The Effect of a Controlled Increment in Dietary Nutrient Intake on Blood and Urine Biochemistry in Children

GUILLERMO ARROYAVE, PH.D.,* MARINA FLORES, M.S.† AND MOISÉS BÉHAR, M.D., M.P.H.‡

THE MOST commonly used methods for the biochemical evaluation of nutritional status are based on (1) measurement of levels of certain nutrients or their metabolites in blood, blood serum or blood cells, and (2) estimation of the urinary excretion of these nutrients or their metabolites.[1] Interpretation of the results obtained using these methods depends on the relationship between nutrient intake and the biochemical measurements in question. Several investigators have studied this relationship in human subjects by administering varying amounts of one nutrient while keeping the rest of the diet constant. The studies of Horwitt et al.[2] and Bessey et al.[3] on riboflavin are classic examples in this regard. Although this approach has yielded much useful information, it does not consider the complex interrelationships among nutrients which may exist when the intakes of not one but several of them are changed.

The objective of the present work was to investigate the effect of a general dietary improvement on a number of biochemical measurements in the blood and urine of a group of institutionalized children.

MATERIALS AND METHODS

The study was conducted on a total of thirty-six children in a privately supported charitable orphanage in Guatemala City. The children in this institution were selected for study because their diet was as poor or poorer than that found in low socioeconomic groups of the region. For the clinical-biochemical study, eleven names were selected at random from the total list of orphans. A medical examination of the subjects revealed the absence of any specific chronic disease.

Before any experimental changes were introduced, a dietary survey was carried out in the institution in order to determine the current nutrient intake of the group. The total amount of raw food available for all the children was weighed every day for a period of five days, and the edible portions were estimated. The nutrient content of the daily total diet on a per capita basis was calculated using the INCAP Food Composition Table.[4] The average nutrient intake per child per day was then compared with Recommended Dietary Allowances of the National Research Council adapted to Central American populations.[5]

A clinical-nutritional examination was completed for each subject, recording a total of sixty-five clinical signs as well as height, weight and skinfold measurements. Height was determined without shoes against a measuring tape fixed to a wall, weight was measured without clothes using a springless scale; skinfold thickness was determined on the back of the upper portion of the right arm over the triceps at the mid-level between the tip of the acromial process of the scapula and the tip of the elbow, and on the scapular site.[6]

A 10 ml. venous fasting blood sample was obtained from each of the selected children, and urine collections were made for 24-hour periods. The blood samples were analyzed for hemoglobin (by the acid-hematin method),[7] serum vitamin A and carotene,[8] vitamin C,[9] free and total riboflavin,[10] pseudocholinesterase,[11] total proteins[12] and total red blood cell riboflavin.[10] In addition, the serum proteins

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* Chief, Division of Physiological Chemistry; †Dietary Research Section; ‡ Director.

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TABLE I
Food Consumption per Child per Day Before and During the Investigation

Food Groups	Before Investigation	Consumption (gm.) During 1st Mo. of Investigation	During 2nd Mo. of Investigation
Dried whole milk	8	...	...
Skim milk	12	52	54
Eggs	..	45	54
Meats	17	70	80
Leguminous seeds (black beans)	82	34	36
Fresh vegetables	7	110	166
Fruits	20	52	69
Musaceous (platnain, banana)	34	82	65
Starchy roots	4	19	12
Cereals			
Corn "tortilla"	92	352	356
Rice	33	28	30
Wheat bread	47	...	...
Oat meal	..	3	...
Spaghetti	..	...	...
Sugar	31	46	48
Fat*	3	25	36

* Before the investigation, lard, and during the investigation, vegetable oil and hydrogenated vegetable fat.

were fractionated by paper electrophoresis in barbital buffer pH 8.6 at an ionic strength of 0.075 using 110 v. for 16 hours. The protein fractions were estimated colorimetrically after staining with Amido Black 10B and elution in 0.01 N sodium hydroxide. The urine specimens were studied for creatinine,[13] urea nitrogen,[14] thiamine[15] and riboflavin. Riboflavin was measured by an adaptation of the method of Burch et al.[10]: A 20 µl. sample of urine diluted with 1 ml. of 0.8 per cent sodium chloride and 3 ml. of 13 per cent trichloroacetic acid was treated with 2 µl. of 4 per cent potassium permanganate and 1 minute later with 2 µl. of 3 per cent hydrogen peroxide. An aliquot of 0.8 ml. was mixed with 0.2 ml. of 4 molar dipotassium phosphate and the fluorescence measured in a Farrand fluorometer before and after the addition of 10 µl. of 10 per cent sodium hydrosulfite in 5 per cent sodium bicarbonate. A standard solution of riboflavin (0.2 µg. per ml.) and water blank were treated in the same manner throughout.

On the basis of these exploratory findings, menus were planned for a period of ten weeks, following as closely as possible the food pattern of the previous diet but increasing the nutrient intake levels to satisfy dietary allowances. The serving of meals was carefully supervised.

The daily nutrient intake of the children on the new diet was determined twice during the study, using the same technics of the initial survey. Each observation period covered nine days. At the end of the ten week period of study, the clinical-nutritional examination by the same clinician, and the biochemical studies of blood and urine were repeated.

RESULTS

Dietary Study

Evaluation of the diet consumed before the experiment revealed that these children were receiving limited amounts of milk, meat, vegetables and fruits. In terms of nutrient content, this diet showed marked deficiencies, according to recommended allowances,[5] in vitamin A, riboflavin and vitamin C. The intakes of the rest of the nutrients and of calories were also low, and only iron intake appeared to reach the recommended allowances.

Table I presents the kind and quantity of food given to the children before the investigation and after the diet was altered to increase nutrient intake. The amount of dried skim milk was increased from approximately 12 gm. to about 50 gm. per child per day. Eggs were introduced into the diet, each child receiving about one egg per day. Meat, vegetables, fruits and fats were given in greater amounts. The portions of black beans given to the children were reduced, and wheat bread was replaced by tortillas which were offered in liberal amounts.

The caloric and nutrient contents of the diet before and during the investigation are given in Table II. The total intakes of calories increased from 1,000 to about 2,000 per day. Intake of animal protein increased approximately fourfold, changing the proportion of animal protein to total protein from 24 per cent to about 54 per cent. Compared with the previous intake, the amount of fat supplied during the experimental period was doubled. Calcium intake was raised from 492 to approxi-

TABLE II

Mean Intake of Calories and Nutrients per Child per Day Before and During the Investigation

Nutrient	Consumption					
	Before Investigation		During 1st Mo. of Investigation		During 2nd Mo. of Investigation	
	Total	Per Cent*	Total	Per Cent*	Total	Per Cent*
Calories	1,082	57	1,898	100	2,072	109
Protein (gm.)						
Total	27.9	63	67.6	113	72.1	120
Animal	9.1	...	35.7	...	39.2	...
Fat (gm.)	15.1	...	39.1	...	51.5	...
Carbohydrate (gm.)	204.4	...	325.2	...	337.6	...
Calcium (mg.)	492	49	1,303	130	1,347	135
Iron (mg.)	11.7	117	22.6	226	24.4	244
Vitamin A (I.U.)	900	26	3,763	108	4,093	117
Thiamine (mg.)	0.70	70	1.01	101	1.09	109
Riboflavin (mg.)	0.58	39	1.61	107	1.73	115
Niacin (mg.)†	5.30	56	10.58	111	11.66	123
Vitamin C (mg.)	14	23	85	142	116	193

* Per cent of recommended dietary allowances.[5]

† Includes only preformed niacin. If "niacin equivalents" are used, that is, 1 mg. of niacin is added for each mg. of tryptophan, the intakes become 11.57, 22.48 and 24.44, respectively.

TABLE III

Heights and Weights of Children at Beginning of Study

Child	Age (yr.)	Height (cm.)	Weight (kg.)	Standard Height*		Standard Weight*	
				5th Percentile	50th Percentile	5th Percentile	50th Percentile
R.C.P.	6	102.0	17.8	108.7	116.6	16.4	20.3
M.A.B.	7	105.0	18.6	113.8	122.2	18.3	23.1
J.G.G.	7	98.0	18.6	113.8	122.2	18.3	23.1
J.C.G.	7	103.0	17.3	113.8	122.2	18.3	23.1
H.S.S.	7	107.0	17.4	113.8	122.2	18.3	23.1
F.M.C.	7	103.5	16.0	113.8	122.2	18.3	23.1
R.D.M.	8	112.2	21.8	119.1	128.3	20.2	26.1
P.A.A.	8	123.3	24.0	119.1	128.3	20.2	26.1
C.A.F.	8	117.5	22.7	119.1	128.3	20.2	26.1
J.M.V.	9	124.5	24.4	124.0	134.1	21.8	29.3
R.C.C.	12	135.2	34.1	135.9	148.8	27.3	39.1

* From FALKNER, F. *Pediatrics*, 29: 467, 1962.[16]

mately 1,300 mg. per day and vitamin A from 900 to about 4,000 I.U. The intake of vitamin C was 14 mg. before the diet was improved and at least six times higher when the improved diet was instituted. The intake of riboflavin was almost tripled and that of niacin doubled, while the thiamine intake was raised only about 45 per cent.

Clinical Examination

Table III shows the individual body heights and weights of the subjects at the time the

TABLE IV

Physical Measurements of Children Before and After Dietary Supplementation in Eleven Subjects

Measurement	Before Supplementation*	After Supplementation*	Increment	Standard Error of the Difference
Body weight (kg.)	21.2	21.8	0.61†	0.17
Body height (cm.)	111.9	111.9	0.0	0.19
Skinfold (mm.)				
Mid-arm	6.2	7.0	0.8‡	0.29
Scapular	3.7	3.7	0.0	0.35

* Mean values.
† Significant at the 1 per cent level.
‡ Significant at the 5 per cent level.

study was begun. Standard figures[16] are also included for comparison. All are far below the fiftieth percentile; in fact, all but two are below the fifth percentile. The weights are also below the fiftieth percentile, but only three are lower than the fifth percentile figure. The standard figures used are those for white North American children since no separate standards for Guatemalan children have been prepared. INCAP's experience has shown, however, that the height and weight measurements of well nourished children of the area are within the values considered normal for North American children.[17]

The average increment during the study period and the standard error of the increment for each of the physical measurements con-

TABLE V

Changes in Frequency of Nutritional Clinical Signs in Eleven Children Receiving an Improved Diet

Clinical Signs*	Present Initially	Disappeared or Decreased in Intensity	Appeared or Increased in Intensity	No Change
Eyes				
Palpebral conjunctival injection	2	2	2	...
Conjunctival thickening	4	1	6	3
Bulbar conjunctival injection and circumcorneal injection	8	2	4	4
Circumcorneal and scleral pigmentation	2	...	2	...
Superficial corneal opacities and corneal scars	3	2	...	1
Corneal vascularization	1	1	...	...
Face				
Malar hyperpigmentation	...	...	1	...
Nasolabial dyssebacea	1	1	...	...
Diffuse depigmentation	2	1	1	1
Lips				
Cheilosis	4	4	...	...
Gums				
Spongy, bleeding	3	3	1	...
Tongue				
Hyperaemic and hyperthrophic fungiform papillae	2	1	...	1
Atrophic filiform papillae	2	2	...	...
Geographic tongue	1	1	...	...
Glands				
Parotid enlargement	1	1	...	...
Thyroid enlargement	1	1	...	...
Skin				
Xerosis	2	2	...	...
Follicular hyperkeratosis	8	6	2	...

NOTE: The signs listed are those which were observed at least once during the study and those which belong to group 1 of the WHO classification, that is "signs known to be of value in nutrition surveys."[18]

* Nomenclature and description of signs according to the Expert Committee on Medical Assessment of Nutritional Status. World Health Organization Technical Report Series No. 258. Geneva, 1963. WHO.[18]

sidered are given in Table IV. No change in height could be detected, perhaps because of the lack of sensitivity of the method and the short period of time of the study, while there was a significant increase in body weight ($p < 0.01$) which did not differ from the expected age specific (eight years) increment on the basis of the standard of comparison employed. The standard increment in body weight for that age is 2.9 kg. per year or 0.56 kg. in ten weeks[16]; the average weight increment in this study was 0.6 kg. for the ten week period of observation.

The skinfold thickness over the triceps increased significantly ($p < 0.05$), while it did not change on the subscapular area. This is in support of the conclusion of the World Health Organization (WHO)[18] that "the balance of evidence at present favors measurement over the triceps as an index of nutritional status."

The results of the clinical examination are given in Table V. The nomenclature recommended by WHO is used.[18] The signs listed in the table are those which (1) were observed at least once during the study, and (2) belong to group 1 of the WHO classification, that is, "signs known to be of value in nutrition surveys." The two signs which are suggestive of riboflavin deficiency (nasolabial dyssebacea and cheilosis) disappeared or decreased in intensity after consumption of the improved diet. Spongy bleeding gums, suggestive of ascorbic acid deficiency, were clearly present in three children, and in all three it decreased in severity. In one of the subjects, however, this sign was observed only on the examination conducted at the end of the study. Of the two skin signs associated with vitamin A nutrition, xerosis was present initially in two children and absent at the second examination, while follicular hyperkeratosis, which was present in eight subjects, disappeared or decreased in severity in six and increased in severity in two.

The only child with slight thyroid enlargement initially showed a normal thyroid after being on the improved diet for ten weeks. Parotid enlargement was also found only in

TABLE VI
Biochemical Measurements in Blood of Ten Children Before and After Diet Supplementation

Constituent (per 100 ml.)	Before Supplementation*	After Supplementation*	Difference	Standard Error of the Difference
Blood				
Hemoglobin (gm.)	12.6	11.5	1.1†	0.25
Serum				
Total proteins (gm.)	7.39	7.20	0.19	0.08
Albumin				
Alpha$_1$ globulin (gm.)	4.24	4.22	0.02	0.03
Alpha$_2$ globulin (gm.)	0.90	0.94	0.04	0.05
Beta globulin (gm.)	0.82	0.88	0.06	0.05
Gamma globulin (gm.)	1.43	1.16	0.27†	0.08
Pseudocholinesterase (ΔpH units)	1.18	1.16	0.02	0.16
Vitamin A (μg.)	19.8	21.3	1.5	2.10
Carotene (mg.)	33.3	78.4	45.1†	5.56
Vitamin C (mg.)	0.5	1.1	0.6†	0.10
Free + flavin adenine mononucleotide riboflavin‡ (μg.)	0.7	1.7	1.0§	0.30
Total riboflavin (μg.)	2.8	3.8	1.0†	0.24
Red blood cells				
Total riboflavin (μg.)	16.0	19.6	3.6†	0.88

* Mean values.
† Significant at the 1 per cent level.
‡ In the determination of free riboflavin by the method used, the small amount of flavin adenine mononucleotide is included.
§ Significant at the 5 per cent level.

Table VII
Biochemical Measurements in Urine of Ten Children Before and After Dietary Supplementation

Constituent (per 24 hr.)	Before Supplementation*	After Supplementation*	Difference	Standard Error of the Difference
Creatinine (gm.)	0.26	0.30	0.04†	0.018
Urea nitrogen (gm.)	2.1	4.3	2.2‡	0.50
Riboflavin (mg)	0.13	0.20	0.07†	0.019
Thiamine (mg.)	0.35	0 24	0.11‡	0.030

* Mean values.
† Significant at the 5 per cent level.
‡ Significant at the 1 per cent level.

one child on the first examination and in none on the second. This sign has been generally associated with protein-calorie malnutrition. The other sign suggestive of the same deficiency is diffuse depigmentation of the skin; in one of the two children who were so afflicted, its severity decreased, in the other it increased. Finally, atrophic filiform papillae, suggestive of niacin deficiency, disappeared after the period on the improved diet in the only two children in whom it was present initially.

Biochemical Findings

The results of the biochemical determinations in blood and urine are given in Tables VI and VII.*

The average hemoglobin value decreased significantly ($p < 0.01$) from 12.6 to 11.5 gm. per 100 ml. of blood. Unfortunately hematocrit and red blood cell counts were not determined and, therefore, the hematologic significance of the drop is obscure.

There was no significant change associated with the diet improvement in serum albumin (+ $alpha_1$ globulin), $alpha_2$ globulin and beta globulin, while the gamma globulin fraction decreased significantly ($p < 0.01$) accounting for most of the drop in serum total proteins from 7.39 to 7.20 gm. per 100 ml. It is to be noticed that the values for the serum protein electrophoretic fractions were normal even before the dietary treatment, with the possible exception of an initially elevated gamma globulin level. This finding suggests that if there was a deficiency of protein in the period before the experiment, it was not sufficiently severe to alter these serum proteins. The drop of gamma globulin from 1.43 to 1.16 gm. per 100 ml. can most probably be explained as a result of the medical supervision and improved hygiene in the group of children during the experimental period. The dietary improvement had no effect on the activity of serum pseudocholinesterase. Although serum pseudocholinesterase is reduced in children with kwashiorkor, it seems to decrease only when malnutrition reaches the acute clinical stage.[19] In this respect, it is similar to serum albumin with which it has been well correlated.[20]

Carotene, ascorbic acid and free riboflavin in serum, as well as the riboflavin content of the red blood cells, increased significantly reflecting the improved intakes during the trial period. The average serum level of ascorbic acid before the experimental period was 0.5 mg. per 100 ml. corresponding to an average intake of 14 mg. daily, which meets only 23 per cent of the recommended dietary allowances. Only one child had a value within the limits of the "low" group of the interpretative guide of ICNND[21]; all others had values in the "acceptable" or "high" ranges. Of the three children who had marginal gingivitis initially with easy bleeding, two had the lowest values of the entire group (0.1 and 0.2 mg. per 100 ml.), but one of them had 0.5 mg. In one child who had marginal gingivitis only at the end of the study, the serum level at the start was one of the lowest (0.2 mg.); after the period of supplementation there was a definite increase to an obviously adequate level (0.7 mg.) which was, nevertheless, the lowest for the whole group. At the end of the experimental period the average value had increased to 1.1 mg. per 100 ml. of serum.

The serum carotene values ordinarily have no nutritional significance *per se*, since low values are compatible with adequate vitamin A nutrition when most of this nutrient comes from the preformed vitamin present in animal

* It was possible to carry out the biochemical study in only ten of the eleven children.

foods. When the diet is mostly of plant origin, however, as was the case before the experimental period of the study, an initial average of 33.3 mg. per 100 ml. may be considered low. After the period on the improved diet, all subjects showed a significant increase in serum carotene values resulting in a final average of 78.4 mg. per 100 ml.

The free plus flavin adenine mononucleotide (FMN) riboflavin in serum increased more than twofold (0.7 to 1.7 mg. per 100 ml.) while the bound riboflavin did not change significantly. The increase in free riboflavin is the direct consequence of the higher intake of this vitamin during the experimental period. The initial average values for both free and total serum riboflavin fall well within the limits for persons with relatively ample intakes of this nutrient. The red blood cell riboflavin, which, according to Bessey et al.[3], is the best indicator of riboflavin nutrition, was, on the average, somewhat low initially but increased significantly ($p < 0.01$) to normal values after the dietary treatment. This indicates that the average intake before the experimental period was not sufficient to maintain the red blood cells with adequate levels of flavin adenine dinucleotide. Cheilosis was apparent in the three children who had the lowest values before the diet change (9.3, 12.5 and 15.2 mg. per 100 ml.). The only other child with cheilosis had the second lowest red blood cell riboflavin value of the remaining seven children. No correlation could be established between either free or total serum riboflavin and red blood cell riboflavin.

Before the experimental diet, the average serum vitamin A level was low (19.8 µg. per 100 ml.) and there was no significant increase after the period of the improved diet (21.3 µg. per 100 ml.). Observation of the individual values (Fig. 1) reveals, however, that the vitamin A concentration increased in the four children who had the lowest levels before the experimental period (8.9 to 15.3 µg.) and that the levels in the rest of the children, who had vitamin A concentrations from 19.1 to 30.6 µg. per 100 ml., either did not change or decreased. The marked drop in one child (J.G.G.) is particularly surprising. There

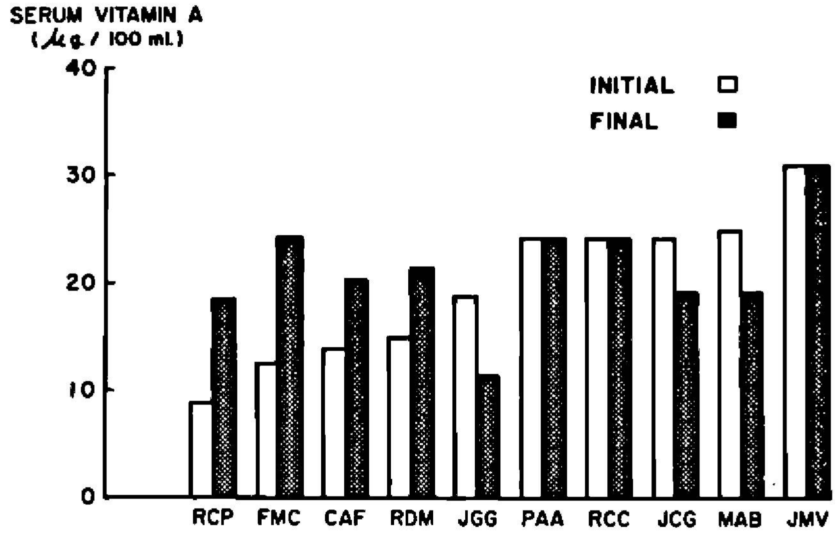

FIG. 1. Serum vitamin A concentration of children before and after an experimental dietary improvement. (The subjects are arbitrarily arranged in increasing order of initial vitamin A serum levels.)

was no evidence of fever or any other condition which could explain this finding. Although the increase in the four lowest values is understandable, it would have been expected that the values around 20 to 25 µg. would also increase after two months of a controlled daily intake of approximately 4,000 I.U. per capita. Values of about 40 µg. of vitamin A per 100 ml. of serum have been observed in children belonging to the upper income group in Guatemala.[22] Uptake of the vitamin by the liver cannot account for this lack of increase; if such were the case, the children with the lowest values would not have demonstrated an increase either.

It may be postulated that children who have subsisted for most of their lives on diets deficient in fat have lost some of their capacity for serum transport of lipids and lipidic compounds. This hypothesis would help to explain the previously reported lack of response in serum vitamin A concentrations observed in children of the rural low socioeconomic group in Guatemala who were receiving up to 15,000 I.U. of vitamin A daily for as long as seventeen weeks followed by 40,000 I.U. daily for thirteen weeks; in these trials the serum levels did not reach values higher than 30 to 35 µg. per 100 ml.[23] Similarly, Méndez et al.[24] have also noticed in Guatemalan children of low socioeconomic status, a surprisingly small increase in serum cholesterol concentrations after eight weeks on a United States-type diet which is much richer in fat and cholesterol than their customary diet. Even a high cholesterol intake derived from additional eggs for four weeks increased serum cholesterol in these children only slightly to levels certainly much lower than those reported for populations who have always subsisted on diets rich in fat.[25]

No individual relationship was found between the presence of xerosis or follicular hyperkeratosis of the skin and the serum vitamin A levels, although, in general, the severity and prevalence of these signs decreased after the ten week period of study.

The urinary excretion of creatinine, urea nitrogen, riboflavin and thiamine are given in Table VII. There was a small but significant increase in the creatinine output per 24 hours ($p < 0.05$) which cannot be quantitatively attributed to increase in muscle mass because of the possible effect of dietary creatine and creatinine.[26] The average amount of urea excreted was significantly larger at the end of the experimental period ($p < 0.01$), obviously reflecting the much higher protein intake.

The urinary excretion of riboflavin also increased significantly ($p < 0.05$), but the observed difference was not as large as could be expected from an estimated threefold increase in riboflavin intake. This apparent inconsistency may be partly explained by the fact that a marked increase in protein intake also took place. It has been demonstrated in studies with experimental animals[27–29] and with human subjects[30,31] that the urinary excretion of riboflavin decreases when the intake of protein is increased, and that the proteins retained in the body result in an increased retention of riboflavin. Another possibility is that the experimental diet was much richer in fat than the diet consumed before the experiment. In animal experiments it has been demonstrated that riboflavin requirements are higher with a fat-rich diet.[32,33]

The results of the determination of urinary thiamine are interesting: despite an increase in the estimated daily per capita intake from 0.7 mg. to 1.0 mg., the average urinary excretion of thiamine was significantly reduced ($p < 0.01$) from 0.35 mg. to 0.24 mg. per 24 hours. Since the requirement for this vitamin is related to calories and particularly to carbohydrates in the diet,[34] it is likely that the increase in calorie (about 100 per cent increase) and in carbohydrate intake (about 65 per cent) resulted in an increment in thiamine demand proportionally larger than the increment in thiamine intake (about 45 per cent). It may be noted that the thiamine per 1,000 calories supplied during the experimental period was less than before supplementation was instituted.

COMMENTS

Despite the extreme care exercised in the dietary investigation, it is realized that the figures derived for mean intakes are at best an indication of the general levels of intake characteristic of the corresponding periods. Although at the individual level these figures have limited meaning, they do reflect with certainty the marked increment in nutrient intake for the group of children as a whole. Under these circumstances, individual correlations of intake with biochemical values were not expected. It was, however, a reasonable assumption that the biochemical measurements were going to reflect the increased intake of specific nutrients for the whole group with corresponding increases in the average values for the biochemical indices studied.

This was, in fact, the case with carotene, ascorbic acid and free riboflavin in serum, as well as with red blood cell riboflavin. Nevertheless, the fact that this was not the case in all instances illustrates the complications which arise in the interpretation of biochemical findings in relation to nutrition when changes in the intake of various nutrients are introduced simultaneously; these complications are probably caused by the interrelationships among nutrients in the organism. The most conspicuous examples in the present study are the decrease in thiamine excretion in the urine despite an increase in intake, and the unexplained lack of response of the serum vitamin A values to a marked increase in vitamin A intake. A relationship was observed between the clinical and the biochemical data in regard to riboflavin and ascorbic acid, but its significance could not be established in view of the small number of observations.

SUMMARY

The study was designed to investigate the effect of a general dietary change on a number of biochemical measurements in the blood and urine of a group of children. The subjects were institutionalized in a home for poor children, in which the socioeconomic situation and the diet were as poor as among low socioeconomic groups in the Central American region. The experimental plan included an initial dietary survey, clinical examination and biochemical determination of hemoglobin, serum vitamin A, carotene, vitamin C, riboflavin, pseudocholinesterase and plasma protein fractions, as well as riboflavin in packed red blood cells. Creatinine, urea nitrogen, thiamine and riboflavin were estimated in urine. The preliminary dietary evaluation revealed marked deficiencies in intake of all nutrients when compared to the National Research Council Recommended Dietary Allowances. The children were then given a controlled diet which raised their nutrient intake to a level sufficiently high to satisfy at least National Research Council recommendations. In the case of vitamin A and vitamin C, this represented approximately a fourfold increase and about a threefold increase for riboflavin. After ten weeks on this improved diet the clinical and biochemical studies were repeated *in toto*. Several of the biochemical components of blood responded as expected with increases which reflected the higher experimental nutrient intakes, namely, carotene, vitamin C and riboflavin in serum as well as total riboflavin in the red blood cells. The serum vitamin A responded differently. The average level before the experimental diet was low and showed practically no increase during the study. Individually the serum concentration of vitamin A increased in four children, did not change in three and decreased in three. The unexplained discrepancy with the dietary data is evident since the corresponding vitamin A intake calculated per capita was four to five times higher during the experimental period than before the improved diet was instituted. Urinary excretion of thiamine decreased despite the increased intake. The significance of this phenomenon is discussed. Riboflavin and urea excretions in urine increased.

REFERENCES

1. Arroyave, G. Biochemical evaluation of nutritional status in man. *Fed. Proc.*, 20 (supp. 7): 39, 1961.
2. Horwitt, M. K., Harvey, C. C., Hills, O. W. and Liebert, E. Correlation of urinary excretion of riboflavin with dietary intake and symptoms of ariboflavinosis. *J. Nutrition*, 41: 247, 1950.
3. Bessey, O. A., Horwitt, M. K. and Love, R. H. Deprivation of riboflavin and blood riboflavin levels in man. *J. Nutrition*, 58: 367, 1956.
4. Flores, M., Flores, Z., García, B. and Gularte, Y. Tabla de Composici.n de Alimentos de Centro América y Panamá, 4th ed. Guatemala, 1960. Instituto de Nutrición de Centro América y Panamá.
5. Instituto de Nutrición de Centro América y Panamá. Recomendaciones nutricionales para las poblaciones de Centro América y Panamá. *Bol. Ofic. san panam.*, supp. 1, p. 119, 1953.
6. Brozek, J. (ed.). Body Measurements and Human Nutrition. Detroit, 1956. Wayne University Press.
7. Wintrobe, M. M. Clinical Hematology, 3rd ed. Philadelphia, 1951. Lea & Febiger.
8. Bessey, O. A., Lowry, O. H., Brock, M. J. and López, J. A. The determination of vitamin A and carotene in small quantities of blood serum. *J. Biol. Chem.*, 166: 177, 1946.
9. Lowry, O. H., López, J. A. and Bessey, O. A. The determination of ascorbic acid in small amounts of blood serum. *J. Biol. Chem.*, 160: 609, 1945.
10. Burch, H. B., Bessey, O. A. and Lowry, O. H. Fluorometric measurements of riboflavin and its natural derivatives in small quantities of blood serum and cells. *J. Biol. Chem.*, 175: 457, 1948.
11. Reinhold, J. G., Tourigny, L. G. and Yonan, V. L. Measurement of serum cholinesterase activity by a photometric indicator method. Together with a study of the influence of sex and race. *Am. J. Clin. Path.*, 23: 645, 1953.
12. Lowry, O. H. and Hunter, T. H. The determination of serum protein concentration with a gradient tube. *J. Biol. Chem.*, 159: 465, 1945.
13. Clark, L. C., Jr. and Thompson, H. L. The determination of creatine and creatinine in urine. *Anal. Chem.*, 21: 1218, 1949.
14. Consolazio, C. F., Johnson, R. E. and Marek, E. Metabolic Methods, p. 141. St. Louis, 1951. C. V. Mosby Co.

15. GYÖRGY, P. (ed.). Vitamin Methods, vol. 2, p. 289. New York, 1951. Academic Press, Inc.
16. FALKNER, F. Some physical growth standards for white North American children. *Pediatrics*, 29: 467, 1962.
17. MÉNDEZ, J. and BEHRHORST, C. The anthropometric characteristics of Indian and urban Guatemalans. *Human Biol.*, 35: 457, 1963.
18. Expert Committee on Medical Assessment of Nutritional Status. World Health Organization Technical Report Series No. 258. (Geneva, 21–27 August 1962.) Geneva, 1963. World Health Organization.
19. ARROYAVE, G., FELDMAN, R. and SCRIMSHAW, N. S. Serum cholinesterase levels of Central American children in relation to nutritional status. *Am. J. Clin. Nutrition*, 6: 164, 1958.
20. FABER, M. Relationship between serum cholinesterase and serum albumin. *Acta med. scandinav.*, 114: 72, 1943.
21. United States Interdepartmental Committee on Nutrition for National Defense. Manual for Nutrition Surveys. Washington, D. C., 1957. U. S. Government Printing Office.
22. BÉHAR, M., ARROYAVE, G., FLORES, M. and SCRIMSHAW, N. S. The nutritional status of children of pre-school age in the Guatemalan community of Amatitlán. II. Comparison of dietary, clinical and biochemical findings. *Brit. J. Nutrition*, 14: 217, 1960.
23. ASCOLI, W., SCRIMSHAW, N. S. and BRUCH, H. La vitamina A y su relación con la hiperqueratosis folicular y el bocio endémico en niños escolares de Ciudad Vieja, Guatemala. *Rev. col. med. Guatemala*, 12: 30, 1961.
24. MÉNDEZ, J., SCRIMSHAW, N. S., FLORES, M., LEÓN, R. DE and BÉHAR, M. Factors influencing serum cholesterol levels of Central American children. II. The effect of gross dietary changes. *Am. J. Clin. Nutrition*, 9: 148, 1961.
25. MÉNDEZ, J. Effect of dietary cholesterol upon serum lipids in rural Guatemalan Indian children. Submitted for publication.
26. BLEILER, R. E. and SCHEDL, H. P. Creatinine excretion: variability and relationships to diet and body size. *J. Lab. & Clin. Med.*, 59: 945, 1962.
27. SARETT, H. P., KLEIN, J. R. and PERLZWEIG, W. A. The effect of the level of protein intake upon the urinary excretion of riboflavin and nicotinic acid in dogs and rats. *J. Nutrition*, 24: 295, 1942.
28. SARETT, H. P. and PERLZWEIG, W. A. The effect of protein and B-vitamin levels of the diet upon the tissue content and balance of riboflavin and nicotinic acid in rats. *J. Nutrition*, 25: 173, 1943.
29. ADRIAN, J., LUNVEN, P. and TERROINE, T. Influence de la valeur protéique de la ration sur le taux urinaire et hépatique de la riboflavine, de la niacine et de la vitamine B_6. *Arch. sc. physiol.*, 15: 321, 1961.
30. OLDHAM, H.; LOUNDS, E. and PORTER, T. Riboflavin excretions and test dose returns of young women during periods of positive and negative nitrogen balance. *J. Nutrition*, 34: 69, 1947.
31. SMITH, J. M., LU, S. D. C., HARE, A., DICK, E. and DANIELS, M. The effect of nitrogen intake upon the urinary riboflavin excretion of young male adults. *J. Nutrition*, 69: 85, 1959.
32. SHAW, J. H. AND PHILLIPS, P. H. The pathology of riboflavin deficiency in the rat. *J. Nutrition*, 22: 345, 1941.
33. MANNERING, G. J., ORSINI, D. and ELVEHJEM, C. A. Effect of the composition of the diet on the riboflavin requirement of the rat. *J. Nutrition*, 28: 141, 1944.
34. SEBRELL, W. H., JR. Thiamine. XII. Requirements and factors influencing them. In: The Vitamins, Chemistry, Physiology, Pathology, vol. 3, p. 478. Edited by Sebrell, W. H., Jr. and Harris, R. S. New York, 1954. Academic Press, Inc.

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