# The Absorption of Vitamin A from Colostrum by Lambs

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The experiments of Dann (1932) and others indicated that in man, rat, cattle and other species, vitamin A under ordinary dietary conditions is not readily transferred from the mother to the foetus. Underwood & Curnow (1943) and Barcroft & Eden (unpublished) have shown that this conclusion also applies to the sheep, the concentrations of vitamin A in the blood and the liver of the foetuses always being much lower than those found in the ewe.

Dann (1933) also found that cow's colostrum usually contains large amounts of vitamin A. Sheep colostrum has been examined by several workers (Underwood & Curnow, 1944; Barron, 1942; Peirce, 1947; Satterfield, Clegg & Holmes, 1944) who have reported concentrations of 200-3600 i.u./100 ml. at parturition. These high values were found to fall during the following few days, until the values for milk were reached which were about one-tenth of the figures given above.

Until recently no thorough investigation had been made of the vitamin A status of newborn lambs. The present work was therefore carried out in order to find the levels of vitamin A present in the lamb shortly after birth, and the effect of the ingestion of colostrum during the first few days of life. The results obtained have been in good general agreement with those of Peirce (1947) in Australia, published since this work was concluded.

#### EXPERIMENTAL

### The estimation of vitamin A

Serum (2 ml.) was extracted according to the method of Yudkin (1941), which consists in precipitating the proteins with alcohol and then extracting with light petroleum. Colostrum was extracted by a modification of the method of Olson, Hegsted & Peterson (1939). The early samples of colostrum were diluted with water so as to reduce their viscosity. Samples of 1-5 ml. were made up to 5 ml. with water in a small graduated separating funnel and were shaken with 0.5 ml. of concentrated ammonium hydroxide and then with 5 ml. of ethanol. For the extraction of fats 7.5 ml. of ether were added, the funnel was shaken for about 30 sec., 2.5 ml. of light petroleum were then added and the funnel was shaken again. The ethereal layer was siphoned off into another separating funnel, and the extraction was repeated three times with the same quantities of ether and light petroleum. The combined extracts were allowed to stand for any particles to settle out and were washed three times with 10, 30 and 30 ml. of water, respectively. The water was drawn off and the extract again allowed to stand for a further 30 min. The extract was dried by filtering through anhydrous sodium

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sulphate, and the solvents were evaporated under reduced pressure. The residue was saponified by adding 0.2 ml. of saturated potassium hydroxide solution and 1 ml. of ethanol and heating on a boiling water-bath for 1 min., 8 ml. of water were then added and the solution was extracted three times with 10 ml. portions of ether. The combined ethereal extracts were washed, dried and evaporated as before, and the residue was made up in chloroform.

The blue colour intensity of the antimony trichloride reaction for the estimation of vitamin A was measured in an absorptiometer, similar to that described by Evelyn (1936), using a single barrier-layer photoelectric cell (Evans Electroselenium Ltd.) with a Wratten no. 29 filter.

Ogal cells (Tintometer Ltd.) of 1 cm. width and 4 ml. volume were used for the solutions. The variable aperture between the lamp and cell was so adjusted that the cell could be used when it contained only 0.5 ml. of liquid.

The antimony trichloride used for the Carr-Price reaction was purchased from British Drug Houses Ltd. ready dissolved in chloroform for vitamin A tests. To every ml. of reagent one drop of acetic anhydride was added so as to prevent clouding which might be caused by traces of moisture.

The galvanometer pointer was adjusted to 80 (full-scale deflexion) with the Ogal cell in position and containing 0.5 ml. of antimony trichloride-acetic anhydride reagent. The chloroform extract (0.1 ml.) was introduced rapidly with quick stirring. When the stirring rod was withdrawn the 'hesitation point' in the galvanometer reading was noted. A duplicate reading was then made and the mean taken.

Vitamin A acetate having the value  $E_{1 \text{ om.}}^{1}$  325 m $\mu$ . = 1480 was used to calibrate vitamin A, the factor for converting the *E* values into i.u./g. being taken as 1600.

### Carotene

As sheep's blood and colostrum do not contain more than traces of carotenoid pigments (Peirce, 1946), they were not estimated in the present work.

## Sampling of blood

The animals were cross-bred Suffolk ewes of 2 years or older. They were kept on pasture, and, in addition, received an adequate ration of hay and roots, because the weather was very cold and the fields were covered with snow during most of the investigation.

Colostrum and blood samples were withdrawn from five ewes and their young immediately after birth and before suckling had started. Unfortunately, the lambs of the remaining four ewes had suckled before the first collections were made, but in no instance was the first sample withdrawn later than 12 hr. after suckling had started. Apart from the withdrawal of specimens, the lambs were allowed to suckle under normal conditions.

#### RESULTS

The results obtained are given in Table 1, and in Fig. 1. As judged by measurements of vitamin A in the serum, the ingestion of colostrum has obviously considerable influence on the vitamin A status of the lamb.

The initial levels of vitamin A in newborn lambs were very low, about 20 i.u./ 100 ml., but within 12 hr. after birth, after colostrum had been given, much higher levels (90 i.u./100 ml.) were observed. In some instances the vitamin A concentration in the blood of the lambs exceeded that of the ewe. This increase, however, was only temporary, and after the 2nd day the levels in the lamb serum fell until the values observed were little different from those at birth. Peirce (1947), whose results appeared since this work was concluded, made similar observations on the change of vitamin A blood levels of lambs, but found that vitamin A settled at a higher level after the peak absorption.

The vitamin A level in the serum of the ewes remained fairly constant during the experimental period. The range of values,



Fig. 1. Concentration of vitamin A in colostrum and blood serum of mother and young. O ----O Colostrum; ●---● ewe's blood; △---△ lamb's blood. Each point represents average values; for individual values see Table 1.

47-112 i.u./100 ml., agrees with that observed by Peirce (1946).

### DISCUSSION

Since specimens of liver could not be collected it was necessary to rely on blood-serum values to show that vitamin A had been absorbed. It is not possible, therefore, to calculate the total amount which was absorbed and stored by the lamb. As far as one can judge from the blood-serum levels, however, it seems that the absorption was rapid and efficient. This contention is supported by the results of Peirce (1947) who found an increase in the liver stores with increasing age.

Although the total volume of colostrum secreted by sheep is small (about 200 ml.), the total vitamin A secreted nevertheless compares favourably with that of other species. Table 2 gives values obtained by various workers for cow, goat, sheep, human and pig colostrum. For the purpose of comparison, when values were not expressed in i.u./100 ml. by other workers, they have been recalculated on this basis. Average values have also been calculated where not given.

Generally the vitamin A concentration in milk was only about one-tenth of that of colostrum.

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	<b></b> .	Vitamin A (i.u./100 ml.)							
Ewe no.	after suckling had started (hr.)		Serum						
		Colostrum	Ewe		Lamb I	Lamb II	Lamb III		
I	0	2070	99		24				
	48	570	112		60				
	96	510							
2	ο	2000	78		23	Not estimated			
	24	780	74		75	Not estimated			
	72	160	89	t	45	Not estimated			
3	0	1340	63		17	21	14		
	24	570	61		168	225	Died		
	48	85	60		34	42	Died		
4	0	1680	50		16	18			
	24	220	47		47	61			
	48	120	48		26	23	_ <del>_</del>		
5	0	2080	57			All three stillbor	n		
6	12	250	67		174	_			
7	12	770	78		71	87			
8	12	1500	100		101	56			
	24	620	67		61	50			
	48		81		34	40	—		
	96	120	109		33	43			
9	12	200	81						
	24	210	64		56	58			
	72	110	64		33	25			
Means	0	1830	69		19				
	12	680	76		90		—		
	24	480	71		85				
	48	260	62		33		—		
	72	130	76		34				

# Table 1. Concentration of vitamin A in colostrum and in blood serum of ewes and lambs

Table 2. Comparison of carotene and vitamin A in the colostrum of different species

Species	Vitamin A (i.u./100.ml.)		Carotene (i.u./100 ml.*)		
	Range	Mean	Range	Mean	Author
Cow	110-1060	1680	70-7600	440	Blakemore, Eden, Kodicek & Moore (unpublished)
	308–3060	1520	300-1480	690	Henry, Houston & Kon (1940)
	0-1860	633	63-1140	423	Luecke, Duncan, Ely, Jones, Greene & Tull (1947)
Ewe	988–1680	1287	Not estimated		Underwood & Curnow (1944)
	1220-3570	2300	Not estimated		Peirce (1947)
	134 <b>0-</b> 2080	1830	Not estimated		Present investigation
Sow	103- 462	247	Not estimated		Braude, Kon & Thompson (1946)
		287	Not estimated	_	Braude, Coates, Henry, Kon, Rowland, Thompson & Walker (1947)
	36- 340	168	15-61	40	Luecke et al. (1947)
	240- 610	472	Not estimated		Thomas, Loosli & William. (1947)
Goat	650-2760	1210	Not estimated	—	Thomas et al. (1947)
Woman	210- 550	400	138-600	386	Lesher, Brody, Williams & Macy (1945)

• Values given in  $\mu g$ . have been multiplied by 1.66 to convert them into i.u.

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#### SUMMARY

1. The vitamin A content of sheep's colostrum was estimated by the antimony trichloride method during the first 4 days after parturition, and simultaneous measurements were made on the blood serum of the ewes and lambs.

2. The colostrum contained 1340-2080 i.u./100 ml. of vitamin A in the specimens collected during the first 12 hr. after parturition. The concentration then fell, till values of 85-510 i.u./100 ml. were reached after 48 hr.

3. The level of vitamin A in the serum of lambs at birth was 15-24 i.u./100 ml. as compared with 47-112 i.u./100 ml. for the ewes. After the ingestion of colostrum the level in the lambs' serum rose to maximum values of 47-225 i.u./100 ml., but some days later subsided towards the original level.

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