# THE EFFECT OF DIETARY SUPPLEMENTATION AND THE ADMINISTRATION OF VITAMIN $B_{12}$ AND AUREOMYCIN ON THE GROWTH OF SCHOOL CHILDREN*

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The vast amount of experimental work on the effects of diet on the growth of laboratory and domestic animals together with the large number of sound clinical studies leave no doubt as to the basic importance of nutrition as a major factor in the growth and development of children. Nevertheless, due to the difficulties of controlling the multiple environmental factors in human growth studies, the length of time such investigations require, the expense involved and the difficulty of evaluating genetic influences, many important questions regarding the role of nutrition in human growth and development are still unanswered.

One of the most intriguing of the unsolved problems as well as far reaching in its consequences is the role in human growth of vitamin $B_{12}$ and other substances with animal protein factor activity. Animals fed on vegetable protein rations almost invariably grow better when one of these

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substances is added. The essential amino acids can, of course, be supplied by vegetable proteins in suitable combinations, but do children also need animal protein or one of the substances with so called animal protein factor activity for satisfactory growth?

Since large areas of the world are not at present able to furnish animal protein for human nutrition in quantities judged desirable, this question has great international, social, economic and political importance. If it can be demonstrated that the animal protein factor is *not* needed for good human growth *or* that it can be practically and cheaply supplied in a synthetic form, much benefit should result, especially to the extensive underdeveloped areas of the world in which animal protein production is low and cannot be readily increased.

Surprisingly, direct comparisons of the effectiveness of animal and vegetable protein feedings are very few in number[1-3]. Although these few comparisons fail to show an advantage of animal over vegetable protein, they are of such short duration that they do not justify recommending primarily vegetable diets for permanent use in underdeveloped areas. The published studies[4-8] which suggest a possible stimulatory effect of vitamin $B_{12}$ on child growth are not conclusive. The same must be said of the negative reports[9-11] at least in regard to the application of their findings to all age groups.

The present study was designed to compare the effects of supplementary animal and vegetable protein on the health, growth and development of children living on a diet low in animal protein, although only the height and weight results are discussed in the present paper. Since vitamin $B_{12}$ and the antibiotic Aureomycin act as animal protein factors in animals fed a vegetable protein ration, the effect of the administration of these substances was also included in the investigation.

## Studies in El Salvador

### *Status of the Children*

The children of both urban and rural El Salvador are approximately two years behind those of the United States in height and weight. Their bone ages, as estimated provisionally by the late Dr. Phillip Jeans in his last piece of scientific work before his death, show a similar retardation when compared to the standards of Watson and Lowry[12]. There is no way of knowing at present, whether racial factors are partly responsible for this difference although they are not believed to be the principal cause. Clinical examinations of these children reveal few of the signs usually associated with malnutrition except for the prevalence of mild degrees of xerosis and follicular hyperkeratosis. No suspected cases of rickets or scurvy were encountered. The most striking clinical observation was the discrepancy between apparent age. Approximately 28% of the rural children had mildly enlarged thyroid glands[13]. The rural groups averaged 83% blood type O and the urban groups 73%.

Hematological studies failed to reveal any important deviations from normal values. The serum total protein, riboflavin, vitamin C, vitamin E and alkaline phosphatase levels in these children have been previously reported to be within normal limits[14]. The serum levels of vitamin A and carotene were found to be relatively low, averaging 21.7 and 68 micrograms per cent respectively. Upon first examination 79 per cent of the rural children were found to have Ascaris lumbricoides, 13 per cent Necator americanus and 9 per cent Trichiurus trichiura. The incidence of these parasites was slightly lower in the urban children.

The children were treated for their intestinal parasites at the beginning of each year. All of the children were living at an altitude of approximately 2,000 feet in a semi-tropical climate.

*Experimental Design*

Fifty children in each of two urban schools in the capital city, San Salvador were selected. The children in one of these schools (Colombia) were given a daily lunch containing milk and other sources of animal protein. Those in the other (Roosevelt) received no supplement. These schools were located only a few blocks apart in a poor district of the city.

Thirty-one children in one rural school (Comecayo) received the lunch containing animal protein and 50 children in another (Portezuelo), a quarter of a mile away, received a lunch free of animal protein and based on soya milk (made available by the Soya Food Research Council of the United States). Twenty-four of the children in Portezuelo received in addition 20 micrograms of vitamin $B_{12}$ daily. A third school, Matazano less than a mile away with 22 children, served as a control. The total number of children in each group decreased each year as some families moved to new localities, but new children were also added to some of these groups in subsequent years.

The average diets before and after supplementations were determined during the first year by 7 day dietary surveys on a small random sub-sample in the urban groups and for approximately half of the rural children. An improvement on the animal protein content of the diets of children receiving the lunch containing animal protein from 15 to 32 grams in the urban school (Colombia) and 8 to 23 grams in the rural one (Portezuelo) was effected. Children of the school receiving vegetable protein (Comecayo) averaged 7 grams of animal protein in the initial diets. Children in the urban control school (Roosevelt) averaged 14 grams, and the children in the rural control school (Matazano) 8 grams. The initial diets were also relatively deficient in vitamin A activity (1000-2500 I. U.), riboflavin (.5-.8 mgs.) and calcium (450-650 mgs.). The supplementary feeding improved the situation in regard to riboflavin and calcium but left the diets short of the desired intake of vitamin A.

### *Results*

When the children in the two urban schools, Roosevelt and Colombia, were compared, no effect of the lunch on the rate of gain in height and weight could be detected during any of the three years, Table I. Except for the group in Portezuelo receiving both the vegetable protein lunch and vitamin $B_{12}$, the children in the rural schools did not gain as rapidly in height as those in the urban groups.

## Table I

### Adjusted Gain in Height and Weight of School Children in El Salvador

| School | Treatment | Starting Date | No. Children | Interval in Months | Average Monthly Gain° Height Cms | Average Monthly Gain° Weight Kgs |
|---|---|---|---|---|---|---|
| | | URBAN | | | | |
| Colombia | Animal Protein Lunch | II-1950 | 23 | 31 | .48 | .27 |
| Roosevelt | Control | III-1950 | 30 | 30 | .49 | .26 |
| | | RURAL | | | | |
| Matazano | Control | VII-1950 | 12 | 25 | .41 | .21 |
| Comecayo | Animal Protein Lunch | V-1950 | 22 | 28 | .40 | .20 |
| Portezuelo | Veg. Protein Lunch | V-1950 | 14 | 28 | .40 | .20 |
| Portezuelo | Veg. Protein Lunch + $B_{12}$ | V-1950 | 17 | 28 | .48 | .20 |

Approx. L. S. $D._{05}$ for HT = .11    Approx. L. S. $D._{05}$ for WT = .09

° The data in this and subsequent tables are adjusted by multiple regression methods for differences in initial age, height and weight.

To the intense disappointment of all concerned with the project, the relatively good animal and vegetable protein containing lunches supplied at considerable effort and expense to the children in the two rural schools Comecayo and Portezuelo produced no measurable improvement in the rates of gain in height and weight over those of the rural control group. The addition of vitamin $B_{12}$ appeared to have no effect on weight gain, but appeared to produce a moderate effect on gain in height over the twenty-eight month period. The over-all rate of gain in this group was similar to that found for children in the two urban schools.

*Comment*

After calculation of the diets, a process greatly retarded by the necessity of analyzing many hitherto unstudied local foods and the preparation of a food composition table, it became obvious that the amount of vitamin A received by the children was relatively low even after supplementation. It is possible that this interfered with the growth response of the children to the various supplements, but we have no direct evidence on this point. It should be noted that since the lunch provided substituted for the main meal of the day at home, it was difficult to bring about much quantitative improvement in the diets by these means. We also do not know at this time whether the children responded favorably in ways not measured by the simple determination of weight and height.

The failure to observe clear-cut physical benefits from lunch programs is a familiar experience to workers in the United States(15), but similar negative results in groups of children as apparently retarded as those in the present study were unexpected. Despite the apparent lack of growth response to the lunches supplied, there appeared to be an improvment in rate of gain in height in children receiving vitamin $B_{12}$. This response did not quite reach the 5% level of significance.

## Studies in Guatemala

### *Status of the Children Studied*

The children of rural Guatemala in the 7 to 11 age range were found to be more retarded in height and weight than those of El Salvador, averaging 2 to 4 years behind U. S. standards. The average retardation in their bone ages was 2.5 years. Clinical findings were essentially the same as in El Salvador including the xerosis and frequency of follicular hyperkeratosis. No signs of scurvy or rickets were encountered. The discrepancy between apparent age and chronological age was somewhat more pronounced in Guatemala. Most of the children were racially and culturally Mayan Indian and 89% belonged to blood group O.

Hematological values were not abnormal. Serum total protein, riboflavin, vitamin C, vitamin E and alkaline phosphatase values were within normal limits[(14)]. Although the average serum carotene value of 121 micrograms per cent is higher than reported for El Salvador, the level of vitamin A, 27.2 micrograms per cent, is only slightly higher.

Although Necator americanus was not detected in these children, 84% were found to have Ascaris lumbricoides and 36%, Trichiurus trichiura. The children were treated at least once a year for intestinal parasites. All were living at altitudes between 5,000 and 6,000 feet, in a year round climate comparable to spring in the northern United States.

### *Experimental Design*

Four comparable Indian communities near Antigua, Guatemala, and within an hour's drive of Guatemala City were selected for study.

No attempt was made to substitute a meal for any of the food received by the child at home but rather to supplement the generally skimpy breakfast with a midmorning snack supplying the nutrients considered most likely to be limiting growth. Children in one locality (Santa María) began in 1950 to receive a daily mid-morning snack based on reconstituted powdered dried skim milk provided by UNICEF while those in another (Magdalena) received a similar snack based on the enriched soya milk powder. All of the school children in this latter town were divided randomly into three groups which received in addition capsules containing 50 mgs. of Aureomycin, tablets containing 20 micrograms of vitamin $B_{12}$ or placebos. In the third town, San Antonio, children in a small parochial school and in one of its two public schools constituted control groups and were given placebos. These were considered as one control group in the present analysis. In the fourth town, Xenacoj, the same quantities of vitamin $B_{12}$ and Aureomycin were administered without other supplement. Programs were planned for administration of supplements 6 days a week but were interrupted by special holidays, and vacations from November through January. New groups of children also entered the other schools in subsequent years and were incorporated into the study. Children participating for less than half the possible number of days in any period considered were not included in the tabulations. The number in each original group decreased with each year of the study due to children leaving school or in rare cases leaving the village.

The average diets in the four villages were very similar to those for rural El Salvador, except that the children receiving the vegetable protein supplement in Magdalena proved to have a higher average daily intake of animal protein, 13 grams. Those in the other three Guatemalan villages average 7 grams daily. The supplementary snack in the village receiving animal protein, Santa María, provided approximately 8 grams of animal protein, 14 grams of total protein, 350 grams of calcium, 350 units of vitamin A activity and 0.5 mgs. of riboflavin. The supplementary snack in the village receiving vegetable protein, Magdalena, provided approximately 14 grams of total protein, 200 grams of calcium, 400 units of vitamin A activity and 0.5 mgs. of riboflavin. Thus the supplementary snacks greatly improved the situation in regard to all four nutrients, although the vitamin A activity of the total diets was still not optimal.

*Results*

## Table II

### Adjusted Gain in Height and Weight of School Children in Guatemala

| School | Treatment | Starting Date | No. Children | Interval in Months | Average Monthly Gain Height Cms | Average Monthly Gain Weight Kgs |
|---|---|---|---|---|---|---|
| San Antonio | Control | VI-1950 | 43 | 11.4 | .33 | .17 |
| Santa María | Animal Protein Snack | V-1950 | 81 | 23.5 | .38 | .21 |
| Magdalena | Veg. Protein Snack | III-1950 | 83 | 28.2 | .39 | .18 |
| Magdalena | Veg. Protein Snack + $B_{12}$ | III-1950 | 28 | 25.3 | .43 | .24 |
| Magdalena | Veg. Prot. Snack + Aureomycin | III-1950 | 29 | 21.7 | .42 | .27 |

Approx. L. S. $D._{05}$ for HT = .14 Approx. L. S. $D._{05}$ for WT = .10

In Table 2, the average monthly gains in height and weight for groups starting in 1950 and 1951 are pooled in order to give the largest possible number of cases in each category. The original control group in San Antonio showed an average monthly gain of 0.17 kilos in weight and 0.33 cms. in height for 43 children. The 63 children who received the vegetable protein in Magdalena and the 81 who received the animal protein snack in Santa María showed greater rates of gain in both weight and height than this control group. However, no differences were observed between the groups supplemented with the vegetable and animal protein snacks. The addition of vitamin $B_{12}$ or Aureomycin to the vegetable protein snack in Magdalena appears to have had little if any effect on the rate of gain in height. However, the rates of gain in weight do appear to have been influenced somewhat by vitamin $B_{12}$ and by Aureomycin. Unfortunately the variation of individual measurements is such that these trends may be chance results of sampling.

## Table III

Supplementation of Current Guatemalan Diets with $B_{12}$ and Aureomycin

| School | Treatment | No. Children | Interval in Months | Average Monthly Gain Height Cms | Average Monthly Gain Weight Kgs |
|---|---|---|---|---|---|
| | MAY 1950-SEPT. 1951 | | | | |
| XENACOJ | $B_{12}$ | 16 | 15 | .44 | .23 |
| XENACOJ | Aureomycin | 14 | 15 | .48 | .26 |
| | SEPT. 1951-SEPT. 1952 | | | | |
| XENACOJ | $B_{12}$ | 12 | 12 | .39 | .27 |
| XENACOJ | Aureomycin | 11 | 12 | .31 | .16 |

Approx. L. S. $D._{05}$ for HT = .11 Approx. L. S. $D._{05}$ for WT = .08

The results of the administration of vitamin $B_{12}$ alone and Aureomycin alone in Xenacoj are shown in Table 3. Although the average attendance was only 63 per cent of the total possible for the vitamin $B_{12}$ groups and 71 per cent for the children receiving Aureomycin, due largely to certain political difficulties in the town during 1951, the rates of gain in both height and weight exceed not only those of the children in the original control group in San Antonio but also of the children receiving the animal and vegetable protein snacks in Santa María and Magdalena.

Publication of these seemingly conclusive positive results with vitamin $B_{12}$ and Aureomycin has been delayed for several reasons. Since the control group in San Antonio was made up of children in an entirely different locality, there remained the possibility that environmental factors were producing higher growth rates in all of the children in the town of Xenacoj quite independently of the experimental treatment.

The figures for the twelve months from September of 1951 to September of 1952 give support to the conclusion that vitamin $B_{12}$ and Aureomycin did have a positive effect on height gains in Xenacoj. During this period the INCAP workers were completely withdrawn from the town and no attention or treatment was given to any of the groups. The rate of gain in height of both the former vitamin $B_{12}$ and the former Aureomycin groups dropped. In the former Aureomycin group this drop was sharp and highly significant for both rate of gain in weight and height. In contrast, the rate of gain in weight appeared to increase in the former $B_{12}$ group.

In Santa María, the town in which the animal protein snack was administered, the group in 1950 grew at a rate even lower than the control group in San Antonio, despite the supplementary food provided. In the subsequent two years, the rates of increase in height and weight of this group increased significantly. The groups beginning in 1951 and 1952 did not demonstrate such an initial period of poor response.

*Comments*

The revisits of the nutritionists to the families in Xenacoj after the $B_{12}$ and Aureomycin had been administered for several months gave some indication that the appetites of at least some of the children receiving these supplements had increased. This may help to explain the apparent growth response even though no food supplement was given.

Whether the initial poor response in Santa María to the animal protein snack was partly associated with the widespread distrust of milk as a food in this town and the conviction on the part of many of the children and their parents that they were having digestive difficulties caused by the milk cannot be stated with certainty. The report of Widdowson[16] describing the reversal of expected effects from supplementary feeding in two German orphanages due to the adverse psychological influence of a harsh superintendent clearly demonstrates the possible importance of such factors in the response to supplementary feeding programs.

In the second year of the study, a relatively large group of new children entered the Xenacoj school most of whom should have been enrolled previously. While thus not strictly comparable to children in the groups originally selected for study, their growth was observed. They were expected to furnish valuable supplementary information on the performance of control groups. When the observations on 70 of these children during the years 1951 and 1952 were tabulated on the same basis as those in tables 2 and 3, the rate of height gain was found to be .32. This is the same as that observed in the San Antonio control group. The rate of weight gain, however, was found to be .27, almost twice that observed for San Antonio children. In fact, it was as great or greater in this special Xenacoj group than was observed in any of the Guatemalan groups studied. This high rate of weight gain in a group of children in Xenacoj receiving no treatment except the placebo capsules during the first half of the observation period, suggests that considerable caution must be used in interpreting weight gain differences among towns.

We should like to believe that under the conditions of the study in Guatemala, the vegetable and animal protein snacks were equally effective. The size of the least significant differences and the divergent weight gain of the special group in Xenacoj warn that such a definite conclusion must be deferred until further data are available. It is tempting to dismiss the failure of vitamin $B_{12}$ and Aureomycin to produce a more definite increase in the rate of gain in height, when given in addition to the vegetable protein snack, as due to the fact that the children were already responding maximally to the basic supplement. If so, would this be for or against the concept of a human animal protein factor requirement? The animal protein content of the basic diet in Magdalena was found to be higher than in the other towns. It is possible that the average of approximately 13 grams daily was a sufficient quantity of animal protein to permit a near maximal growth response without added vitamin $B_{12}$, when other needed nutrients were supplied.

## Individual Growth Responses

Occasional individuals showing highly significant increases above the average growth performance occurred in all groups. They contribute disproportionately to the average and standard deviation of the data and have proved a major complicating factor in the drawing of specific conclusions by statistical evaluation of the data. Obviously, a great deal remains to be done in the analysis of our records on an individual rather than an average basis. However, it is clear that the selection of *any* group for *any* kind of treatment would include a number of individuals who would respond spectacularly. For evaluations of this type to be based on the number of individuals showing clearly positive growth responses, a control group of equal size and equivalent initial status, receiving some kind of comparable placebo treatment would seem to be absolutely essential.

## Discussion

In this initial presentation of the results it is obviously impossible to enter upon a detailed analysis of the multiple factors involved. It has seemed more important to give as accurate a picture as possible of the various trends observed. In doing so we have mentioned data which seem divergent and for which we have no satisfactory explanation, as well as results which seem to help in answering the fundamental questions which the project was designed to study.

When control and experimental groups can be drawn randomly from children in the same school and all groups can receive apparently equal treatment, the results of a single trial can be viewed with considerable confidence. Unfortunately comparisons of animal and vegetable protein cannot be set up in this way. It seems obvious that when a study cannot be conducted in this ideal fashion, the differences in growth rates between control and experimental groups in any single trial, even though appearing to be significant, may be due to factors other than the treatment administered.

Unless a study has true controls, positive conclusions seem to us to be justified only when repeated trials combining different groups give results in the same direction. By this criterion part of the data presented in this report, while highly suggestive, cannot be regarded as conclusive until additional studies are carried out. Nevertheless, the data presented provide grounds for reasonable optimism concerning the potentially positive effects of vitamin $B_{12}$ and Aureomycin on growth under the type of conditions prevailing in rural Central America. If the positive effect of these agents is confirmed by further studies under similar conditions, serious consideration must be given to providing the minimum quantity of animal protein necessary for satisfactory growth in underdeveloped areas or possibly the enrichment of vegetable diets by vitamin $B_{12}$ in some other manner.

## Summary

School children 7 to 11 years of age in El Salvador and Guatemala in the lower and lower middle economic levels, were found to be two to four years behind U. S. children in height, weight and bone age. The diets were generally low in animal protein, vitamin A, riboflavin and calcium. The number of children analyzed in the experimental groups varied from 12 to 81.

Under the experimental conditions prevailing in El Salvador, no significant improvement in the rates of gain in height and weight over a three year period resulted from the administration of a lunch rich in animal protein to the children in one rural and one urban school. A similar lunch in one rural school containing only protein from vegetable sources also produced no change in these rates. The addition of 20 micrograms daily of vitamin $B_{12}$ produced no effect on weight but a distinct increase in height which, however, did not reach the 5% level of significance. Children receiving placebos in one urban and one rural school served

as control. There was doubt regarding the adequacy of the vitamin A intake even in the groups receiving the experimental lunch.

In Guatemala, animal and vegetable protein containing snacks were offered during two and a half years to school children in two rural villages and placebos were administered to children in a third village. Increases in the rates of gain of height were observed but the variations were such that this could have been the chance result of sampling.

One-third of the children in the village receiving vegetable protein were given in addition 20 micrograms of vitamin $B_{12}$ and one-third 50 mgs. of Aureomycin. These supplements brought an apparent increase in the rate of gain in weight but had only a very slight effect on the rate of gain in height. None of the differences reached the 5% level of significance.

Children in one rural Guatemala village were given either 20 micrograms of vitamin $B_{12}$ or 50 mgs. of Aureomycin without other supplementation. Their rates of increase in both height and weight over an 18 month period proved to be significantly greater than those observed for the control group in another rural village. All treatment and attention was suspended for the succeeding twelve months during which the rates of gain in height and weight of the children formerly receiving Aureomycin dropped significantly. The rate of gain in height of the group formerly receiving vitamin $B_{12}$ also dropped although less markedly, but its rate of gain in weight increased. A separate group of slightly older children in Xenacoj receiving no supplement and studied during the last two years of the project showed a rate of gain in height similar to that of the original control children, but a rate of gain in weight as high or higher than that of any other group.

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