C H A P T E R II

MATERIALS AND METHODS

MATERIALS AND METHODS

As stated earlier the main object of these investigations was to investigate the efficacy of a school lunch based on locally available and cheap foods in improving the nutritional status of school boys. Additional investigations were carried out as specified.

The following investigations were carried out:

(1) Data were collected on the dietary intake and clinical and biochemical status of poor school boys aged 7-12 years in a selected village.

(2) Comparative data were obtained on apparently well-nourished upper class children.

(3) Some of the children in the village were fed diets formulated in this laboratory for a period of six months and an assessment made of the effects of the same on growth as well as clinical and biochemical status. These studies were repeated in a modified form in a subsequent year.

(4) The above data were compared with those obtained on children fed a school lunch under 'CARE' programme for two years.

(5) The school lunch formulated provided vitamin A in the form of carotene and no animal food other than 3 g of skim milk powder. Additional studies were therefore made of the availability of carotene in leafy vegetables and the effects of vitamin B_{12} supplementation.

Subjects

For the comparative studies on boys belonging to the low income group in rural areas and high income group in urban Baroda, fifty-two boys aged 6-12 years in the village of Raipura and twenty-one belonging to upper class families in Baroda were investigated.

For the studies on the impact of the school lunch programme in Raipura forty boys in the fed group and twelve boys in the control group were investigated in the first series. In the second series twenty five boys in the fed group and twelve boys in the control group were used.

For the studies on the impact of the CARE lunch programme fifty subjects each were used in the fed and control groups. Body weight and height were measured in a larger sample of 750 subjects.

In addition, 21 subjects were used in the studies on the comparative utilization of carotene in leafy vegetables and vitamin A palmitate and twenty subjects for the studies on the effects of vitamin B_{12} supplementation.

Some details of the above studies and the parameters measured in the same are shown in Tables 13 and 14. Further details will be given in the appropriate context.

Dietary intake

In all the above studies information was obtained on dietary intake by the modified oral questionnaire method, supplemented by more systematic measurements in the case of children.

In the studies on adults the subject was asked to give information on the quantities of different foods consumed and the nutritive value of the diet calculated from previous data collected on the raw food equivalents of cooked foods by weighing the ingredients and the foods before and after cooking. Extensive information of this type has been obtained on both rural, urban poor and urban

Table 13: Details of various experiments

Groups	Place	No. of subjects	Period of treatment (months)	Experimental treatment	Parameters measured
School boys					
I Experimental	Raipura	40	6	School lunch	Body - Height and weight Blood - Hemoglobin Serum - Protein, albumin, vitamin C and carotene Urine - Vitamin B_1, B_2, creatinine and nitrogen.
Control	Raipura	12		None	
II Experimental	Raipura	25	5	School lunch	Body - Height and weight. Urine - Vitamins B_1, B_2, creatinine and nitrogen. Wrist X-rays Salivary amylase
Control	Raipura	12		None	
III Experimental	Devagad Baria	50	1-2 years	Receiving lunch under CARE assistance	Body - Height and weight. Blood - Hemoglobin Serum - Protein, albumin, vitamin C and vitamin A. Urine - Vitamin B_1, B_2, creatinine and nitrogen Wrist X-rays
Control		50		None	
Adult Men					
IV Utilization of carotene	Baroda	21	3	* a) Leafy vegetable b) Vitamin A palmitate c) None	Blood - Hemoglobin Serum - Protein, albumin, carotene and vitamin A.
V Effect of vitamin B_{12}	Baroda	20	3	a) Vitamin B_{12} b) Vitamin B_{12} + intrinsic factor c) None	Blood - Hemoglobin Serum - Total protein, albumin

* Given along with a basal lunch.

Table 14: Analysis of blood and urine

Parameter measured	Method used
Blood	
hemoglobin	Evelyn and Malloy, 1938.
Serum	
total protein	Biuret method (Reinhold, 1953 as described by Varley, 1958) modified to microscale.
albumin	Paper electrophoresis and elution (Varley, 1958).
carotene and vitamin A	Neeld Jr. and Pearson (1963).
ascorbic acid	Lowry, Lopez and Bessey (1945).
Urine	
creatinine	Alkaline picrate method (Hawk, Oser and Summerson,1954).
thiamine	Thiochrome method (Methods of Vitamin Assay, 1951).
riboflavine	Fluorimetric method (Methods of Vitamin Assay, 1951).
vitamin C	Dinitrophenyl hydrazine method (Roe and Kuether, 1943).
nitrogen	Micro-Kjeldahl method (Hawk, Oser and Summerson,1954).

upper class families in the various projects carried out in the department including the present investigation. In other studies in this laboratory as well as in the present studies, analysed values were found to agree well with calculated values for most nutrients (Rajalakshmi and Ramakrishnan, 1968). Further, the customary lunch was prepared in the laboratory and served ad libitum and their intake compared with information previously recorded.

In the case of children the mothers were questioned on how much of the different foods the child ate at different times of the day (They were asked to show the approximate quantity) and the food intake calculated therefrom. As the diets were monotonous (dal, chapati (i.e, home made unleavened bread) vegetable, rice, kodri, kadi and tea) and standard cooking practices were used in most households it was possible to do this without much difficulty.

More intensive studies were carried out in the case of 12 subjects in Raipura. Aliquot portions of all the food consumed by these subjects were collected over a five day period by field workers who were at the homes of the children from the first meal in the morning to the last meal in the night. Data were also obtained on the quantities of raw ingredients used for cooking and quantities of cooked foods during this period. The diets were analysed for protein, calcium and iron.

<u>Formulation of a school lunch based on locally available sources</u>:

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

The deficiency in calories can be presumed to be atleast in part due to poor appetite caused by an unsatisfactory diet. It seemed possible on the basis of previous experience that a balanced lunch provided ad libitum would help to bridge the calorie gap, whether it is caused by poor appetite or poor availability of food.

As the diets provided 10% calories in the form of protein, increasing calorie intake and improving the quality of protein in the diet were considered as adequate measures.

Studies in this laboratory and elsewhere have shown that the quality of cereal proteins can be corrected by appropriate combinations of cereals or millets and legumes which are known to be of complementary amino acid composition. In previous studies in this laboratory, bengalgram was found to be as effective as milk powder, fish flour or lysine in improving the protein value of wheat (Table 11).

A lunch based on a cereal-legume combination was therefore proposed. Wheat was the most obvious choice for the cereal to be used as it was available in ration shops at a cheap price at the time when the investigations were started and is eminently suitable as it forms part of the customary diet, has a high protein content and lends itself to incorporation in a variety of dishes. Peas were chosen as they were the cheapest legume available in the market at that time. In other studies in this Department peas were found to be as effective as bengal gram in improving the nutritive value of kodri (Table 15). Peas compare with bengalgram in nutritive value as shown below. In fact peas have a higher lysine content according to food tables published by the ICMR.

Table 15: Data on biological evaluation of different supplements to Kodri.

	Supplement used					
	None	Redgram	Bengalgram	Mothbean	Cowgram	Peas
Weight grain in four weeks (g)	-2	16	25	30	25	33
PER	-0.2	0.84	1.25	1.36	1.19	1.65
*Xanthine oxidase	824	468	405	429	459	331
*Succinic dehydrogenase	1143	480	452	446	474	403
Blood hemoglobin (g%)	9.1	12.1	13.3	13.4	13.4	13.3

Rajalakshmi and Majmudar (1966)

*Expressed as time taken in seconds for the reduction of methylene blue by 150 mg of liver under the assay conditions.

Aykroyed *et al.* (1966)

	per 100 g				Lysine g/g of nitrogen
	Protein (g)	Calcium (mg)	Thiamine (mg)	Riboflavine (mg)	
Bengalgram	20.8	58	0.48	0.18	0.49
Peas	19.7	75	0.47	0.38	0.98

On the basis of the studies cited earlier it seemed worthwhile to use this legume and also popularise it. A combination of wheat and peas was therefore used in the first series. In the second series a combination of wheat and bengalgram was used as at the time bengalgram was provided at a cheap price in fair price shops.

Vitamin A deficiency was sought to be corrected by including 30 g of leafy vegetables. The leafy vegetables used were spinach, fenugreek and amaranth. In addition 30 g of potatoes or other vegetables and 30 g of seasonal fruit were also included.

The "B" vitamin content of the diet was sought to be increased by the above measures as well as the frequent use of procedures such as sprouting and fermentation. Both these processes have been found to be associated with an increase in vitamins (Table 16) and other favourable changes such as reduction in phytate and increase in amino nitrogen and free sugar.

About 30 g of curd prepared from 3 g of milk powder were diluted and given as butter milk. The milk powder used (supplied by the UNICEF) was reinforced with vitamin A and provided about, 180 i.u. of the vitamin. In addition it can be expected to provide about 40 mg of calcium and 0.1 µg of vitamin B_{12}. About 20 g of oil were used in the preparation of lunch.

Thus in the first series of investigations (1965-66) the lunch provided included wheat, peas, leafy vegetables, other vegetables, seasonal fruit, butter milk and oil. The menu provided was varied within this frame work to make for variety and to permit the use of procedures such as sprouting and fermentation.

During the second series of investigations organized in 1966-67, the following modifications were made. Fruit was omitted to make the diet more practicable. The omission was not expected to affect nutritive value as the lunch included leafy and other vegetables and the vitamin C status of the subjects was not unsatisfactory.

Table 16: Change in vitamin content during sprouting and fermentation

	mg per 100 g of raw material										
	Thiamine			Riboflavine			Ascorbic acid		Nicotinic acid		
	raw	sprouted	sprouted and fermented	raw	sprouted	sprouted and fermented	raw	sprouted	raw	sprouted	sprouted and fermented
Wheat	0.45	0.70	0.72	0.14	0.22	0.31	0	10.0	5.4	7.8	9.7
Maize	0.43	0.47	0.48	0.11	0.17	0.24	0	10.0	1.5	2.2	3.0
Bengalgram	0.32	0.38	0.39	0.50	0.87	1.10	0	59.0	2.0	3.1	4.1
Greengram	0.45	0.60	0.61	0.40	0.60	0.81	0	82.0	2.4	3.6	4.7

Annual Report of PL 480 Project FG-IN-402 (1970)

Bengalgram, which was cheaply available at the time in fair-price shops, was substitued for peas. Attempts were also made to increase the calcium content of the diets. Whenever fermented cereal-legume preparations, namely, dhokla and poora, were used, lime was added to the fermented batter so as to increase its calcium content. The amount added was adjusted so that the resulting pH did not exceed 6.5 and this prevented the loss of vitamins (Table 17). The buttermilk given daily was also reinforced with lime-powder. These procedures resulted in increasing the overall calcium content of the lunch by about 150 mg.

Conduct of the school lunch programme:

The first series (1965-66)

The co-operation of villagers and the teachers of the school was obtained for organizing a school lunch programme. A kitchen and dining hall had been previously established near the school. As mentioned earlier the lunch was based on a combination of wheat and peas. Diet surveys made in the village showed that cereals and legumes were consumed roughly in the ratio 12:1. They are produced in the ratio 8:1 whereas it would

Table 17: Effect of lime powder incorporation in acid foods on thiamine and riboflavine content

Foodstuff	Amount of lime powder added (mg/100g)	pH	Thiamine µg/100g	Thiamine Percentage loss	Riboflavine µg/100g*	Riboflavine Percentage loss
1. Dhokla	0	5.0	422 (410-434)	-	430 (410-450)	-
"	500	6.3	402 (398-406)	4.7	420 (410-430)	2.3
2. Idli	0	4.7	490 (477-498)	-	495 (470-510)	-
"	450	6.0	443 (440-447)	9.5	446 (420-460)	9.9
3. Khaman	0	5.2	630 (628-632)	-	886 (874-888)	-
"	350	6.5	617 (615-619)	2.5	818 (816-820)	7.6
4. Sambhar	0	5.6	589 (580-598)	-	746 (739-756)	-
"	150	6.0	554 (550-558)	5.6	696 (630-702)	6.8
5. Butter milk (curd mixed with water in the ratio)	0	4.1	10 (9-12)	-	33	-
1:4	280	5.5	10	0	31	6

*On dry weight basis for 1,2 and 3 and wet weight basis for 4 and 5.

Values are means with range shown in parentheses.

Rajalakshmi and Ramachandran (1967)

seem desirable to have them in the ratio 4:1. However, the former ratio may be more realistic and may even be adequate as peas contain more lysine and produce more growth than bengalgram when added to kodri. The diet includes not only legumes but also some quantities of milk and greens. It seemed worth while to investigate from the standpoint of practical nutrition whether a greater proportion of pulse made a significant difference in nutritive value. Previous studies conducted from this point of view proved equivocal. Animal studies in this laboratory have shown that at both levels, the nutritive value of wheat is improved, the improvement being greater with the higher proportion of pulse, as can be seen from the following data:

Diet	Weight gain (g) during 8 weeks
Wheat alone	61
Wheat + Bengalgram (8:1)	99
Wheat + Bengalgram (4:1)	115

As mentioned earlier, the main dish was based on a cereal-legume combination with or without leafy vegetables. The vegetables were prepared separately when they were not included in main dish. Fruits and butter milk were provided in the quantities specified.

A variety was provided within the above frame work by varying the recipes used as shown in Tables 18 and 19.

The nutrient content of the lunch provided and the extent to which they meet the recommended allowances are shown in Table 20. The main dish made of wheat and peas was provided <u>ad libitum</u>. A known quantity was given in the first serving and records kept of further servings. The nature of the main dishes prepared made this relatively easy.

A matched group of school children who were not attending the lunch served as a control.

<u>Comparative study on CARE fed children at Devgadh Baria</u>

Devgadh Baria is a tribal area 100 miles from Baroda. The people in this area are "Bhils", an aboriginal tribe. They live on maize and jowar and their economic status is very poor. The children in some schools in this area are being fed a school lunch sponsored by CARE. The lunch consists of 'Upma' prepared from 60 g cracked wheat, 30 g of skim milk powder and 15 g soya bean oil. Attempts

Table 18: Composition of the lunch provided.

Foodstuff	Amount (g)	Preparation	Weight of cooked food(g)
Wheat + Peas	150-200	Roti	240 - 270
		Pooda	420 - 470
		Dhokla	320 - 380
		Dhebra	260 - 300
Oil	15-20	used in cooking	
Leafy greens	30	Vegetable dish	70 - 80
Potatoes	30		
Butter milk (Prepared from 3 g skim milk powder)	100	Buttermilk	
Fruits (Papaya, banana, or chiku)	30	served as such	

Table 19: Preparation of the menu.

Item	Method
Roti	Dough prepared from wheat and pea flour and baked like chapaties.
Dhebra	A dough prepared from wheat and pea flour and finely chopped greens and shaped into balls, rolled out with a rolling pin and pan-fried with a little oil like parathas.
Dhokla	Coarsely ground wheat and peas mixed with water and salt to form a thick batter and the same fermented overnight. The fermented batter steamed, cooled and cut into pieces and seasoned.
Poora	A thick pancake-type batter prepared from wheat and pea flour. The batter fermented overnight and pan-fried like dosas.
Vegetable	Chopped greensand cut potatoes were cooked together in just enough water with salt, spices and oil added. On 'dhebra' days, some other vegetable was substituted for greens as greens were already incorporated in dhebra.
Butter milk	Milk prepared from skim milk powder and set as curd and churned and diluted to butter milk.

Table 20: Cost and nutritive value of the lunch provided at school as compared to Recommended Daily Allowances (RDA).

Nutrient	Amount consumed per day	Percentage of RDA	RDA	Source
Calories	920	47	1800-2100	ICMR (1968)
Protein (g)	28	78	33-41	ICMR (1968)
Calcium (mg)	230	40	500-700	FAO (1962)
Iron (mg)	16	90	15-20	ICMR (1968)
Vitamin A value (i.u.)	1500	75	1600-2400	ICMR (1968)
Thiamine (mg)	1.0	100	0.5 mg per 1000 calories	Rajalakshmi & Ramakrishnan (1968)
Riboflavine (mg)	0.8			
Cost	0.37*			

*Based on the values obtained on 1965.

were made to measure the impact of this programme by comparing the nutritional status of fed and control children in the area. Children in schools where the programme had been in operation for at least two years and whose attendance was more than 70% during this period were considered as fed children. Children in schools in neighbouring villages where no such programmes were in operation were taken as controls. There were no differences either in socio-economic status or in dietary patterns at home between the two groups. The chief difference was the willingness or otherwise of the village communities to make a very nominal contribution towards the administrative cost of the lunch programme.

Studies on the availibility of carotene from leaf greens

After the conclusion of the lunch programme in Raipura it was found that although the severity and incidence of clinical vitamin A deficiency had decreased in the 'fed' children the symptoms had not altogether disappeared. This could have been due to several reasons including a previous state of depletion as the amount required for treating a deficiency may be expected to be more than that required for preventing it. Also, the lunch was fed on 150 days out of a total period of 180 days and the number of days when the children were actually fed was reduced further by absenteeism as the average attendance was only about 75%. However in retrospect it was felt that the amount of leafy vegetables provided was not adequate.

The lunch given included 30 g of leafy vegetables containing 2100 I.U. which would provide about 1400 I.U. of carotene after allowing for cooking losses. If allowance is also made for holidays the figure will have to be downgraded to about 1200 I.U. Absenteeism would reduce it still further to about 1000 I.U. The home diet provided about 600 I.U. in the case of the fed children so that their over all carotene intake was 1600 I.U. per day. According to the FAO committee (1967) boys in the age group studied would need about 1200-1500 I.U. of vitamin A in the form of retinol on the basis of upper class body weights so that at least twice this amount would be required in the form of carotene. Thus the lunch fell short of a reasonable dietary allowance. It was felt that about 50-60 g of leafy vegetable would have been more adequate.

The question also arises as to how efficiently the carotene in leafy vegetables is utilized. In this connection the FAO committee assumes that 1 μg of retinol is equivalent to 0·6 μg of carotene. Animal studies carried out in this laboratory suggest that the utilization of carotene from commonly used leafy vegetables is much more efficient and that about 50 g of leafy vegetable may be equivalent to 2000 I.U. of retinol.

The validity of the above suggestions was investigated in adult male volunteers. It was considered that if 50 g are sufficient for adults they should be quite sufficient for children, as assuming no great differences in the utilization of carotene between school boys and adults.

Twenty one adult men were given a lunch in the laboratory similar to the one they would get at home for a period of three months. The lunch provided roti, dal, rice, potatoes. In addition the experimental group was given 50 g of leafy vegetables and the two control groups were given an equal quantity of other vegetables such as potato and brinjal containing only negligible amounts of carotene. In one of the two control groups the subjects were given 2000 I.U. of vitamin A in oil in the form of palmitate. A teaspoon of oil containing 2000 i.u. of palmitate was served over rice at the time of consumption (People in this region are accustomed to take rice with oil if they can afford it). An equal quantity of oil was served as such without the addition of vitamin A to the other groups. Except for this and leafy vegetables, the other foods were served ad libitum. Records were kept of vegetables and fruits consumed by the subjects at other times of the day. The quantity of foods consumed by the subjects at lunch was also unobstrusively recorded.

The leafy vegetables included were amaranth, spinach and fenugreek. The carotene content of the same was analysed a few times in fresh and cooked samples. In the latter case analysis was also made at the time of consumption. Description of subjects and treatment is given in Table 13.

Effect of vitamin B_{12} supplementation

In the studies on school children the response of blood hemoglobin levels to the dietary treatment was not as much as was expected. The lunch included only 3 g of skim milk powder and one possibility to consider is lack of Vitamin B_{12}. However, ordinary diets in this country generally include no more milk than what was available to the children and very little of other animal foods in spite of which megaloblastic anemia is seldom found. Nevertheless, it seemed desirable to investigate the effects of supplementation with vitamin B_{12} on blood hemoglobin in a selected group of subjects. Further, it has been alleged that clinical symptoms such as mental confusion may be found even in the absence of anemia. Moreover, subclinical deficiency may possibly reduce the concentration of hemoglobin without affecting cell-size. Twenty subjects with low socio-economic status were studied in these experiment. Initial data were obtained on hemoglobin, total protein and albumin. The subjects were divided into 3 groups matched for age, body weight and initial hemoglobin levels. One group was given 5 μg vitamin B_{12} per day, another group was given 5 μg vitamin B_{12} along with 5 μg intrinsic factor. The third group was kept as control. Blood was collected after 3 months of treatment. Details of the experiment are given in Table 13.

MEASUREMENT OF PARAMETER

Height and weight

Erect body length was taken with the subject's heals, 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 on the 'Detecto' weighing machine (Detecto, U.S.A.). The subjects in Raipura wore a towel whose weight was less than 0.25 kg. The weights of the upper class subjects in Baroda were taken in ordinary clothes (shorts and shirts) whose weight was less than 0.50 kg. The weighing machine was checked by calibration with standard weights frequently.

Clinical Status

The nutritional assessment schedule suggested by the ICMR (1948) was used (Appendix I) for the clinical examination which was done in the case of subjects in Raipura by Dr. K. Bagchi, Nutrition Adviser, Government of India who was not aware of the group to which the subject belonged. In the case of the other groups the same criteria were used by the investigator.

Analysis of urine

Urine samples were collected in the morning at 6 O'clock. The subject was asked to void the urine in a brown bottle containing about one gram of oxalic acid.

The bottle was shaken well and the volume of urine measured. Five ml of the same were transferred to tubes containing TCA and kept in ice. The remaining samples were also kept in ice after addition of toluene. They were transported to the laboratory as soon as possible and taken up immediately for the estimations of thiamine and riboflavine. A known amount of the remaining sample was stored in the cold room at 5° after further addition of toluene, and the same was used for the estimation of creatinine, and nitrogen. The methods used are given in Table 14.

Analysis of blood

Blood samples (0.3 to 0.5 ml) were collected in the fasting state from the middle finger of the left hand by the finger-prick method. For estimation of blood hemoglobin 0.01 ml blood collected in a hemoglobin pipette were immediately transferred to a test tube containing 4 ml of 1% ammonia solution. The samples were kept in ice and analysed within 4 hours. For other estimations the blood was collected in clean capillary tubes and the serum separated.

For the estimation of vitamin C, 0.02 ml of serum was immediately transferred to a microtube containing

0.1 ml of 4% TCA. The sample was frozen and the estimation was carried out the next day. For carotene, vitamin E, and vitamin A estimations, 0.06 ml of the serum was transferred to microtubes and frozen. The analyses were carried out within two weeks. For the estimation of total serum protein, albumin and alkaline phosphatase the serum samples were preserved at 4° and the estimations carried out within a week.

The biuret method for serum protein estimation was modified to use with 0.005 ml of the serum. This modified procedure enabled the collection of blood with a finger-prick which was less traumatic to the subjects. The validity of the micromethod used was ascertained by comparisons of the values obtained in the micro and macromethods. Some comparisons are shown below:

Samples no.	Macro method	Micromethod
1	6.00	6.05
2	6.20	6.02
3	6.50	6.50
4	6.15	6.12

During the second series of investigations in Raipura blood collections had to be abandoned as the same threatened to jeopardise the success of the programme but measurements of height, weight and analyses of urine were carried out. In addition radiological examination of the right hand (palm and wrist) was made. In view of the difficulty in making blood collections, the possibility of using salivary amylase as an index of nutritional status was explored. In this connection, serum amylase is found to be lowered in protein deficiency so that we may expect a similar effect on salivary amylase (This suggestion arose out of discussions with Sir David Cuthbertson when he visited the department in 1966). Salivary amylase was estimated by method of Bernfeld (1955).

The subject was given a piece of candy (25 mg) and asked to retain the same in the mouth for 1 minute in order to facilitate salivary secretion. Saliva was collected in clean beakers and transferred to clean tubes. The tubes were kept in ice and samples were assayed within 4 hours. The samples were diluted 100 to 200 times with 0.02 M phosphate buffer, pH 6.9.

0.5 ml of diluted saliva was incubated for 3 minutes at 37°C with 0.5 ml of substrate solution (1 g of soluble starch, in 100 ml of 0.02 M phosphate buffer, pH 6.9, containing 0.0067M NaCl). The reaction was stopped by the addition of 1 ml of dinitrosalicylic acid reagent (1 g of 3,5-dinitrosalicylic acid in 20 ml of 2N NaOH and 50 ml water, and 30 g of sodium potassium tartrate made upto 100 ml with distilled water). The assay tubes were heated for 5 minutes in a boiling water bath, cooled and 10 ml of water added. The optical density of the solutions were read off in a Klett Summerson Colorimeter using 54 filter. No enzyme was added in the case of blank. Maltose was used as standard.