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

II. THE CAROTENE AND VITAMIN A CONTENT OF COW'S COLOSTRUM.

By WILLIAM JOHN DANN¹.

*From the Nutritional Laboratory, University of Cambridge, and Medical
Research Council.*

(Received October 12th, 1933.)

IN an earlier paper [Dann, 1932, 2] an account was given of experimental work devised to ascertain the channels through which young mammals receive supplies of vitamin A up to the time of weaning. The placenta, the colostrum and the milk are the three available channels; but of their relative importance and the factors controlling the efficiency of each we know almost nothing. The experiments previously described were made upon rats and rabbits, and unfortunately in these species it was not possible to distinguish between the colostrum and the milk. It is therefore necessary to examine the colostrum and milk of other species in order to find their relative importance in supplying vitamin A to the suckling. At the same time it is to be expected that variation will occur between different species, and so it will be necessary to examine the colostrum of a number of species before drawing any general conclusions about the importance of colostrum as a vitamin A carrier. In this paper a preliminary study of cow's colostrum is described.

EXPERIMENTAL.

The experimental animals. The work to be described consists of a series of measurements of the vitamin A and carotene content of the colostrum and milk of individual cows of a herd of dairy shorthorns belonging to Cambridge University Farm. The composition and management of the herd have been described by Mansfield and Garner [1931], and for the present purpose it is only necessary to recapitulate the following details of the feeding of the animals. The herd grazes collectively in summer and in the winter receives hay collectively in covered yards. During the winter roots are given to the animals individually and all the year round concentrated foods are given individually to each animal in proportion to its milk yield. The roots given are marrow kale, sugar beet pulp or mangolds; the concentrates vary according to the market price of the constituents, but a typical concentrate contains crushed beans, flaked maize, crushed oats, ground nut cake, cottonseed cake, bran and steam bone flour. Thus the carotene of the cow's diet (on which it is wholly dependent for carotene and vitamin A in the colostrum and milk) is chiefly supplied by the grass or hay, to a small extent by the roots, and much less still by the concentrates.

In the management of lactation it must be noted that the cows are allowed a dry period of two months before calving, and after birth a calf is allowed to suck

¹ Medical Research Scholar of the Worshipful Company of Grocers.

CAROTENE AND VITAMIN A OF COW'S COLOSTRUM 1999

for four days before it is weaned and the parent milked. Throughout lactation the cows are milked morning and evening and the best milkers in the afternoon

Fig. 1. The vitamin A concentration in colostrum and milk of individual cows. In each diagram the columns refer to successive samples of colostrum and milk obtained at 12-hourly intervals; the left-hand column represents the first sample taken just after calving. The height of the closed columns indicates the amount of vitamin A in B.U. (on the scale given) contained in 10 g. of the sample. Columns not closed at the top indicate that the 12-hourly sample was not assayed, except where the figure 0 is placed above the column to show that no measurable amount of vitamin A was contained in 10 g. of the sample.

also. All samples examined in this work were taken at morning or evening milking time except the first sample from each animal, which was taken within an hour after calving.

Examination of the samples of colostrum and milk. Each sample of colostrum and milk was examined by colorimetric methods for carotene and vitamin A. So far as the writer is aware, no method of separating the vitamin A from milk in a way suitable for subsequent application of the Carr-Price test has been

Fig. 2. The carotene concentration in colostrum and milk of individual cows.
Arrangement of diagrams exactly as in Fig. 1.

described. In extracting the carotene and vitamin A it was however found possible to apply the simple alkali digestion method used for animal tissues, with slight modification. The strength of potash used in digesting tissues (usually about 5 %) is insufficient to digest the proteins of milk in a reasonable time. As it has been shown [Dann, 1932, 1] that even strong alcoholic

potash does not destroy vitamin A over long periods of time, the effect of strong aqueous potash in digesting the milk samples was tried. This has proved quite satisfactory, and tests have shown that no measurable destruction of vitamin A occurs over digestion periods up to twelve hours under the conditions described. The following procedure was used throughout. 10 g. of the sample were weighed into a 150 cc. conical flask and 10 cc. of 66 % KOH (*w/v*) added, after which the flask was placed in an oven at 100° and left for 2–4 hours, when digestion had usually proceeded sufficiently far. The flask was then cooled and its contents poured into a separating funnel together with 10 cc. of alcohol and 30 cc. of ether. On shaking the flask, the fat, carotene and vitamin A present in the digest passed into the ether layer, which was then separated, washed, dried and evaporated in the usual way. The resultant fatty residue was dissolved in chloroform and its carotene content determined by direct measurement of the yellow colour of the solution in a Lovibond tintometer. This procedure estimates xanthophyll and carotene together, but in the light of recent work [*cf.* Gillam *et al.*, 1933] it is clear that the error in carotene determination in butter or milk-fat caused by the presence of the xanthophyll is on the average about 7 %; it is therefore not worth while separating the xanthophyll as the error inherent in the colorimetric method used is greater than that due to the presence of the xanthophyll. The vitamin A content of the chloroform solution was determined by the method of Carr and Price, the depth of blue colour produced with SbCl_3 in the Lovibond tintometer being estimated. In order to make sure that the error due to the presence of xanthophyll was unimportant in carotene estimations on colostrum, the xanthophyll content of three samples of colostrum was determined by the method of Gillam *et al.* The first samples of colostrum obtained from Maud 6th, Cantab Lily and Russet 8th yielded a mixture of pigments in which the xanthophyll accounted for 7.6, 6.9 and 7.2 % respectively of the total colour. The ratio of xanthophyll to carotene therefore appears to be the same in colostrum as in milk.

In this way a large number of samples have been examined and the data are presented graphically in Figs. 1 and 2. In Fig. 1 the vitamin A content of each sample is given in blue units (B.U.) of vitamin A and in Fig. 2 the carotene contents are given in yellow units (Y.U.) of carotene. For definition of these units see Moore [1929].

Biological confirmation of the high vitamin A activity of some samples of colostrum. It is evident from Figs. 1 and 2 that some samples of colostrum were extremely rich in vitamin A and carotene as compared with later milk, and to leave no doubt about the results obtained by colorimetric measurements two of the richest samples were tested biologically. The following method was employed to check the colorimetric estimations. A rich sample was chosen—that yielded by the cow Foggathorpe 12 hours after calving, which had been found to contain 200 B.U. of vitamin A and 40 Y.U. of carotene in 10 g. 400 g. of this sample were weighed out and digested with concentrated potash as described above. The extraction of the fatty residue containing the carotene and vitamin A was then performed, and the residue was saponified by boiling in 25 cc. of 5 % alcoholic potash. The unsaponifiable matter was extracted and dissolved in coconut oil, sufficient of the oil being added to make 50 g. of solution. This stock solution was then examined in order to find what proportion of the colorimetrically determined carotene and vitamin A had been extracted from the colostrum and transferred to the coconut oil solution. 1 g. of the solution was weighed out, saponified and the residue dissolved in chloroform and examined as already described for carotene and vitamin A. It was found that 1 g. of the

solution contained 166 B.U. of vitamin A and 33 Y.U. of carotene, corresponding to a total of 8300 B.U. of vitamin A and 1650 Y.U. of carotene in the whole of the solution. The 400 g. of colostrum initially contained 8000 B.U. of vitamin A and 1600 Y.U. of carotene, figures identical within the limits of error of the determinations with those calculated for the whole of the stock coconut oil solution prepared, so that the colorimetrically determined carotene and vitamin A had been transferred quantitatively to the coconut oil solution.

The day doses of the stock coconut oil solution to be given in the biological test were calculated following the demonstration of Moore [1933] that the growth-promoting activities of carotene and vitamin A are approximately the same, weight for weight. The coconut oil solution contained 166 B.U. vitamin A and 33 Y.U. carotene per g., or $10/3$ B.U. vitamin A and $2/3$ Y.U. carotene per drop. (For dosing, dropping pipettes which delivered $20 \text{ mg.} \pm 1 \text{ mg.}$ of solution in each drop were used.) From the best figures available [cf. Moore and Woolf, 1932; Carr and Jewell, 1933], 1γ vitamin A = 4 B.U. and 1γ carotene = 2 Y.U., so that one drop of the coconut oil solution contained $5/6 \gamma$ vitamin A + $1/3 \gamma$ carotene, roughly equivalent to $7/6 \gamma$ of vitamin A. The minimum dose of carotene or vitamin A required to secure growth throughout a 10-week test under conditions employed in this laboratory was shown by Moore [1933] to be about 0.5γ , therefore the dosing solution (prepared from the stock coconut oil solution weekly) was made by diluting the stock solution to $8/3$ times its own

Fig. 3. Growth curves of animals used in biological test. The rats used were three trios of matched bucks. One drop of dosing solution is equivalent to 60 mg. of the second sample of colostrum obtained from the cow Foggathorpe.

weight with coconut oil, producing a dosing solution containing the equivalent of $7/16 \gamma$ vitamin A per drop. Two levels were tested, 1 drop and 2 drops daily, and the weight curves of the animals appear in Fig. 3. These show that 1 drop of the dosing solution daily is barely sufficient to promote growth throughout

the 10-week period, while 2 drops daily suffice for good growth. The growth-promoting activity of the solution thus tallies closely with that to be expected from the carotene and vitamin A content estimated colorimetrically and confirms the remarkable richness of the colostrum sample. The equivalent of 120 mg. of this colostrum daily promoted rapid growth in the rat. The richness of the sample obtained from the cow Janet 3rd immediately after calving was also confirmed in the same way.

DISCUSSION.

Little previous work has been recorded on the vitamin A content of cow's colostrum. Drummond *et al.* [1921] found that "the concentration of vitamin A in the fat of colostrum is decidedly higher than in the fat of later milk," but their results give no quantitative information about their relative vitamin A contents and no other data appear to have been published.

The results tabulated above give a quantitative statement of the vitamin A and carotene content of a series of samples of colostrum from individual cows and heifers of a well controlled herd. Among the cows examined, the late milk contained generally 2-5 B.U. of vitamin A per 10 g., but many samples of colostrum contained over 100 B.U. (the highest 280 B.U.) of vitamin A per 10 g. The colostrum of every cow and heifer was richer in vitamin A than its later milk; taking a value of 3 B.U. per 10 g. as an average for the vitamin A content of the later milk of cows of this herd, then the richest samples from 4 animals contained 10-20 times, from 7 animals 30-60 times and from 3 animals 60-100 times as much vitamin A as the "standard milk." For every animal except one the colostrum had a maximum vitamin A content immediately after birth or in the next 12 hours, and the vitamin A content fell rapidly for a few days and then more slowly for a further 5-10 days until it reached the value for milk. Similarly the carotene content of early colostrum from each animal was higher than that of later milk and was very variable. The carotene and vitamin A contents of the best sample of colostrum from each animal are given in Table I in terms of the concentrations in the "standard milk."

Table I.

Maximum carotene and vitamin A concentrations of samples of colostrum of each animal in terms of the concentrations in "standard milk" of the herd. (Standard milk contains 3 B.U. vitamin A and 2 V.U. carotene in 10 g.)

Animal	Date of calving	Cow or heifer	Vitamin A concentration of richest sample of colostrum	Carotene concentration of richest sample of colostrum
Maud 6th	Dec. 1	H	70	60
Janet 2nd	Dec. 4	C	50	68
Cantab Lily	Dec. 10	H	93	53
Flora 3rd	Dec. 17	C	33	33
Star 3rd	Dec. 24	C	42	15
Lily	Feb. 6	C	10	4
Star 4th	Feb. 10	C	10	2
Russet 8th	Feb. 17	H	42	12
Constance 2nd	Feb. 27	H	42	20
Cantab Maud	Mar. 7	C	13	4
Janet 3rd	Mar. 12	H	50	7
Daisy 5th	Mar. 21	C	17	10
Foggathorpe	Mar. 25	C	66	20
Maud 4th	May 6	C	42	10
"Standard milk"			1	1

An interesting point in the carotene and vitamin A economy of the cow is revealed in Table I, which shows that although colostrum very rich in vitamin A was produced by animals calving at the end of the winter feeding period, no animal calving later than December 10th gave a colostrum very rich in carotene. Thus although the cow depends for its vitamin A on transforming the carotene of its diet [Moore, 1932], it is able to secrete vitamin A in large amounts in colostrum at a time when its diet is poor in carotene, so that it is evident that the colostrum is enriched with the vitamin at the expense of a large store in the body, presumably in the liver. A further point of interest is that the heifers generally produced richer colostrum than the cows. The average vitamin A content for 10 g. of the colostrum present at birth was: for heifers 157 B.U. (varying from 100 B.U. to 210 B.U.) and for cows 66 B.U. (varying from 10 B.U. to 150 B.U.). This distinction between the colostrum of cows and heifers is presumably due to the heifer having greater reserves of vitamin A because the reserves have not been depleted during previous lactation as with the cows. The figures for carotene content of the colostrum cannot be compared in this way because the season exerts a marked effect, and it happened that the heifers calved earlier in the season than the cows.

The importance of colostrum in supplying a reserve of vitamin A to the newborn calf is seen from the table and figures to be considerable. Kuttner and Ratner [1923] have stated that at calving the mother's udder contains from 2 to 20 lbs. of colostrum which is ingested reflexly by the calf. In view of this, the following calculation may be applied to the shorthorn cows studied. Suppose that a typical cow of the herd studied has 10 lbs. of colostrum available for the calf containing 100 B.U. per 10 g. (10 of the 14 animals gave colostrum richer than this) then the total amount of vitamin A in the colostrum available is approximately 45,000 B.U. In addition, if calving occurs during or soon after the close of the summer feeding period, there may be 45,000 Y.U. of carotene in the colostrum available at birth. Thus the calf at birth is endowed with a large reserve of vitamin A and, according to season, possibly with carotene also: a reserve which it would require weeks to build up if colostrum were no richer in vitamin A than milk.

The relative importance of colostrum in supplying vitamin A to the calf is well illustrated by figures for the cow Star 3rd. This cow gave colostrum containing 125 B.U. vitamin A per 10 g. and assuming the average quantity of 10 lbs. of colostrum, the vitamin A store available for the calf would amount to 55,000 B.U. Now this particular calf was killed by accident during birth, so that the carcass was available for examination. The liver weighed 800 g. and contained 75 B.U. of vitamin A per 100 g., or a total store of 600 B.U. The calf was born with 600 B.U. and 55,000 B.U. of vitamin were immediately available for it: this perhaps may be taken as a measure of the relative importance of the placenta and colostrum in providing the young of this species with a reserve of vitamin A until more figures can be obtained.

Further work on these lines is in progress.

SUMMARY.

1. A convenient method is described for extracting carotene and vitamin A from milk and colostrum samples.
2. This has been applied to colostrum and milk from fourteen cows of a dairy shorthorn herd.
3. The vitamin A concentration in colostrum may be from ten to one hundred times greater than in later milk from the same cow independently of season.

4. The carotene content of colostrum may be as much as 70 times greater than in later milk unless calving occurs during late winter feeding.

5. The colostrum of heifers is generally richer in vitamin A than the colostrum of cows (on the average over twice as rich).

6. The importance of colostrum as compared with the placenta and milk as a source of vitamin A for the young calf is demonstrated. On the first day of life the calf receives supplies of vitamin A greater than the later milk could give in 20-50 days.

My warmest thanks are due to the Director of the Cambridge University Farm (Mr Mansfield) and his staff, without whose continued ungrudging assistance and interest this work could not have been contemplated.

REFERENCES.

Carr and Jewell (1933). *Nature*, **131**, 92.

Dann (1932, 1). *Biochem. J.* **26**, 666.

— (1932, 2). *Biochem. J.* **26**, 1072.

Drummond, Coward and Watson (1921). *Biochem. J.* **15**, 540.

Gillam, Heilbron and Morton (1933). *Biochem. J.* **27**, 878.

Kuttner and Ratner (1923). *Amer. J. Dis. Child.* **25**, 413.

Mansfield and Garner (1931). *Cambs. Milk Recording Society Ann. Rep.* 1930-31, p. 45.

Moore (1929). *Biochem. J.* **23**, 1268.

— (1932). *Biochem. J.* **26**, 1.

— (1933). *Biochem. J.* **27**, 898.

— and Woolf (1932). *Lancet*, ii, 13.