VITAMIN B_{12} CONTENT OF MILK AND THE HAEMATOLOGY OF LACTATING MOTHERS

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Haematological status and the vit. B_{12} content of the breast milk of women in the early lactation period has been studied. It is observed that irrespective of the haematological values, the vit. B_{12} in milk is maintained at a fairly steady level. All the vit. B_{12} in the milk is in the bound form. No change in the vit. B_{12} binding capacity of the milk is seen amongst the lactating women exhibiting varying degrees of anaemia. The implications of these findings are discussed.

The early needs of nutrient requirements of the new born are met in mammals by milk specifically elaborated after birth. Much attention has been paid to the chemical composition of milk. Of particular interest is the observation that nutrition status of the mothers' play an important role in maintaining levels of the vitamins in the milk. This is particularly true in the case of vitamins of B group, whereas with fat soluble vitamins such as vitamin A, nutrition of the mother does not seem to play a prominent role (Gopalan and Belavady, 1961).

Early workers have attempted to compare the vitamin B_{12} levels of lactating mothers' milk with those of other species (Gregory, 1954; Gregory and Heldsworth, 1955; Srinivasamurthy et al., 1949, 1953). Attempts to correlate the nutritional status with the vit. B_{12} levels of human milk are meagre. Deodar and Ramakrishnan (1959) have observed that as the socio-economic status of mother rises, the vit. B_{12} content of the breast milk also tends to increase. This might imply that deficient nutrition of the lower class may contribute to the low concentration of this vitamin in milk. But the nutritional intake by itself cannot give a complete picture of vit. B_{12} deficiency.

A slight degree of anaemia during pregnancy is a natural occurrence particularly in the last trimester. The haematological values show considerable variation during this period—a slow fall in values of haemoglobin, red cells and cell volume being recorded (Das Gupta, 1954). After normal delivery, not associated with any complications or post-partum haemmorhages, the same type of qualitative and quantitative changes as seen during later stages of pregnancy continue, till normalcy is reached (Dickman and Wagner, 1934). Whether this type of anaemia during early lactation period reflects the vit. B_{12} content of breast milk is not known.

The importance of vit. B_{12} in every major class of metabolism is fairly well established (Netravali *et al.*, 1961). For infants the vit. B_{12} in the breast milk is the only source of this vitamin.

This aspect of the interrelationship between the haematological status of the mothers and the vit. B_{12} content of their breast milk has now been studied and reported in this communication. The results will also provide the data on the composition of the human milk as it relates to vit. B_{12} of Indian women, as such reports are very few. The study also includes the vit. B_{12} binding capacity and the presence or absence of free vit. B_{12} in the milk.

METHODS

Collection of milk samples :-The milk samples were collected from 15 mothers admitted to Sir Haji Ismail Sait's Gosha Hospital, Bangalore and the Corporation Maternity Hospital, Guttahalli, Bangalore. The samples were obtained in the morning in sterile conical flasks before feeding the babies, by manual expression of the breast by mothers between the 4th and 7th day after delivery.

Collection of Blood :—From the same mothers who had donated milk, blood was collected in sterile citrated tubes. The determinations of RBC, Hb, PCV and the calculation of MCV, MCH and MCHC were according to standard procedures.

Estimation of vit. B_{12} :—Total vit. B_{12} : Microbiological assay technique (turbidimetry) using *L. leichmanii* 313 as test organism was adopted for the determination of vit. B_{12} (Pharmacoepia of India, 1955). The test samples were prepared by extracting the milk sample with acetate buffer pH. 4.6 (0.1 M) and digesting with cyanide activated Papain (BDH). This digestion would release the bound vit. B_{12} and make it available for the test organism. The samples were filtered and suitably diluted before adding to the assay tubes.

Free vit. B_{12} :—The samples were subjected to similar procedure as above, except that enzyme digestion was omitted. Hence the bound Vit. B_{12} was not released for the assay organism.

Determination of binding capacity :—5 ml. of milk samples were mixed with 2.5 ml. of vit. B_{12} containing 0.01 μg/ml. and the mixture was incubated for 2 hours at 37° C. This procedure ensured the addition of vit. B_{12} in excess to the binding capacity of milk as determined by preliminary experiments. After incubation, the samples were treated with acetate buffer which now extracts only free vit. B_{12}. They were filtered, suitably diluted and used for assay procedure.

Recovery of added vit. B_{12} :—A few recovery experiments were carried out by adding known volume of vit. B_{12} solution and assaying for the vit. B_{12} after papain digestion.

RESULTS AND DISCUSSION

Haematology of lactating mothers :—The average values of 15 subjects are presented in Table I. The haemoglobin ranges from 7.5 to 15g. % : the

erythrocyte counts from 2.19 to 3.65 million/cu. mm. and the packed cell volume from 27 to 35%. While considerable data are available on the haematology during normal pregnancy, informations in the haematology during early lactation are very little. Since the haemodilution caused during the pregnancy continues for about 8 weeks after the delivery, the values for different constituents are also considered to be similar to those obtained during the last trimester. Our values of Hb and RBC during early lactation compare well with those observed by Das Gupta and Chatterji (1953) for urban women during the last trimester.

TABLE I

Average Values of Haematological Data, Vitamin B_{12} Content and Vitamin B_{12} Binding Capacity of Milk for the entire series

Particulars		Values Av. ± S.D.
Haemoglobin gm./100 ml.	...	11.17 ± 2.3
Erythrocytes million/cu. mm.	...	3.05 ± 0.39
Packed cell volume %	...	30.5 ± 3.2
Mean corpuscular volume (MCV) cu. μ	...	101.12 ± 2.32
Mean corpuscular haemoglobin (MCH) $\mu\mu$	...	36.38 ± 1.14
Mean corpuscular haemoglobin concentration (MCHC) %	...	36.37 ± 1.21
Vitamin B_{12} in milk. mγ/ml.	...	0.063 ± 0.007
vitamin B_{12} binding capacity of milk mγ/ml.	...	4.27 ± 0.41

A further analysis indicates that out of 15 cases, values of red cells and Hb in 7 persons were found to be higher than 3.0 million/cu. mm. and 11.5 g% respectively, that is, more than the average of entire group. These seven have been grouped separately (A) and the rest with less than the average values are group (B). These individual values are shown in the Table II. These two groups are referred to as slightly anaemic and more anaemic respectively according to the haematological picture.

TABLE II

Average Haematological Values, Vitamin B_{12} Content and Binding Capacity of Milk of Individuals Belonging to Two Different Groups

Particulars	Group A Av. ± S.D.	Group B Av. ± S.D.
Haemoglobin gm./100 ml.	13.2 ± 1.03	9.38 ± 1.52
Erythrocytes milion/cu. mm.	3.36 ± 0.17	2.78 ± 0.31
Packed cell volume %	32.7 ± 1.48	28.5 ± 2.95
Mean corpuscular volume (MCV) cu. μ	97.48 ± 1.87	103.06 ± 4.23
Mean corpuscular haemoglobin (MCH) $\mu\mu$	39.33 ± 1.22	33.82 ± 1.7
Mean corpuscular haemoglobin concentration (MCHC) %	40.48 ± 1.29	32.8 ± 2.55
Vitamin B_{12} in milk mγ/ml.	0.065 ± 0.07	0.062 ± 0.008
Vitamin B_{12} binding capacity mγ/ml.	4.34 ± 0.39	4.2 ± 0.60

A comparison between the haematological values of normal non-pregnant women and the present findings in lactating mothers clearly reveal the latter to be exhibiting anaemia of macrocytic hyperchromic character similar to the type seen in the later stages of pregnancy. Though observed in both groups, this is very prominent in group A. In group B, while the mean corpuscular volume is definitely high, the mean corpuscular haemoglobin concentration is the same as seen in non-pregnant women. The anaemia in these individuals (group B) appears to be of macrocytic normochromic type.

Vit. B_{12} content of milk :—As is evident from the results, vit. B_{12} content of milk does not seem to have direct relationship with haematological status of mothers. The higher and lower values have been observed in both groups A and B and the averages for the two groups, as also for the entire group were almost similar (i.e. about 0.06 mγ/ml). Thus it would appear that irrespective of haematological status of mothers, vit. B_{12} in milk tends to remain the same. This regulatory mechanism of mammary glands in controlling and maintaining a steady level, is indicative of the importance of the vitamin to the developing child. In this connection the observations of early workers that there is preferential transfer of vit. B_{12}

from mothers to the growing fetus is noteworthy (Boger, *et al.*, 1956). The serum vit. B_{12} levels of mothers are lower than those of infants, indicating that the transfer of vit. B_{12} is at the cost of mother. The present observations also indicate and lend support to the view that there is a preferential transfer of vit. B_{12} to the infants through milk, probably by depletion of the reserves of mothers, if the external source is limited.

Incidentally, it may be observed that the vit. B_{12} of milk reported in the present series averaging 0.065 mγ/ml. (ranging from 0.054 - 0.075) is below the values reported by others among western women, 0.2 mγ/ml. (Gregory, 1954; Gregory and Holdsworth, 1955). They are also slightly less than those reported by Deodar and Ramakrishnan among women of Baroda (0.08 - 0.11 mγ/ml.) but seem to be comparable with values of Srinivasa Murthy *et al.* in Bangalore (0.055 - 0.16 mγ/ml.). Since the technique adopted and the nutritional status of mothers differ, it is difficult to have a critical comparative evaluation.

There was no free vit. B_{12} found in the milk. This confirms the early observations of Gregory and Holdsworth (1955).

The binding capacity of the milk samples ranged from 3.31 - 4.91 mγ/ml. averaging at 4.3 mγ/ml. No appreciable difference in the binding capacity is seen between groups A and B. The chemical constituents of milk protein that bind the vit. B_{12} are not well defined. Since the binding capacities do not change very much among the individuals studied, it may be assumed that the concentration of these binding factors in the milk do not vary with the haematological and nutritional status of mothers.

About 15-23% of the added vitamin B_{12} could not be recovered (Table III). The recoveries in the assays of milk reported by Srinivasa Murthy *et al.* were within 15% of added vitamin. Failure to recover quantitatively the added vitamin to serum has also been reported by others (Mulgoankar and Srinivasan, 1958).

TABLE III

Recovery of Added Vitamin B_{12} to Human Milk

Nos.	Added Vitamin B_{12} mγ/ml.	Vitamin B_{12} in milk mγ/ml.	Vitamin B_{12} found mγ/ml.	Recovery mγ/ml.	Percentage Recovery
1	5	0.056	4.18	4.12	82.5
2	5	0.071	3.96	3.89	77.8
3	5	0.075	4.34	4.26	85.1
4	5	0.069	4.16	4.09	81.8

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