless

we can exercise actual or statistical controls for all familial characteristics which may influence mental development.

When accurate information on home diet is obtained through dietary surveys, the provision of a dietary supplement does permit the differentiation of the effects of nutrition from the effects of social and familial factors which may independently affect either the home diet or mental development or both. This differentiation of social and familiae from nutritional influences on behavior can be made, however, only if the supplementation insures good nutrition through making up for inadequacies in the home diet.

Dietary surveys indicated that the mean village intakes of calories, proteins, niacin, riboflavin, vitamin A, and iron were inadequate for all age groups in this study (table 3). A supplement combining a high protein-vegetable mixture with powdered skim milk was formulated (table 4). The effects were tested first on rats with favorable results (Erdmenger, Gonzaga, Souza, Salomon, Bressani, Arroyave and Habicht, 1970), and then provided to preschool children.

Theoretically, the addition of the supplement to the home diet provides all children with adequate protein intake (table 5). However, our dietary surveys indicated that even this supplemented diet remained inadequate in calories, riboflavin, niacin, vitamin A, and iron for a majority of the children (table 5) as measured against current recommended allowances (NAS/NRC, 1968). To determine whether this was, in fact, a physiological inadequacy for these nutrients, a biochemical survey (Habicht, Schwedes, and Arroyave, 1970) was conducted. We compared two-year-olds matched for frequent attendance at supplementation centers (N=19) with those attending a center where a supplementation surrogate is distributed (N=15). This surrogate contains only 70 Kcal. of sugar per cup. We found no anemia and

TABLE 3

Percentage of children whose home nutrient intake is equal to or above the recommended allowances* Conacaste Village, Guatemala (1969)

Age in months	0-11	12-23	24-35	36-47	48-59	60-83
Proteins	15	40	48	33	67	63
Calories	0	14	36	14	17	0
Riboflavin	29	7	4	0	6	0
Niacin	29	29	12	6	6	0
Vitamin A	15	40	40	60	61	25
Iron	0	0	0	7	6	12

*Recommended allowances:
Calories: by height NAS/NRC (1968)
Others: by age

TABLE 4
Formulation and composition of food supplement

<u>Formula of supplement per 100 ml</u>		
Ground whole cooked corn	4.35	gms
Cotton seed flour	2.85	gms
Torula yeast	0.22	gms
Calcium carbonate	0.08	gms
Dry skim milk	12.00	gms
Sugar	5.00	gms
Vitamin A	338	IU
<u>Composition of supplement per cup (180 ml)</u>		
Protein	10.8	gms
Fat	0.9	gms
Carbohydrate	27.3	gms
Thiamin	0.36	mg
Riboflavin	0.54	mg
Niacin	1.2	mg
Vitamin A	610	IU
Calcium	393	mg
Iron	1.3	mg
Phosphorous	313	mg
Potassium	2.88	mg
Sodium	2.52	mg
Total calories: 162		

TABLE 5
Percentage of children
with adequate nutrient intake* if all children attend Supplementation Center
Conacaste Village, Guatemala

Age in months	0-11	12-23	24-35	36-47	48-59	60-83
Proteins	100	100	100	100	100	100
Calories	15	47	76	73	67	50
Riboflavin	57	27	8	0	6	13
Niacin	57	40	16	7	17	0
Vitamin A	70	46	64	73	72	38
Iron	0	0	0	56	18	25

*Defined as in table 3.

no vitamin A or niacin deficiencies. Thus, in spite of the fact that the estimated intakes of these children were below the recommended allowances, there was no biochemical evidence of deficiency of these nutrients even in the unsupplemented children. Only indices of riboflavin and protein nutrition reflected inadequate intake. The protein indices revealed adequate protein intake for all the supplemented children. The urea/creatinine ratios were all above 11.5, and the serum albumins were all above 4.1 gms/100 ml. Over half the unsupplemented children had urea/creatinine ratios below 11.5. Further evidence of the efficiency of the supplement was revealed by the improved growth rates of the supplemented children as compared to the retarded growth of their unsupplemented counterparts.

Because of this biochemical evidence and because of the appearance of occasional protein, calorie and riboflavin deficiency syndromes, we believe protein, calories and riboflavin to be the only limiting nutrients in these villages. However, when these village diets are complemented by a protein and calorie supplement, not only riboflavin but also other nutrients such as niacin, iron and vitamin A should also be included in the supplement to be sure that they do not become limiting.

This concern about supplementation causing dietary imbalance applies to the children receiving the "empty" calories of the supplement surrogate as well as to those receiving the supplement itself. Thus, we are planning to include vitamins and iron in both. This seems especially important as there is no practical way to estimate the home intakes of these nutrients.

Under these circumstances, one's attention may be restricted to protein and calorie nutrition, and children may be ranked and matched between supplemented and unsupplemented villages, with the assurance that no uncontrolled and unmeasurable deficiencies are confusing interpretation.

The above estimates of nutritional intake provide only one part of the information necessary to estimate nutritional status. The nutritional requirements of the individuals must also be known. This is difficult to ascertain, even in metabolic wards, and is impossible in a field study of so many children. Lean body mass is one of the principal factors affecting protein and calorie requirements. The adequacy of nutrition can be estimated as indicated above by growth rate (Jelliffe, 1966).

Anthropometry measures variables which can be used to estimate nutritional requirements (height and weight) and variables which reflect the adequacy of nutrition through growth. The variables in table 6 seemed to us adequate to measure linear growth, bone development, fat deposition, and the growth of

muscle mass. The first two reflect general nutritional adequacy. Fat deposits indicate caloric adequacy of the diet and muscle mass reflects protein adequacy. This presumptive model has proven satisfactory in all areas save estimating muscle mass (table 6). From table 6 and the statistics of the different variables within age groups one may evolve equations, whose sum will be an index of general size (equation A, table 6) or of fat (equation B, table 6). The mean of these indices is zero with a standard deviation of one. When a child's measurements are fitted into the equation, the equation will result in an index of how large or how fat he is in relation to his age group. The relative importance of the variables is indicated by their order in the equation. It would be neater if the weighting given the variables indicated the order of importance, as for the index "fat," and if only those variables which contribute significantly were included. The index "fat" approaches a satisfactory first approximation because it has a subjective internal logic, in that it is negatively related to length, positively related to variables thought to be associated with fat deposits and not related to head circumference.

TABLE 6
Orthogonal quartimax factor loadings of
anthropometric data within age groups

Ages 0 to 84 months, N = 1376				
Variable	Factors			Communality
	1	2	3	
Total weight	.872	.195	-.204	.839
Total height	.928	-.157	.031	.887
Sitting height	.915	-.076	.027	.843
Head circumference	.842	.000	.369	.844
Thorax circumference	.868	.176	.097	.793
Upper arm circumference	.698	.576	-.136	.836
Diameter of the wrist (Bistyloid diameter)	.718	.180	-.569	.872
Arm tricipital skinfold	.257	.882	.135	.862
Subscapular skinfold	.065	.910	-.123	.847
Sum of squares	4.991	2.068	.564	7.624

Indices:

- A. "Size" = .0087 (height in cm) + .0142 (sitting height in cm) + .0278 (weight in Kg) + .0128 (thorax circumference in cm) + .0147 (head circumference in cm) + .0132 (wrist diameter in mm) + .0254 (arm circumference in cm) + .0063 (triceps skinfold in mm) + .0021 (subscapular skinfold in mm) - 2.21 (age in months) - 29.78
- B. "Fat" = .0446 (subscapular skinfold in mm) + .0343 (tricipital skinfold in mm) + .0326 (arm circumference in cm) + .0097 (body weight in Kg) + .0041 (thorax circumference in cm) + .0033 (wrist diameter in mm) - .0023 (total height in cm) - .0018 (sitting height in cm) - .0000 (head circumference in cm) - .0365 (months) - 10.97

We are thus refining our anthropometric method, including other measures of muscle mass, so as to develop an appropriate index of protein nutrition. In future analyses we will include X-ray data which consists of bone age by the Greulich-Pyle and Tanner-Whitehouse methods, cortical thickness, and incidence of bone anomalies of the head and wrist. Once we have defined the vectors of muscle mass and fat, these must be validated by biochemical and physiological methods, probably in metabolic wards.

Another major factor affecting nutrient requirements is disease (Scrimshaw, Taylor and Gordon, 1968; Pollack and Sheldon, 1970). Disease not only influences nutrient intake, but also social stimuli and their psychological integration. Estimates of illness are thus important as one attempts to reduce this disturbing variable to a minimum. The rationale and components of our preventive and curative services are described elsewhere (Habicht, Reyna-Barrios, Guzman, and Gordon, 1970). Here it is sufficient to note that the estimates of disease must concentrate on those aspects of illness which may effect either nutritional status or mental development. These can be summarized as severity and duration. Incidence is secondary in this context, although the problem of incidence may affect the index of severity. This is the reverse of the usual priority of morbidity surveys in which definition of the disease agent is the object. Changes in nutrient intake and deprivation of social and psychological stimuli are also estimated. Each child's health, from birth through seven years of age, as well as his mother's during pregnancy and lactation, is recorded through a bimonthly interview with the mother or guardian. We are at present checking the validity of the interview method. We will then validate the assumptions made about the effects of illness upon nutritional status and mental development.

Another measure of the physiological adequacy of nutrition is provided by biochemistry (Arroyave, 1968; Whitehead, 1969). Longitudinal biochemical surveys in small children do not seem practicable because much information requires drawing blood. The logistics are difficult, the analysis expensive, and above all, the results can probably be achieved otherwise and less expensively. However, biochemical surveys have an important role to play in our experimental design. They are used to validate other methods. Examples have already been given regarding estimates of dietary intake and the adequacy of the supplement and validating the use of anthropometric indices of nutrition.

So far, the methodology we have presented serves, at best, to describe the child after birth, or in the case of nutrient intake, after weaning. But the child has been growing long before then,

and it is essential to have some estimates of his previous health and nutritional status. To this end we have initiated studies of intrauterine and suckling development. We are trying to assess the health and nutrition of the mother, both for its own sake and to establish indices of intrauterine infection and placental function and determine the quantity and quality of breast milk.

Psychological Performance

Psychological performance as the outcome variable generates two major areas of concern: the adequacy of our operationalization of our concepts (i.e., the degree to which our tests are an accurate, thorough and valid measure of the concepts we think we are measuring) and the confounding of our interpretations of measured psychological differences between well and malnourished children by other variables. Most of our comments deal with the second of these concerns; problems encountered in the interpretation of demonstrated differences in psychological performance between well and malnourished children.

Psychological performance has been studied under many guises. It appears variously as intelligence, as psychomotor development, as maturational or developmental indices, and as measures of personality. There is a great diversity among these concepts, and it is an open question as to what should be the focus of the research in this area. Significant findings using some operationalization of any of these concepts as a dependent variable would be interesting, provided, of course, that the tests employed meet accepted criteria for reliability, validity, and cultural appropriateness.

The range of psychological tests which have been employed in nutrition studies is as large as the range of concepts behind them. In some studies, mental development has been operationalized in terms of performance on specific psychological tests (Cravioto, De Licardie and Birch, 1966; Brockman and Ricciuti, 1966), whereas in others, attempts have been made to measure rather comprehensively such concepts as cognitive processes, or intellectual development (Segall, 1970; Klein, Yarbrough, and Habicht, 1970; Cobos and Guevara, 1970). But regardless of how the investigator chooses to conceptualize his dependent variable, one of the most critical aspects of the study of the effects of malnutrition is the selection of the situation and the behavior which reflect the theoretical construct chosen for measurement. Examples of this procedure, are the use of a cross-modal matching task as an index of intersensory development or a memory for digits test as an index of short-term memory.

The complicated problems of test validity and reliability

will be dealt with elsewhere. Therefore, we turn our attention to a second area of concern: that of confounding and the attendant problems of interpretation of experimental findings.

Confounding, our second concern, raises difficult problems of interpretation, and questions as to the structure of personality and mental abilities cannot be avoided. It is a doubly complicated issue because findings are confounded both by such external variables as child-rearing practices and also by the complex psychological inter-relationships within a single child. Thus, any one of several underlying variables may produce similar test results, and this leads to great difficulties in constructing adequate tests of hypotheses.

An example of a case wherein simple statements about observed response differences between well and malnourished children may be misleading comes from our own research (Klein, Gilbert, Canosa and De Leon, 1969). We compared a group of previously malnourished children, who were nutritionally recuperated at the time of testing, with a group of children who had no history of malnutrition, on several tests of short-term memory. Since we had good psychometric evidence that these memory tests were a satisfactory index of the construct of short-term memory, we were inclined to hypothesize that malnourished children had short-term memory defects. Happily, we attempted a replication and extension of our findings in a second study, and we structured this extension in such a way as to allow rejection of our previous findings. In the replication study we found that the previously malnourished group again performed more poorly on several, but not all, of our short-term memory tasks. Furthermore, the general pattern of test differences between the well and malnourished subjects in the second study precluded a simple interpretation of memory deficit. Rather, the differences between the two groups seemed to be related to attentional or motivational factors rather than simple problems of short-term memory. These hypothesized attentional and motivational differences between well and malnourished children are currently being explored by our group as possible mechanisms which mediate the observed performance differences between well and malnourished children.

Problems of interpretation of results also accrue to the confounding of variables external to the child. Take, for example, social structural position. It is by now well recognized that many pioneering studies in malnutrition are hopelessly compromised by failure to adequately control for social class. In our study, we operationalized the concept of social structural position in rather a traditional way by constructing indices which reflected income, education, and family structure. We regressed these in-

dices against psychological performance for a group of children in our sample and found that we could account for a sizeable portion of the variance in psychological test performance: 20% of the variance for a test of vocabulary development, 30% of the variance of a test of perception, and up to 30% of the variance for measures of response speed on tests wherein measured thoughtful responding is conducive to better performance.

The finding that response speed as well as other aspects of test performance vary powerfully with social structural position illustrates two important and related considerations in nutritional research. The first is that there is a wide range of possible mechanisms which mediate performance differences between well and malnourished children. An example of this point comes from a study conducted in Guatemala (Witkop, Baldizon, Castro and Umaña, 1970) wherein children who had been hospitalized and recuperated from kwashiorkor were found to perform significantly more poorly on a test of intersensory integration than a group of controls. Response speed was also found to be significantly related to the number of errors for both the well and malnourished children. When we consider that response impulsivity has been demonstrated to be an important factor in predicting performance on difficult tasks (Kagan, 1966), a clear interpretation of the differences between well nourished and malnourished children requires that these differences be demonstrated independently of individual differences in response speed. Pursuit of this issue may reveal that malnourished children are more impulsive and that their poor performance is due to this dimension of response style and not to a reduced ability to integrate stimuli from two modalities.

The general problem of controls in nutritional studies is the second important consideration highlighted by our findings that test performance varies significantly with social structural position in rural village preschool children. It is widely believed that child-rearing practices central to the development of intellectual performance vary dramatically across social classes and that this accounts for much of the perceived differences in psychological performance (*Perspectives in Human Deprivation*, 1968). Since the nutrition of a child also seems to vary with social structural position, social structural position must be estimated separately. In most investigations, such estimates usually take the form of blocking variables, wherein the investigator groups subjects from families which are generally similar, usually in terms of income and parental education. The disadvantage of this technique is that the investigator is in no position to account for variability within his social structural groups. An obvious alternate strategy is to attempt direct mea-

surements of different child-rearing styles and behaviors and then to relate the family child rearing characteristics to the effects of malnutrition. Such an experimental design will allow the investigator to analyze the possible interactive effects between malnutrition and psychological performance based on the possibility that certain child rearing techniques may function to ameliorate the impact of malnutrition on psychological development.

Conclusion

We began with the public health question about a relationship between nutrition and intelligence. An answer to this, even if uninformed by an understanding of the underlying mechanisms, is greatly desired. In pursuing this question we have been drawn inexorably into precisely those scientific operations required for the elucidation of a model. Because of the complexity of the variables with which we deal, careful definitions are essential; otherwise, unambiguous interpretations are impossible. Similarly, the wealth of conflicting and plausible mechanisms make careful experimental designs unavoidable. It is possible, of course, to list an unending series of "what if" variables and mechanisms but even when we restrict our attention to variables with known associations with nutrition and intelligence, we are left with the situation so complicated that only the elaboration and verification of a model seems to hold the hope for sorting out cause and effect.

Finally, we wish to return to the public health question and emphasize that, even in the presence of scientific findings, there are broader problems of interpretation. Beyond the problem of estimating the magnitude of an effect lies the question of the significance of that effect. For example, in a large cross-sectional study, we found that for many psychological tests in our battery, children who were tall for their age performed consistently better than children who were short for their age. Although no single test was significant, the consistency of the direction of the differences suggested a mild effect. An analysis of the power of our design, however, showed that, with a probability of greater than .99, the differences were on the order of not more than one word in 16. While such a finding may suggest future inquiries, its practical relevance seems small. Not all differences will be so inconsequential, but this will only be clarified when the magnitude of effects and their functional significance are carefully considered.

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