CCXXXIV. THE TRANSMISSION OF VITAMIN A FROM PARENTS TO YOUNG IN MAMMALS.

V. THE VITAMIN A AND CAROTENOID CONTENTS OF HUMAN COLOSTRUM AND MILK.

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FEW data have been recorded on the vitamin A content of human colostrum and milk. Kennedy *et al.* [1923] reported that 10 ml. of milk from women on an adequate diet were a sufficient daily dose for the rat. Macy *et al.* [1927] have shown that 2.5–3 ml. daily of pooled milk from wet-nurses in Detroit provided sufficient vitamin A for growth and reproduction in the rat; and further, in 3 women on an abundant diet studied individually, the milk had a similar vitamin A potency, which could not be increased by supplementing the diet with 15 ml. of cod liver oil daily [McCosh *et al.* 1934]. Debré & Busson [1933] prepared from several samples of human milk the unsaponifiable matter containing the vitamin A and carotene and fed this material to rats on a vitamin A-deficient diet. They found that the unsaponifiable matter from 3 ml. of human milk was not a sufficient daily dose to keep the rats alive, but double this amount supported life and promoted slow growth. None of these workers, however, employed a biological test in which the growth-promoting activity of the human milk was compared directly with that of a standard source; and in view of the results of Coward *et al.* [1933] only a test involving direct comparison with a standard can be regarded as yielding quantitative data. Van Eekelen & de Haas [1934] applied the Carr-Price test for vitamin A and the direct colorimetric test for carotene to the colostrum and early milk of 4 women and to the later milk of 3 other women and concluded that "colostrum contains more carotene and vitamin A than milk excreted later on"; also that the later milk contained about as much carotene and vitamin A as cow's milk. They give no indication of the method by which the samples were extracted, which is a technical point of the first importance, and examination of their protocols shows that large variations of vitamin A content of samples taken from the same women on successive days were found, suggesting a lack of uniformity in the method of collecting the samples. The importance of avoiding this is dealt with below. Menken has published [1934, 1] an average figure obtained by him for the vitamin A and carotenoid contents of 49 samples of human milk; but elsewhere [1934, 2] the same figure is stated to be the average of measurements of colostrum.

In an earlier study the writer has shown [Dann, 1933] that the colostrum of the cow may be as much as one hundred times richer in vitamin A than the later milk of the same animal, and that the colostrum is therefore of considerable value in giving the calf at birth a reserve supply of vitamin A. On the hypothesis of the complementary functions of the placenta and colostrum in supplying certain

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materials to the foetus and new born [Needham, 1931], it would be expected that in the human subject with a more highly developed placental barrier than the cow, the colostrum would play a less important part in providing a vitamin A reserve for the infant.

In order to test this prediction quantitative data are required for the vitamin A contents of the colostrum and milk of a series of women; in this paper such a body of data is presented.

EXPERIMENTAL.

The collection of colostrum and milk samples. The samples examined were all collected from patients in the obstetrical wards of Duke Hospital, Durham, North Carolina. The patients included both white and negro women, from both town and country districts, but all alike were poor and subsisted on the poor diet common to the poor classes in the Southern states. No control of their diet before entering the hospital was possible, except that those patients seen in the pre-natal clinic were advised to take cod liver oil, tomato juice, iron and calcium during pregnancy. In the hospital the patients received a varied and liberal diet arranged by the dietist.

The procedure adopted in collecting the samples of colostrum and milk was arranged so as to avoid as far as possible the influence of known disturbing factors on the concentration of the various constituents. Widdows & Lowenfeld [1933] have shown that the fat content of a sample of milk from a given patient is dependent upon the pressure exerted on the areola and nipple during extraction and inversely dependent upon the quantity of milk in the breast at the time the sample is taken, precisely as is found in milking cattle. Although evidence will be presented below to show that the secretion of vitamin A into the milk does not proceed parallel with the secretion of fat, the vitamin A in the milk is undoubtedly dissolved in or linked to the fat, and it is necessary to adopt a uniform method of extracting samples which will control the effect of pressure and of volume of milk in the breast. All samples were therefore extracted by use of the Abt mechanical breast pump, set to give a maximum negative pressure of 8 lb. per square in. An attempt was made to extract the sample of colostrum from each patient as soon after delivery as was possible, and if the flow had not commenced, attempts were made at intervals until the sample was obtained. The time after delivery until the sample of colostrum could be extracted varied from 4 to 110 hours, but the sample was always taken soon after secretion commenced. The samples of later milk were taken shortly before the patient left the hospital, and were all taken from the full breast approximately 5 hours after the infant had suckled it (this period being possible because the breasts were suckled alternately at successive 4-hourly feedings). It was almost always found to be quite easy to obtain 20 ml. of milk, or between 5 and 20 ml. of colostrum in this way.

Examination of the samples. The volume of the sample was determined and it was digested with concentrated KOH solution. The vitamin A and carotenoids were extracted and measured colorimetrically, the whole process being carried out exactly as previously described for cow's milk [Dann, 1933]. The results are expressed in yellow units (Y) of carotenoids and blue units (B) of vitamin A, calculated by the method of Moore [1929]. As the yellow value of pure β -carotene on this scale is about 2000 Y per mg. and as 1 B of vitamin A is approximately equivalent to 0.6 international unit (i.u.) [Moore, 1936], it is possible to estimate the total vitamin A activity of the sample by using the formula $(5/6 \times \text{yellow units of carotenoids} + 3/5 \times \text{blue units of vitamin A}) = \text{i.u. of vitamin A activity}$. The figures so obtained will be maximum values because the yellow colour measured

in the tintometer is not due to pure β -carotene, but to a mixture of carotenes together with some xanthophyll. An accurate evaluation of the vitamin A potency due to the pigments in each sample could only be made by fractionating the mixture and measuring the amounts of each pigment. In the present work this could not be done owing to the small samples available, which were often completely used up in the Carr-Price test for vitamin A after the total pigment had been measured. It has been established that the pigments of the milk fat of cows generally contain about 93 % of carotene [Gillam *et al.* 1933] but human milk fat may contain a smaller proportion of carotene. Palmer [1914], on the basis of a rough inspection of two samples of human milk, suggested that about half of the pigment present was carotene. On the other hand, Van Eekelen & de Haas [1934] also examined two samples (using the Lovibond tintometer) and found that in one the proportion of xanthophyll was too small to determine, whilst in the other it was approximately one-seventh of the total pigment. Three samples examined by the writer yielded a quantity of pigment sufficient for fractionation, and in these the carotene varied from 75 % to 90 % of the total pigment. It was decided therefore that no uniform correction could be applied to the figures obtained for the carotenoid content in order to allow for the xanthophyll present.

Data recorded. For each patient studied the age and race were recorded, and the number of the birth after which the colostrum was obtained. The date and time of birth and of the collection of the samples of colostrum and milk were also recorded, so that the length of time between parturition and the collection of the samples was known. The data obtained cover 111 women from whom colostrum was taken; milk samples were also collected from all except 7 of the group. Of the group studied 42 were white and 69 were coloured (that is, full-blooded negro, mulatto, octoroon etc.).

DISCUSSION.

Before discussing the experimental results collected in Table I it is necessary to define the use of the term colostrum. Some writers [cf. Hawk & Bergeim, 1931] apply this term to the fluid secreted by a mammalian female for a period of 2 or 3 weeks after parturition, but this usage appears to be indefensible either on etymological or physiological grounds. Colostrum is defined by the Oxford English Dictionary as "the first milk secreted after parturition by a mammal". Moreover, it is known that in species such as the cow where the colostrum differs considerably from milk in constitution (e.g. in protein or vitamin A content) a rapid change in the constitution of the mammary secretion occurs during the first 3-6 days following parturition, to be followed from then until the end of the lactation period by very much more gradual changes. In the human subject there is a much smaller difference between the constitution of colostrum and of milk, but in the one case where a comparatively large difference may occur (in the protein content of the colostrum and milk of primiparae only) Lowenfeld *et al.* [1927] found that the rapid change in composition ceased for all patients examined on or before the sixth day after delivery, which was generally 2-4 days after the first secretion of colostrum. It seems therefore that from the physiological standpoint the term colostrum should be restricted either to the first fluid secreted by the mammary glands after parturition or to all the fluid secreted before the rapid change in composition ceases. It is important to note that while the udder of the cow is filled with colostrum which is suckled reflexly by the calf at birth the secretion of colostrum in the human subject does not commence until after parturition and may be further delayed for several days.

Table I.

Mean vitamin A content, carotenoid content and estimated total biological activity of all samples of colostrum and milk examined and of samples from certain groups of subjects.

	Vitamin A content. (B per 100 ml.)	Carotenoid content. (Y per 100 ml.)	Estimated total biological activity. (i.u. per 100 ml.)
Colostrum of 111 women	629	305	632
Colostrum of 42 white women	488	301	544
Colostrum of 69 coloured women	715	307	685
Milk of 104 women	410	120	346
Milk of 38 white women	415	106	337
Milk of 66 coloured women	407	128	350
Colostrum of 39 women treated with cod liver oil during pregnancy	581	—	—
Colostrum of 72 women untreated	658	—	—
Milk of 35 women treated with cod liver oil during pregnancy	340	—	—
Milk of 69 women untreated	442	—	—

Analysis of the experimental observations. The samples of colostrum were obtained as soon as possible after parturition; that is, as soon as sufficient fluid had been secreted to allow of the collection of 5–10 ml. of colostrum. The period between parturition and the collection of the sample varied in different subjects from 4 to 110 hours. 17 samples were collected within 20 hours of parturition, 26 samples between 21 and 40 hours after, 44 samples between 41 and 60 hours after, 17 samples between 61 and 80 hours after, and 7 samples more than 80 hours after parturition. On plotting the individual figures for the vitamin A content or for the carotenoid content it was found that each of these variables was independent of the interval between parturition and the collection of the sample.

The samples of milk were collected from each subject on the day previous to her departure from the hospital, at intervals ranging from 2 to 14 days after the collection of the colostrum. 15 samples were collected within 5 days after, 68 samples from 6 to 10 days after, and 21 samples more than 10 days after the colostrum. On plotting the *ratio* of the concentration of vitamin A in colostrum to the concentration in milk against the interval between the collection of the samples, no correlation was found, showing that the change in concentration of vitamin A had gone to completion before the samples of milk were collected; the same was true for the carotenoids. (It must be remembered that the change here referred to is the first rapid change in concentration concurrent with the replacement of colostrum by milk. Other more gradual changes in the concentration of these constituents may be expected to occur over the whole period of lactation.)

The age of the subjects varied from 14 to 41 years, and they ranged from primiparae to one 11-parous subject. Here again a plot of the data showed that neither of these factors could be correlated with the vitamin A or the carotenoid content of the colostrum.

In Table I are collected mean values for the vitamin A content, carotenoid content and estimated total biological activity of all samples of colostrum and milk examined, and also mean values for samples obtained from certain specified subgroups of the subjects studied. From these figures it appears that there is no significant difference between the vitamin A contents of the colostrum (or milk) of white women and of coloured women; also there is no difference between the carotenoid contents of the milk of the two groups of women, but possibly the higher

vitamin A content of the colostrum of the coloured women may be significant. This must however remain doubtful until many more data are available on account of the great range of variation for individual samples from members of each group.

Since the vitamin A content and carotenoid content of human colostrum and milk both vary over such wide ranges the mean figures given in Table I are not alone sufficient to describe the experimental findings, and some description of the distribution of the values obtained is necessary. This is contained in the histograms seen in the figures. In Fig. 1 is shown the distribution of values for the vitamin A content, carotenoid content and estimated total biological activity

Fig. 1. Distribution of values found for (a) carotenoid content of colostrum, (b) vitamin A content of colostrum, (c) estimated total biological activity of colostrum.

of the 111 samples of colostrum examined. The vitamin A content varied from 0 to 2625 B per 100 ml.; 78 samples contained less than 800 B and 50 of these had between 200 and 599 B per 100 ml. The carotenoid content varied from 20 to 1540 Y; 86 samples contained less than 800 Y, and 60 of these contained between 100 and 299 Y per 100 ml. The estimated total biological activity ranged from 56 to 2172 i.u. per 100 ml.; 80 samples contained between 100 and 799 i.u. per 100 ml. and 62 of these between 100 and 299 i.u. per 100 ml. Fig. 2 gives corresponding data for the 104 samples of milk examined. Here the vitamin A content varied from 0 to 2000 B per 100 ml.; 86 samples contained less than 800 B and 60 of these contained between 100 and 399 B per 100 ml. The carotenoid content varied from 0 to 800 per 100 ml.; 95 samples contained less than 200 Y and 80 of these less than 100 Y per 100 ml. The estimated total biological activity ranged from 0 i.u. to 1860 i.u. per 100 ml.; 62 samples contained between 100 and 299 i.u. per 100 ml.

In Fig. 3 the distribution of values of the ratio of concentration in colostrum to concentration in milk is given for vitamin A, for carotenoids and for total biological activity. The mean values for these ratios are shown in Table II. It will be seen that the carotenoids are characteristically somewhat more specially concentrated in the colostrum as compared with the milk, than is vitamin A.

Comparison of human with cow's colostrum and milk. The data recorded here afford an interesting contrast to similar data previously obtained for the colostrum

Fig. 2. Distribution of values found for (a) carotenoid content of milk, (b) vitamin A content of milk, (c) estimated total biological activity of milk.

Fig. 3. Distribution of ratios found for (a) carotenoid content of colostrum : carotenoid content of milk, (b) vitamin A content of colostrum : vitamin A content of milk, (c) estimated total biological activity of colostrum to that of milk.

and milk of shorthorn cattle [Dann, 1933]. The mean figures for the cow's colostrum were 1253 B of vitamin A and 447 Y of carotene per 100 ml., giving a total estimated biological activity of 1055 I.U. per 100 ml. For the cow's milk the

Table II.

Mean values of the ratio of concentration in colostrum to concentration in milk for vitamin A and carotenoids, and means of the ratio of estimated total biological activities. The means are calculated to two significant figures, and two cases in which the ratio was infinity have been excluded.

Mean value of ratio for:	Vitamin A	Carotenoids	Estimated total biological activity
For all subjects (102 women)	2.4	4.8	2.7
For 37 white women	2.1	5.0	2.6
For 65 coloured women	2.5	4.6	2.7
For 35 women receiving cod liver oil during pregnancy	2.4	—	—
For 67 women untreated	2.4	—	—

corresponding figures were 30 B of vitamin A and 20 Y of carotenoids per 100 ml., giving a biological activity of 35 I.U. per 100 ml. Thus the samples of human colostrum examined in this study contained on the average about half as much vitamin A and three-quarters as much carotenoids as the cow's colostrum: the total estimated biological activity being about three-fifths as great. On the other hand the human milks were much richer than the cow's milks; they contained on the average about fourteen times as much vitamin A and about six times as much carotenoids, and their biological activity was about ten times as great. The mean value of the ratio of total activity of colostrum to that of milk was 35 for the cows examined, whilst it was 2.7 for the human subjects now studied.

Human colostrum as a source of vitamin A for the infant.

An answer can now be given to the question whether the colostrum of the human subject has any special significance as a source of vitamin A for the infant at birth, as the colostrum of the cow has for the calf. It has been shown [Dann, 1933] that the colostrum of the cow contains from 10 to 100 times as much carotenoids and vitamin A as the early milk; also that the total output of carotenoids and vitamin A on the first day following parturition may be as much as 25 times greater than the daily output in the milk 2 weeks later (unpublished observations by the writer). This is due to the favourable combination of the high concentration of vitamin A in the colostrum and the large volume of colostrum secreted (which may be as great as the daily output of milk later on).

Among the 104 women yielding both colostrum and milk samples the ratio of vitamin A activity of the colostrum to that of the milk was in general much less than the same ratio for the cow: the mean figures being 35 for the cow and 2.7 for the human subject. Also, according to Kuttner & Ratner [1923] the total secretion of colostrum by the woman is less than 100 ml., whilst the daily secretion of milk 1 week after delivery is about 500 ml. [Camerer, 1912]. In the human subject then the colostrum does not usually supply the infant with as much vitamin A as a single day's secretion of early milk, and it appears that for the human infant the colostrum has no special significance as a source of vitamin A.

It is of interest to note that colostrum so rich in carotenoids and vitamin A should be secreted by subjects receiving a poor diet during pregnancy; no figures could be obtained for subjects receiving a good diet during pregnancy, but it is hoped to extend the study to such a group in the course of further work now in progress.

SUMMARY.

1. Single samples of colostrum were obtained from 111 women shortly after secretion commenced and single samples of early milk were obtained from 104 of the women several days after the colostrum was taken. The diet of the group was uncontrolled, but poor, during pregnancy and liberal after delivery.

2. Measurements of the vitamin A content of each sample were made by the Carr-Price method, and the carotenoid content was also measured by means of the Lovibond tintometer. On the assumption that the carotenoid pigment was all β -carotene an estimate was made from these measurements of the total biological vitamin A activity in international units.

3. The vitamin A and carotenoid contents of the colostrum of women of this group are of the same order as for cow colostrum, but the vitamin A and carotenoid contents of the early milk of these women while on a liberal diet are much higher than those of cow's milk.

4. The vitamin A and carotenoid contents of the colostrum in this group could not be correlated with (a) age of patient, (b) number of birth after which colostrum was taken or (c) length of time after birth until colostrum was obtained.

5. The vitamin A content of colostrum was not increased in this group by regular ingestion of cod liver oil during pregnancy.

6. The ratio of concentration of vitamin A in human colostrum to concentration in human milk is small relative to the ratio for the cow; in approximately 70% of the cases examined it was less than 3. As the volume of colostrum in the human subject is also small, it appears that human colostrum has no special function to perform in providing a reserve supply of vitamin A for the infant at birth.

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