CHAPTER V

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A positive relation between dietary and milk composition with regard to protein and fat was inferred from the cross sectional studies detailed in two preceding chapters. It was argued that if such a relation exists we should obtain favourable effects on milk levels of these constituents with dietary supplementation. Longitudinal studies were therefore carried out on changes in the fat, protein, and essential amino acid composition of milk following dietary supplementation.

Subjects of poor nutritional status were chosen for the investigations as the hypothesized relation was found only within certain ranges of dietary intake.

The supplementation was carried out mainly with regard to fat and protein, but in either case, in order to investigate how the results of such supplementation are affected by improvement of the diet with regard to the other constituent, additional supplements were made in some of the experimental groups. In other words, some of the fat supplementation groups were given additional protein and vice versa. The effects of additional vitamin supplementation were also studied. The chief constituent supplemented was started at small doses and increased to progressively larger doses, whereas additional supplements were given at the same dosage level throughout the experiment. The results obtained were compared with those of isocaloric groups. The effects of such supplementation on the 24 hour yield were also studied.

EXPERIMENTAL

Subjects: The seventy subjects used in this study were all between the first and third month of lactation and belonged to families with a monthly per capita income below Rs.10. Their mean dietary intake at the commencement of the experiment was as follows:

Calories	-	1300
Fat	-	18.3 g (15-20)
Protein	-	21.0 g (18-23)
Carbohydrate	-	270 gm
Ascorbic acid	-	1.5 mg
Thiamine	-	0.25 mg
Riboflavin	-	0.18 mg
Pantothenic acid	-	2.2 mg
Nicotinic acid	-	2.4 mg
Cyanocobalamin	-	0.26 mcg
Biotin	-	0.03 mg
Pyridoxine	-	0.35 mg
Folic acid	-	0.30 mg

<u>Supplementation procedure</u>: In the first part of the experiment fat and protein supplementation were started at initially low levels and increased progressively to levels beyond which dietary increases were found from the previous study (Chapter IV) to have no effect on milk levels. The additional supplements were made at constant levels and again their size was determined so as to raise dietary intake to the minimum level corresponding to ceiling levels in milk on the basis of the previous study. Nine groups of five subjects each were used, four for the different fat supplementation treatments, four for the different protein supplementation treatments, and one, as a control group receiving no supplementation. Each dosage level was maintained for a month before change to the next level was made, according to the data shown in Table 15. Five such levels were used.

Two additional groups receiving fat and protein supplementation were compared with those receiving carbohydrate supplementation in the form of glucose at isocaloric levels. A control group received no supplementation. The supplementation was done at two different levels for each group. Protein was supplemented in the form of skimmed milk powder at 20 and 40 g, and fat in the form of butter at 20 and 35 g. Milk yield in the

Table 15

The design of the experiment

Chief constituent supplemented	Dosage level in g					Group* no.	Additional supplements@
	1st	2nd	3rd (months)	4th	5th		
I Fat (in the form of butter)	5	15	25	35	45	1	None
						2	15 g protein.
						3	Standard dose of vitamins.
						4	15 g protein +standard dose of vitamins.
II Protein (in the form of skimmed milk powder)	10	20	30	35	40	5	None
						6	Standard dose of vitamins.
						7	20 g fat.
						8	Standard dose of vitamins. +20 g fat.
III None (control group)						9	

* Each group consisted of 5 subjects.

@ Protein and fat were supplemented so as to raise daily dietary intake to 40 g and 45 g respectively. Vitamins were supplemented in the form of multivitamin tablets (Dumex company) at 1 tablet a day.

control group was compared with that in the protein and fat supplemented groups.

Procedures for the estimation of yield and for the collection and analysis of milk and diet were the same as described in Chapter II. The analysis of milk was done at the end of each supplementation period prior to the introduction of the next dosage level.

RESULTS

The changes in the fat and protein contents of milk with the progress of fat supplementation are shown in Table 16 and Fig. 5 from which it can be seen that the fat content increases with the dose supplemented to a ceiling level at 35 g per day which corresponds to a total intake of 50-55 g of fat per day when we take into account the initial intake of the subjects. No such increase is found in the control group. The increase in fat content is found to be of the same order in all the four groups supplemented suggesting that the fat content of milk is not influenced by additional supplementation of protein and vitamins. Rather, the additional protein supplementation is found to have a small beneficial effect on ~~protein~~ fat content.

It can be seen that the dietary fat intake of 50-55 g corresponding to ceiling levels in milk agrees with the value of 60 g obtained in the previous chapter.

Table 16

Variation in the fat and protein contents* of milk with increasing fat supplementation

Fat supplement@ per day g	Supplementation groups								Control group	
	Fat (5)		Fat +Protein (5)		Fat +Vitamin (5)		Fat +Protein +Vitamin (5)		(No supplementation) (5) **	
	F	P	F	P	F	P	F	P	F	P +
0	3.75	1.09	3.80	0.95	3.85	1.11	3.75	1.03	3.55	1.08
5	4.10	1.06	4.25	1.08	4.15	0.98	4.10	1.15	3.60	1.06
15	4.35	0.98	4.75	1.13	4.50	0.96	4.65	1.15	3.60	0.98
25	4.61	0.99	4.90	1.11	4.65	0.96	4.95	1.13	3.65	0.98
35	4.80	0.99	5.00	1.08	4.70	0.97	5.00	1.14	3.60	0.96
45	4.71	0.98	5.00	1.09	4.65	0.96	5.05	1.12	3.50	0.97

* Expressed in terms of g/100 ml.
@ The initial fat intake before supplementation in the subjects was 15 to 20 g per day. Each level was maintained for a month.
** The numbers in parentheses indicate the number of subjects in each group.
\+ F denotes fat content of milk and P denotes protein.

Similar results are obtained with regard to protein supplementation as can be seen from Table 17 and Fig. 6. The increase in dietary protein is found to be associated with an increase in milk levels upto a dose of 35 g per day which corresponds to a dietary intake of 53 to 58 g of protein per day, further increases having no effect. Here again additional supplementation with regard to fat and vitamins is found to have no effect on protein levels but the former is seen to be associated with increases in fat content.

As might be expected, increase in the protein content of milk is seen to be associated with a general increase in amino-acid content, the increase being statistically significant with regard to histidine, methionine, and tryptophan. As the increase in the case of these three amino-acids is of the same order in all the four protein supplementation groups, the combined mean values for these amino-acids at different levels of protein supplementation are shown in Table 18. It will be recalled that these were the three amino-acids which showed the most significant relation to dietary intake in the cross-sectional studies detailed in the previous chapter. The significant relation with regard to histidine is interesting in the light of the suggestion that this amino-acid is essential for infants though not for adults (Brock, 1960). The values are found to reach ceiling values at the same time as milk protein.

Table 17

Variation in the protein and fat contents* of milk with increasing protein supplementation

Protein supplement@ per day g	Supplementation groups								Control group	
	Protein (5)		Protein +Vitamin (5)		Protein +Fat (5)		Protein +Vitamin +Fat (5)		(No supplementation) (5) **	
	P	F	P	F	P	F	P	F	P	F +
0	0.92	3.90	0.94	4.01	1.06	3.70	1.03	3.55	1.08	3.55
10	1.06	3.75	1.07	4.00	1.15	4.29	1.10	4.05	1.06	3.60
20	1.16	3.90	1.15	3.95	1.20	4.30	1.22	4.05	0.98	3.60
30	1.27	3.80	1.25	4.05	1.28	4.20	1.29	4.15	0.98	3.65
35	1.33	3.85	1.31	4.00	1.35	4.20	1.34	4.15	0.97	3.60
40	1.34	3.85	1.33	4.00	1.35	4.15	1.36	4.20	0.96	3.50

* Expressed in terms of g/100 ml.
@ The initial protein intake before supplementation in the subjects was 18 to 23 g per day. Each level was maintained for a month.
** The numbers in parentheses indicate the number of subjects in each group.
\+ P denotes protein content of milk and F denotes fat.

Table 18

Histidine, methionine and tryptophan[@] contents of milk with protein supplementation

Protein supplement* per day g	Histidine		Methionine		Tryptophan	
	supplementation (20)	control (5)	supplementation (20)	control (5)	supplementation (20)	control (5) +
0	32	31	16	16	10	9
10	35	29	18	16	12	9
20	37	30	20	16	13	9
30	38	29	21	16	15	9
35	39	30	21	16	15	9
40	39	30	22	16	15	9

@ Expressed in terms of mg/100 ml.
* The initial protein intake before supplementation in the subjects was 18 to 23 g per day.
Each level of supplementation was maintained for a month.
\+ The numbers in parentheses indicate the number of subjects in each group.

It may be argued that the effects of protein and fat supplementation on the respective constituent in milk may be due to an increased calorie intake. However, the results of additional supplementation with regard to fat and protein appear to negate this possibility. Nevertheless, additional confirmation in this regard was sought to be obtained by comparing the effects of protein and fat supplementation with those of carbohydrate supplementation at isocaloric levels. The results are presented in Tables 19 and 20 from which it would again appear that the increases are specific to the constituent supplemented.

Our present finding that carbohydrate intake at isocalorie level has no effect on fat content is at variance with that of Polonovski (1933) who found an increase in fat content with the addition of 100 g of glucose daily to the diet. Although a small increase in milk fat content with glucose supplementation was obtained in our subjects, the increase was not found to be statistically significant and was less than 15% of the increase in the fat supplementation group. However, there may be differences in the initial nutritional status of our subjects and those of Polonovski, particularly the carbohydrate/fat ratio of the diet which may perhaps affect the results obtained.

The results of the dietary supplementations on the 24 hour yield of milk are presented in Table 21 from which it can be seen that neither protein nor fat supplementation has any effect on milk yield at the doses and periods of treatment used. It should be pointed out that the results are based on a moderate dietary improvement with regard to only one constituent, viz., fat or protein. We can not rule out the possibility that beneficial results might have been obtained with an over-all improvement in the diet in the face of the observation that the yields reported for Indian subjects are generally low as compared to those reported by Western investigators.

Table 19

Comparative values for the protein content of milk (g %)

with protein and carbohydrate supplementation

Protein* supplementation group (5)	Isocalorie group receiving glucose (5)	Control group (No supplementation) (5) !
0.99 ±0.06	0.99 ±0.09	0.95 ±0.08
1.26 ±0.10	0.99 ±0.10	0.94 ±0.07
1.31 ±0.12	0.98 ±0.08	0.94 ±0.09

Table 20

Comparative values for the fat content of milk (g %)

with fat and carbohydrate supplementation

Fat* supplementation group (5)	Isocalorie group receiving glucose (5)	Control group (No supplementation) (5) !
3.80 ±0.16	3.75 ±0.17	3.65 ±0.15
4.40 ±0.20	3.90 ±0.16	3.70 ± 0.18
4.75 ±0.21	3.90 ±0.18	3.70 ±0.19

* The supplementation was done at two levels, protein at 20 and 40 g., and fat, at 20 and 35 g/day and brought total intakes to levels of 58 and 55 g per day respectively.

! The numbers in parentheses indicate the number of subjects in each group.

Table 21

Effects of dietary supplementation on 24-hour yield* of milk

Experimental group+	Dosage levels@		
	0	I	II
Fat supplementation group	605 +16	595 +15	590 +15
Protein supplementation group	570 +13	585 +13	595 +15
Control group (No supplementation)	560 +12	550 +12	545 +13

* Expressed in terms of ml.
@ The supplementation was done at two levels, protein at 20 and 40 g, and fat, at 20 and 35 g/day and brought total intakes to levels of 58 and 55 g/day respectively.
\+ The number of subjects was five in each group.

DISCUSSION

Thus the results of these studies confirm the existence of a positive relation between dietary and milk levels of fat and protein within certain ranges of dietary intake. It is of interest to note that the levels of dietary intake corresponding to ceiling levels of milk are in agreement with those inferred from the studies detailed in the previous chapter. It may be pointed out that the initial values for the protein content of milk obtained in this study are somewhat low as compared to the value for the first quartile in the previous chapter. However, it will be noted that the mean dietary intake of protein in this study was initially 18 g as compared to 27 g in the previous study. Further, the subjects in this group were from slum areas and belonged to the lowest rung of the economic ladder and their diet was marked by the paucity of good quality proteins.

It will be seen that the additional supplementation, particularly with regard to protein, is associated with relatively small increases in milk levels. This is not surprising in view of the small dose supplemented and the fact that the subjects had undergone the stresses of pregnancy and lactation on a woefully inadequate diet. Even so, the results were found to be quite consistent in that all the subjects showed some improvement in milk levels. Some typical sets of values are given below :

Group 7		Group 2	
Fat		Protein	
Initial	Final	Initial	Final
3.50	4.00	0.97	1.00
3.75	4.00	0.98	1.22
3.25	4.00	0.87	1.01
4.00	4.50	0.92	1.12
4.00	4.25	1.01	1.11

It would appear from the data of Tables 17 and 18 that ceiling levels of milk with regard to fat and protein are attained at a dietary intake of 50-55 g in either case. In this connection, Gopalan (1961) has suggested that the minimum dietary intake supporting ceiling levels in milk may serve as a possible criterion of maternal requirement. From this point of view, a diet containing 50-55 g each of fat and protein plus 270 g of carbohydrate and yielding 1750 to 1800 calories should constitute the minimum requirement in lactation. It is seen that these levels are far below the recommended allowances for lactating women. However, what is minimal level for the maintenance of lactation may not be the optimal level from the point of view of maternal health. It must further be pointed out that while the values arrived at are low in comparison to recommended allowances, the actual intakes of lactating women in this region would appear to be still lower as can be seen from Table 8 (Chapter IV, page 50).

A comparison is made in Table 22 of the available nutrients in 24-hour milk with reported infant requirements at a body weight of about 5 kilograms. It can be seen that the requirements are not met even at this stage. When it is recalled that the infants were fully breastfed beyond the sixth month when their body weights should have been of the order of 16 lbs according to standard norms, or atleast 13-14 lbs according to the data reported for poor class Indian infants by Gopalan (1958), the degree of inadequacy is seen to be much greater.

It will be seen from a comparison of the availability of essential amino-acids on a percentage basis (Table 23) that the quality of proteins in the milk secreted is poor with regard to leucine, valine, phenylalanine, methionine, tryptophan but shows improvement on supplementation, particularly with regard to methionine and tryptophan. Thus supplementation with good quality protein may have a beneficial effect not only on the content of protein in milk but also on its composition with regard to essential amino-acids.

In conclusion, the trend of the investigations outlined in this and preceding chapters has been to suggest that, within certain ranges of dietary intake, there is a positive relation between dietary and milk constitution of fat, protein, and essential amino-acids. This observation

Table 22

Data* on nutritients in 24-hour milk in comparison with recommended allowances

Constituent		Before Supplementation	After Supplementation@	Recommended allowances at 5 kilogram body weight (Holt Jr., 1960; Evans & MacKeith, 1958)
Fat	g	23	29	
Lactose	g	42	42	70 ?
Protein	g	6.0	8.2	10
Calories		400	460	575
Leucine	mg	438	462	770
Isoleucine	mg	432	456	600
Valine	mg	318	336	525
Histidine	mg	186	222	115
Lysine	mg	432	486	525
Phenylalanine	mg	240	264	450
Threonine	mg	270	288	300
Methionine	mg	96	120	170
Tryptophan	mg	57	84	110

* Estimated on the basis of 600 ml of milk yield.
@ Protein supplementation was carried out for 5 months with progressively increasing doses. The details are given in Table 15.

Table 23

Percentage composition of essential amino-acids in breast milk as compared to recommended norms

Amino-acid	Before Supplementation*	After Supplementation*	on the basis of recommended allowances (Holt Jr., 1960)
Leucine	17.8	17.3	21.8
Isoleucine	17.4	17.1	16.8
Valine	12.9	12.6	14.7
Histidine	7.5	8.3	3.2
Lysine	17.4	17.1	14.7
Phenylalanine	9.7	10.0	12.6
Threonine	10.9	10.8	8.4
Methionine	3.9	4.5	4.7
Tryptophan	2.4	3.1	3.0

* Protein supplementation was carried out for 5 months with progressively increasing doses. The details are given in Table 15.

underscores the possibility that, even when lactation is maintained on an inadequate diet, the quality of the milk secreted may suffer as a result with detriment to the requirements of infant nutrition, and points out the need for dietary improvement during lactation from the standpoint of both maternal and infant welfare.

SUMMARY

Longitudinal studies were carried out in subjects of poor nutritional status on changes in the fat, protein, and essential amino-acid composition and 24-hour yield of milk following dietary supplementation. The supplementation was carried out with regard to fat, and protein, singly and in combination, and with and without added vitamins. The results obtained were compared with those of an isocaloric group supplemented with equivalent amounts of carbohydrate.

The supplementation studies showed that fat and protein contents of milk increase to a ceiling level with the dose supplemented till dietary levels of 50-55 g are reached in regard to either. The increase in protein content was found to be associated with a general increase in essential amino-acids, the same being significant with regard to histidine, methionine, and tryptophan.