C H A P T E R II

MATERIALS AND METHODS

CHAPTER II

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The aim of this experiment was to get a comparative data on the adequacy of dietary intake and nutritional status in the school boys aged 7 - 12 years in the lower and upper classes and the extent to which the boys in the lower class are affected by differences in dietary intake resulting from economic condition.

Sixty boys from the village of Raipura, thirty boys from the village of Bhaili and twenty-one boys from upper class families in Baroda were selected for the present studies comprising a total of 111 subjects in all. The boys in Raipura also formed subjects of either the experimental group fed a school lunch or a control group not fed the lunch. They were re-investigated after five months of treatment.

The following parameters were used for the assessment of their nutritional status :

(a) dietary intake;

(b) physical status;

(c) clinical examination;

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(d) analysis of 24 hours urine, for the creatinine, thiamine, riboflavine, ascorbic acid, N'-methyl nicotinamide and nitrogen;

(e) hemoglobin content of blood and composition of serum with regard to total protein, albumin, carotene, vitamin E, ascorbic acid, calcium and phosphorus;

(f) activity of alkaline phosphatase (E.C., 3.1.3.1.).

The methods used for the measurement of the above parameters are discussed below :-

(a) Dietary intake

Dietary intake was estimated by getting data on the food consumption of the family to which the subject belonged and the food intake of the subjects.

In the case of Raipura, the family survey included not only the families of the subjects studied but also of other families.

The amount of food consumed by the family was first estimated by the oral questionnaire method and the general pattern of food consumption was ascertained (Appendix I). The housewives were asked to show the amounts of different foodstuffs such as foodgrains, legumes, oil etc. used by them on an average per day and the volume of the same measured with standard measures and converted to weight equivalents.

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All the raw ingredients used for cooking or consumption and the cooked foods were weighed for atleast one day.

The food intake of selected subjects was measured by collection of aliquot portions of all the foods consumed over a period of five days in the villages of Raipura and Bhaili and over a twenty four hour period in the case of upper class subjects in Baroda.

The amounts of raw ingredients in the cooked foods were calculated from the data obtained on the weight of raw and cooked foods. The nutrient contents of the diet were calculated therefrom using the food tables published by the ICMR (1966).

(b) Physical statuse

Erect body length was taken with the subject's heels, buttocks, and upper back in contact with an upright board having an inlaid millimeter scale and a sliding horizontal that rests on vertex.

Weight was taken without shoes by using a weighing machine (Detecto, U.S.A.). Some of the initial measurements were however done with a bathroom scale before the Detecto weighing machine became available. The subjects in Raipura and Bhaili wore a towel whose weight was less than 0.25 kg. The weight of the upper class subjects in Baroda were taken in ordinary clothes (shorts and shirts) whose weight was less than 0.50 kg.

(c) Clinical examination

The nutritional assessment schedule suggested by ICMR (1948) was used (Appendix II). The clinical examination was done in the case of the subjects in Raipura before and after treatment by Dr.K.Bagchi, Nutrition Adviser, Government of India.

The identification of intestinal parasites in feces was carried out by the methods of Craig and Willis-Malloy (Kolmer and Boerner, 1949).

(d) Analysis of 24 hours urine

For analysis of urine all the urine voided between 8.00 a.m. on the collection day to 8.00 a.m. on the following day was collected in brown glass bottle containing 2 g of oxalic acid and 10 ml of toluene. The bottle was shaken well and the volume of urine measured. A known volume was stored in the cold room at $5^{o}C$ and different aliquots of the same were used for the estimation of creatinine, thiamine, riboflavine, ascorbic acid, N'-methyl nicotinamide and nitrogen.

(e) Analysis of blood

For blood analysis, 5-10 ml of fasting blood(venous) was drawn and serum was separated after taking 20 cmm blood for the estimation of hemoglobin.

The methods used for the determination of blood, serum and urine constituents are given in Table 14, and those for the serum enzymes in Table 15.

Formulation and evaluation of a low cost balanced school lunch

A school lunch so as to correct the basic deficiencies in the diet of the children was planned on the basis of foods locally available. The cost of the school lunch was not expected to exceed very much that of the home lunch. The deficiencies in the diet were found on the basis of diet survey and clinical examination. The most common deficiencies in the order of magnitude were vitamin A, riboflavine, calories, protein and calcium.

The lunch was planned so as to provide one third of the day's requirement of calories, half of the requirement of protein and more than eighty per cent of the requirement of vitamin A. Protein deficiency was sought to be corrected by adequate combination of cereals or millets and legumes which are known to be of complementary amino acid composition and that of vitamin A by the regular inclusion of the carotene rich vegetables and fruits. The riboflavine content of the diet was increased by the inclusion of leafy vegetables and the frequent use of procedures such as sprouting and fermentation. These additions were expected to increase the calcium content of the diet as well.

Only foodstuff locally grown were used in the programme. One dish meal incorporating cereal, legume and leaf greens were worked out. The cooking procedures used required simple equipment available in the most village homes and were part of common culinary practice in this region.

Previous studies carried out with rats suggested that a combination of cereal and legume in the ratio 4:1 was satisfactory with regards to amino acid composition and nutritive value. A preliminary diet survey in the village showed that they were consumed in the ratio of 12:1. In the school lunch cereals and pulses were, therefore, used in two proportions, 8:1 and 4:1. The former was closer to the ratio in which they were produced in this country and represented an improvement over existing levels of intake. The latter was used as a desirable one. Half the children receiving the lunch were fed the former combination and the other half the latter.

The main dish was based on cereal with or without legumes. A legume dish was prepared separately. It was prepared only if the amount incorporated in the cereal-legume dish was less than the amount intended. Vegetables, fruits and butter-milk were provided in specified quantities. A variety was provided within a certain framework which was to allow about 15-30 g of pulses, 30 g of leaf greens or other carotene rich

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vegetables, 30 g of other vegetables and 30 g of locally available fruit in season for every 120 gm of cereal or millets. Inspite of the variation in the menu, the protein, calorie and calcium contents were held reasonably constant and minimum amount of carotene was provided.

The lunch provided is compared with the home lunch in Table 16. Its nutritive value and extent to which it meets the recommended allowances are shown in Tables 16a,b and c. Appendices III and IV give the menus used and the cooking procedures employed.

Table 14. Methods used for the chemical determinations

(A) Blood and serum analysis

Parameter measured	Method used	Amount used for estimation
Blood		
Hemoglobin	Oxyhemoglobin (Evelyn and Malloy, 1958)	20 cmm blood
Serum		
Total protein	Biuret (Reinhold, 1953; Cf.Varley, 1967)	0.4 ml serum
Carotene and Vitamin E	Quaife et al, (1949)	60 cmm serum
Ascorbic acid	Lowry et al, (1945)	20 cmm serum

(B) Urine analysis

Parameter measured	Method used	Amount taken for analysis
Creatinine	Alkaline picrate (Hawk et al, 1954)	1 ml of urine was diluted to 100 ml and different aliquots used.
Thiamine	Fluorimetric (Methods of vitamin Assay, 1951)	25 ml of urine was hydrolayed, volume made upto 100 ml and 25 ml was used for estimation.
Riboflavine	Fluorimetric (Methods of vitamin Assay, 1951)	25 ml of urine was hydrolysed, volume made upto 50 ml and 10 ml was taken for estimation.
N'-methyl nicotinamide	Fluorimetric (Carpenter and Kodicek, 1950)	2 ml of urine was diluted to 10 ml and used.
Ascorbic acid	Dinitrophenyl hydrazine (Roe and Kuether, 1943)	4 ml of urine was diluted to 12 ml with 8 ml of 5% trichloro acetic acid and used for estimation.
Nitrogen	Microkjeldahl (Hawk et al, 1954)	5 ml of urine was digested and diluted to 50 ml and 5 ml of the same used for estimation.

Table 15. Methods used for the enzyme estimations

Details of assay system	Alkaline Phosphatase (E.C.,3.1.3.1)
Reference	Bessey et al, (1946)
Buffer	Glycine-sodium hydroxide, pH 10.4; 5 µmoles
Substrate	1.33 µmoles of disodium nitrophenyl phosphate, pH 10.4
Enzyme source	0.005 ml serum
Other components	Magnesium chloride, 0.05 µmole (final volume, 0.105 ml)
Temperature and period of incubation	37° for 30 minutes
Initiation of reaction	Addition of serum
Termination of reaction	Addition of 0.5 ml of 0.02 N sodium hydroxide
Blank	Add 0.1 ml of concentrated hydrochloric acid after the first reading is taken and read again.
Parameter measured	Amount of nitrophenol liberated

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Table 16. Lunch provided at home and school

Home	School
Rotla	A cereal-legume combination (main dish) such as khichri, Rotla, Debra, Poora and Dokla
Dal (liquid)	Leafy or carotene rich vegetables as such or combined with other vegetables and legumes.
Vegetables	Other vegetables
	Seasonal fruits
	Buttermilk

Table 16a. Composition of the lunch provided at home and School

Foodstuff	Amount (g)		
	Home lunch	School lunch	
		A	B
Cereals and millets	115	165	145
Pulses	14	21	37
Milk *	Nil	20	20
Leafy vegetables	Negligible	24	24
Other vegetables	22	38	38
Fruits	Nil	27	26
Fats and oils	4	20	18
Sugar and Jaggery	Nil	1	1

* Equivalent of buttermilk provided

Table 16b. Nutrient content of the lunch provided at home and school

Nutrients	Home lunch	School lunch	
		A	B
Calories	490	900	860
Protein(g)	15	24	26
Calcium (mg)	55	190	190
Iron (mg)	8	17	17
Vitamin A(i.u.): as preformed vitamin	Nil	20	20
as carotene	200	3010	3010
total	200	3030	3030
Thiamine (mg)	0.43	0.66	0.68
Riboflavine (mg)	0.18	0.33	0.36
Ascorbic acid (mg)	6	30	30
Nicotinic acid (mg)	4	6	6

A-Cereal-pulse ratio, 8:1. B-Cereal-pulse ratio, 4:1

Table 16C. Cost and nutritive value of the lunch provided at school as compared to Recommended Daily Allowances (RDA)

Constituent	Amount consumed per day	Percentage of RDA	RDA	Source
Calories	880	51	1580	Aub and DuBois, (1917)
Protein(g)	25	49	57	FAO, (1957)
Fat (g)	20	-	-	-
Calcium (mg)	190	38	600-700	FAO, (1962)
Iron (mg)	17	68	20-30	ICMR, (1966)
Carotene(i.u.)	3030	above 100	2650-3500	FAO (1967)
Thiamine (mg)	0.67	80	0.84	Mitchell, (1964)
Riboflavine(mg)	0.34	29	1.16	Mitchell, (1964)
Vitamin C (mg)	30	above 100	15	Mitchell, (1964)
Niacin (mg)	6	54	11	Mitchell, (1964)
Cost (Rs.)	0.25*			

* Based on the values obtained on 1965

(a) The calorie requirements were calculated from the age, sex, height and weight of the upper class subjects using Aub-Dubois standard for B.M.R. and adding an appropriate allowance for activity (Table 25, Chapter III). The dietary standards used for the other nutrients were selected on the basis of their suitability for Indian conditions.

* The cost of the homelunch was Rs.0.13 and that of the whole day diet was Rs. 0.35.