Serum Cholinesterase Levels of Central American Children in Relation to Nutritional Status

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PRIOR to 1948 limited information was available to suggest a direct relationship between cholinesterase serum levels and nutritional status.[1-3] More recently, McCance *et al.*,[4] and Hutchinson and co-workers[5] reporting on the Wuppertal Studies, have demonstrated a significant decrease in serum cholinesterase activity with severe undernutrition. With re-feeding, the values increased toward, and even above, normal. Studies of children with kwashiorkor[6-8] indicate that serum cholinesterase activity is low and rises with treatment parallel to the clinical improvement. There are, however, no reports as to whether cholinesterase activity decreases before clinical signs of kwashiorkor develop.

Investigation of nutritional deficiency diseases in Central America[9] has indicated that the incidence of kwashiorkor, a severe form of protein malnutrition, is greatest between the ages of one and four years and that moderate protein deficiency (prekwashiorkor) is widespread in this age group.[10,11] If serum cholinesterase activity were a sensitive measure of protein malnutrition, low values should be found often among poorly nourished children of this age. In order to investigate this possibility, serum cholinesterase activity was measured in groups of children differing markedly in nutritional and socio-economic background. Children of both preschool (one to six years) and school age (seven to twelve years) were included in the study.

ECONOMIC AND SOCIAL STATUS OF THE GROUPS IN SURVEY

School Children

Four groups of school children aged seven to twelve years were examined. Those from the Guatemalan schools served simultaneously as subjects for a study of serum cholesterol levels.[12] The Nicaraguan children were part of a clinical nutrition survey of families in a poor suburb of Managua.[13]

Group I, Guatemala Upper Income Urban: The school children in this group came from relatively well-to-do families.* Their fathers were business and professional men in Guatemala City. Only children having at least one Guatemalan parent were included.

Group II, Guatemala Lower Income Urban: These children came from families who lived in a relatively poor district of Guatemala City. Their fathers worked as mailmen, policemen, truck and bus drivers, or as unskilled or semi-skilled laborers.

Group III, Nicaragua Lower Income Urban: The children included in this group had very similar socio-economic backgrounds to those included in Group II, but their families lived in a relatively poor district of Managua, Nicaragua.

Group IV, Guatemala Lower Income Rural: The families of the children included in this

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group lived in and around the rural village of San Miguel Dueñas, located about 35 miles from Guatemala City. The fathers of most of these children were agricultural laborers on nearby farms and coffee plantations or cultivated their own small plots of land.

Preschool Children

Central American children of both sexes from one to six years of age were chosen as follows:

Group V, Guatemala Upper Income Urban: The preschool children in this group came from families with the same favorable socio-economic status as those of the school children of Group I. All were attending a private nursery school in Guatemala City.*

Group VI, El Salvador Lower Income Urban: The parents of the children included in this group earned very low salaries working as unskilled or semiskilled laborers. They lived in a very poor district of San Salvador, El Salvador.

Group VII, Nicaragua Lower Income Urban: In this group were included the children from the families of Group III who were not yet attending school.

Groups VIII and IX, Guatemala Lower Income Urban: The parents of the preschool children of these groups lived on two coffee plantations in Guatemala and worked as agricultural laborers. They had a very low rural standard of living.

Group X, Kwashiorkor: The children in this group were suffering from severe protein malnutrition (kwashiorkor), and were sampled on the day of admission to the hospital.

Dietary Status

No precise individual dietary information was available for most of the children studied, but the diets of their parents, or of adults of comparable socio-economic groups, have been determined by daily dietary interviews for periods of seven days. The total intake of calories, protein and fat, as well as the proportions of animal and vegetable protein and fat are summarized in Table I.

These data indicate basic differences in the dietary patterns characteristic of the socio-economic levels, as exemplified by the daily intake of calories, total protein and fat, and particularly by the amounts of the two latter nutrients of vegetable or animal origin. They also illustrate the close correspondence between the proportion of the total dietary proteins which are of animal origin and the economic and social status.

METHODS

Approximately eight children of each sex were selected at random for each year of age from seven through twelve, in each of the four school-age groups. Preschool children were also studied in each year of age from one through six. About 0.1 ml of blood was obtained by a fingertip puncture and the serum promptly separated and frozen. The samples were analyzed within two weeks after collection.

The total cholinesterase activity was determined by a micro-adaptation of the method of Reinhold *et al.*[19] using 0.02 ml aliquots

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TABLE I

Characteristic Dietary Estimates of Adults of Different Socio-Economic Status

Socio-economic status	Calories	Protein g/day			Fat g/day		
		Animal	Vegetable	Total	Animal	Vegetable	Total
Guatemala upper income urban[14]	2,462	39	30	69	46	56	102
Guatemala lower income urban[15]	1,585	10	37	47	13	14	27
Nicaragua lower income urban[16]	1,928	26	36	62	—	—	53
El Salvador lower income urban[17]	1,784	25	35	60	—	—	48
Guatemala lower income rural[18]	2,283	6	61	67	9	14	23

of serum. The results are expressed in Michel Units.[20] Determinations were run in duplicate, using 0.01 per cent phenol red as the indicator of pH change.

Replicate aliquots of a quantity of pooled sera from normal adults were analyzed with each group of samples studied. The average value of 15 independent determinations in the pooled specimen was 1.11 ± 0.08 Michel Units. This value, as well as the averages for the groups studied, is higher than previously reported values for normal adults obtained by the original Reinhold *et al.*[19] method. The discrepancy may be explained by the fact that in the modified technic employed in the present study, the tubes were allowed to incubate only until the indicator showed that they had reached a pH of about 7, instead of incubating them for a fixed period of one hour. The modification introduced results in an improvement of the method, since it gives a better measure of the initial velocity of the reaction, which is in turn proportional to the enzyme concentration. With samples of normal activity the pH decreases in one hour to a point where the optical density change is very small per unit of pH change, and under these conditions the method becomes insensitive. In addition, it is known that the activity drops with decreasing pH.

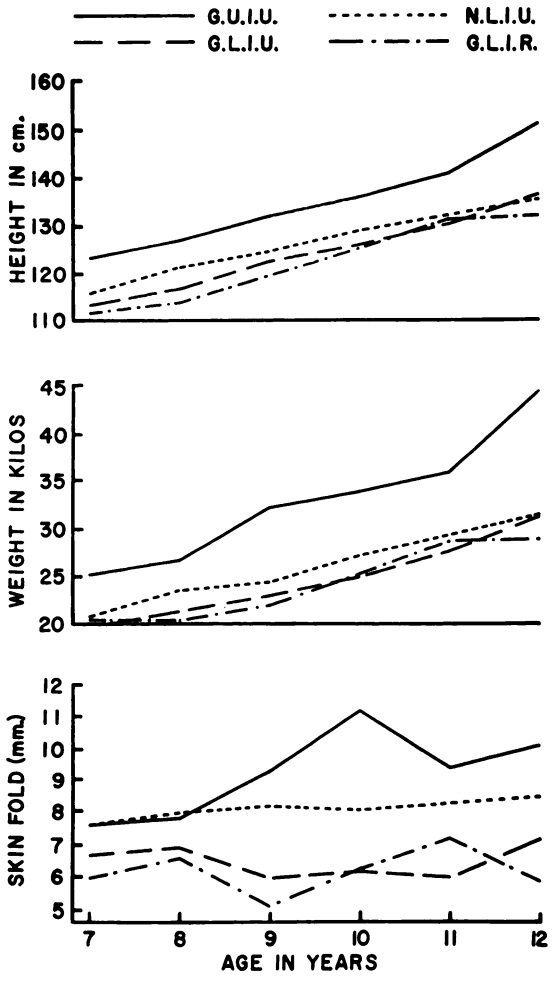

Fig. 1. Physical measurements of the experimental groups of school children. G.U.I.U., Guatemala Upper Income Urban (Group I); N.L.I.U., Nicaragua Lower Income Urban (Group III); G.L.I.U., Guatemala Lower Income Urban (Group II); G.L.I.R., Guatemala Lower Income Rural (Group IV).

Height, weight, and skin-fold thickness of the children were determined at the time of taking the blood samples. The measurements of the thickness of the skin-fold of the posterior surface of the upper arm were made as recommended by Brožek *et al.*[21]

RESULTS

The height, weight, and skin-fold thickness for the four groups of school-age children are portrayed graphically in Figure 1 and illustrate the differences in these measurements among the different socio-economic groups. It should be noticed that in general the "upper income urban" children are taller and heavier and have more subcutaneous fat than the other urban and rural groups; the latter show the lowest figures for the nutritional status indices employed.

The serum cholinesterase activity values are summarized in Table II. The standard deviations and the standard errors were similar in the different groups. Even though some differences occur among them in the average cholinesterase activity levels, no tendency of the enzyme levels to be related to the socio-economic standard or to the nutritional status of the children is suggested by the data. The marked decrease in serum cholinesterase activity characteristic of the severe protein malnutrition of kwashiorkor is illustrated in Table II by the low average value for 18 cases.

TABLE II

Serum Cholinesterase Values in Children of Different Socio-Economic Status

No. of group	Group description	Number of children	Serum cholinesterase (Michel Units ΔpH/hr	
			Mean ± S.E.	S.D.
	School children:			
I	Guatemala upper income urban	88	1.12 ± 0.02	0.19
II	Guatemala lower income urban	88	1.02 ± 0.02	0.17
III	Nicaragua lower income urban	35	1.03 ± 0.04	0.23
IV	Guatemala lower income rural	89	1.18 ± 0.02	0.23
	Preschool children:			
V	Guatemala upper income urban	19	1.28 ± 0.05	0.23
VI	El Salvador lower income urban	39	1.20 ± 0.04	0.27
VII	Nicaragua lower income urban	42	1.10 ± 0.03	0.19
VIII	Guatemala lower income rural	30	1.21 ± 0.06	0.32
IX	Guatemala lower income rural	48	1.00 ± 0.02	0.16
X	Kwashiorkor	18	0.30 ± 0.04	0.15

DISCUSSION

In view of the finding that the levels of serum cholinesterase are markedly reduced in severe protein malnutrition[6–8] (see Table II) it was not unreasonable to expect that low values might be found among lower income rural children, since in these groups kwashiorkor or the clinical stage just preceding the syndrome (prekwashiorkor) is common.[10] The fact that the values encountered were not low strongly suggests that the decrease in cholinesterase to the low levels found in kwashiorkor is a relatively sharp fall rather than a gradual one, and that it probably occurs only after clinical signs of protein malnutrition have begun to develop.

The data presented indicate that serum cholinesterase levels are not a sensitive means for evaluating nutritional status, since the large differences observed in this study between the upper and lower income groups, both in urban and rural areas, were not reflected in consistent differences in enzyme levels. On the other hand, the physical measurements of height, weight, and skin-fold thickness did show a close, direct relationship to the adequacy of the dietary patterns. Therefore, it is reasonable to consider them a more sensitive measure of mild to moderate nutritional deficiency than serum cholinesterase activity. This is the same conclusion reached by Saunders *et al.*[22] in studies of United States high school children where, however, the range of malnutrition was not nearly so great.

SUMMARY

The levels of serum cholinesterase activity were studied in four groups of school children (age seven to twelve) and in five groups of preschool children (age one to six) from different socio-economic and nutritional backgrounds. The following values for school-age children were found: Guatemala upper income urban, 1.12 ± 0.02; Guatemala lower income urban, 1.02 ± 0.02; Nicaragua lower income urban, 1.03 ± 0.04; and Guatemala lower income rural 1.18 ± 0.02. For preschool children the levels obtained were: Guatemala upper income urban, 1.28 ± 0.05; El Salvador lower income urban, 1.20 ± 0.04; Nicaragua lower income urban, 1.10 ± 0.03; Guatemala lower income rural, first group. 1.21 ± 0.06, and second group 1.00 ± 0.02. The cholinesterase activity showed no tendency to be related to the socio-economic background or the nutritional status of these children. Eighteen children age one to five with the severe protein deficiency of kwashiorkor showed a serum cholinesterase value of 0.30 ± 0.04. It is concluded that serum cholinesterase is not a sensitive measure of protein deficiency and drops only when the deficiency is so severe as to be clinically apparent.

REFERENCES

1. MILHORAT, A. T.: The cholinesterase activity of the blood serum in disease. *J. Clin. Investigation* 17: 649, 1938.
2. PEÑA YAÑEZ, A. and ZALDUA, B.: Cholinesterase in chronic malnutrition. *Medicina, Madrid* 11: 441, 1943.
3. FABER, M.: The relationship between serum cholinesterase and serum albumen. *Acta Med. Scandinav.* 114: 72, 1943.
4. MCCANCE, R. A., WIDDOWSON, E. M., and HUTCHINSON, A. O.: Effect of undernutrition and alterations in diet on the cholinesterase activity of serum. *Nature* 161: 56, 1948.
5. HUTCHINSON, A. O., MCCANCE, R. A., and WIDDOWSON, E. M.: Serum cholinesterase, in Studies of Undernutrition, Wuppertal, 1946–49. *Med. Res. Council* (London), *Special Report Series* 275, 1951, p. 216.
6. WATERLOW, J.: Liver cholinesterase in malnourished infants. *Lancet* 1: 908, 1950.
7. DEAN, R. F. A. and SCHWARTZ, R.: The serum chemistry in uncomplicated kwashiorkor. *Brit. J. Nutrition* 7: 131, 1953.
8. SCRIMSHAW, N. S., BÉHAR, M., ARROYAVE, G., VITERI, F., and TEJADA, C.: Characteristics of kwashiorkor (Síndrome Pluricarencial de la Infancia). *Fed. Proc.* 15: 977, 1956.
9. AUTRET, M. and BÉHAR, M.: Síndrome policarencial infantil (Kwashiorkor) and its prevention in Central America. *FAO Nutritional Studies No. 13*, Food and Agriculture Organization of the United Nations, Rome, 1954.
10. SCRIMSHAW, N. S., BÉHAR, M., PÉREZ, C., and VITERI, F.: Nutritional problems of children in Central America and Panama. *J. Pediat.* 16: 378, 1955.
11. SCRIMSHAW, N. S., BÉHAR, M., VITERI, F., ARROYAVE, G., and TEJADA, C.: Epidemiology and prevention of severe protein malnutrition (Kwashiorkor) in Central America. *Am. J. Pub. Health* 47: 53, 1957.
12. SCRIMSHAW, N. S., BALSAM, A., and ARROYAVE, G.: Serum cholesterol levels in school children from three socio-economic groups. AM. J. CLIN. NUTRITION 5: 629, 1957.
13. ARCE PAIZ, A. and GUZMÁN, M.: Estudios clínico-nutricionales en poblaciones de Nicaragua. II. Barrio de San Luis, Ciudad de Managua. In preparation.
14. FLORES, M., CRUZ, E., and MENESES, B.: Estudios de hábitos dietéticos en poblaciones de Guatemala. VIII. En un grupo de mujeres pertenecientes a un alto nivel económico. *Rev. Colegio Médico Guatemala* 7: 99, 1956.
15. FLORES, M., GARCÍA, B., CRUZ, E., MENESES, B., MAZARIEGOS, A. de, and SIERRA, A. de: Estudios de hábitos dietéticos entre pre-escolares del Area de Demostración Sanitaria de Amatitlán, Guatemala. INCAP Report L-69, 1957.
16. FLORES, M.: Estudios dietéticos en Nicaragua. II. Barrio de San Luis, Ciudad de Managua. *Edición especial del Boletín Sanitario de Nicaragua*, Nicaragua, 1956, p. 31.
17. SOGANDARES, L., GALINDO, A. P. de, and MEJÍA, H. P.: Estudios dietéticos de grupos urbanos y rurales de la República de El Salvador. *Suplemento No. 1 del Boletín de la Oficina Sanitaria Panamericana*, Publicaciones Científicas del Instituto de Nutrición de Centro América y Panamá, 1953, p. 27.
18. REH, E., CASTELLANOS, A., and BRAVO de RUEDA, Y.: Estudios de hábitos dietéticos en poblaciones de Guatemala. VI. Estudio de la dieta y de las condiciones de vida existentes entre los trabajadores de una plantación azucarera de Guatemala. *Bol. ofic. san. panam.* 37: 32, 1954.
19. REINHOLD, J. G., TOURIGNY, L. G., and YONAN, V. L.: Measurement of serum cholinesterase activity by a photometric indicator method. Together with study of influence of sex and race. *Am. J. Clin. Path.* 23: 645, 1953.
20. MICHEL, H. O.: An electrometric method for the determination of red blood cell and plasma cholinesterase activity. *J. Lab. & Clin. Med.* 34: 1564, 1949.
21. BROŽEK, J., and KEYS, A.: The evaluation of leanness-fatness in man: Norms and interrelationships. *Brit. J. Nutrition* 5: 194, 1951.
22. SAUNDERS, J. P., SANDSTEAD, H. R., BUTLER, R. E., and MICKELSEN, O.: Relation of serum cholinesterase to nutritional status of adolescents. *J. Nutrition* 47: 191, 1952.