2. The separation proved to be satisfactory either for artificial mixtures or biological extracts fortified with vitamin A alcohol or ester.

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Hydrolysis and Esterification of Vitamin A during Absorption

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The absorption of vitamin A by the lymph stream has been demonstrated in several species, including man (Drummond, Bell & Palmer, 1935), dogs (McCoord, Breese & Baum, 1943), rats (Popper & Volk, 1944; Radice & Herraiz, 1947), sheep (Eden & Sellers, 1949), and bovines (Eden & Sellers, 1949; Goodwin & Gregory, 1948). Further studies on the mechanism of vitamin A absorption have been made by Gray, Morgareidge & Cawley (1940) who have shown that vitamin A esters are hydrolysed in the intestinal tract of the rat. In the present work the fate of free and esterified vitamin A during absorption by bovines and sheep has been studied.

EXPERIMENTAL

The procedure adopted was to determine the relative concentration of vitamin A alcohol and ester in the intestinal content, mucosa, lymph and blood after dosing with one or other form of the vitamin. The interval between dosing and slaughter was chosen as 4 hr., as previous experiments (Eden & Sellers, 1949) had shown that, although the rate of absorption of vitamin A was variable, it most commonly reached its maximum then.

The experimental animals were ten adult sheep and eleven calves. All the sheep and seven of the calves were about a year old; four newborn calves

were also studied, to see if absorption was the same in them as in older animals.

Preparation of dose. Vitamin A ester concentrates (from fish-liver oils) in which over 95 % of the vitamin was esterified, were obtained from Distillation Products Inc. Vitamin A alcohol was prepared by saponification of halibut-liver oil, and was compared chromatographically with a crystalline preparation of vitamin A alcohol obtained from Roche Products Ltd. Over 95 % of the vitamin A in the liver oil unsaponifiable fraction was free alcohol. The materials were made up for dosing by emulsifying the vitamin A with reconstituted separated milk and arachis oil in a Waring blender. The animals received about 5000 i.u. of vitamin A/kg. of body weight, and the total volume of the dose was 200–400 ml. according to the size of the animal.

Collection of materials for estimation. Before dosing and at slaughter, samples of jugular blood were collected. At slaughter the intestinal lymph glands were removed, sliced and centrifuged, the supernatant liquid drawn off and its vitamin A content determined. The intestinal contents were obtained from the duodenum and the jejunum. These portions of intestine were cut open and washed repeatedly with 0.9 % saline in order to remove any adhering contents. The samples of mucosa were removed with a scalpel from the connective and muscular tissue.

Chemical methods

Methods of extraction. Plasma (6 ml.), or lymph (1 ml.) were treated according to the method of Yudkin (1941), which consists in precipitating the proteins with ethanol and extracting the vitamin with light petroleum (b.p. 40–60°). This method was slightly modified for the extraction of intestinal contents and mucosa. The intestinal contents (3 ml.) were diluted with an equal volume of distilled water, 3 ml. of ethanol were added, then the mixture was extracted with 12 ml. of light petroleum. After shaking and centrifuging, 10 ml. of light petroleum were withdrawn and the sample re-extracted with another 10 ml. of light petroleum. Samples of intestinal mucosa (4 g.) were weighed into 50 ml. thick-walled glass beakers, cut up finely, and then ground with a little quartz in presence of 4 ml. of ethanol. The

contents of the beaker were transferred to a 25 ml. centrifuge tube and the beaker was rinsed twice with 2 ml. of distilled water. To the centrifuge tube 12 ml. of light petroleum were added and the rest of the procedure was the same as that used for plasma.

Separation of vitamin A alcohol and ester. A chromatographic separation was effected by a modified method of Hoch & Hoch (1946). A small column of Al_2O_3 was used, and the conditions were so adjusted that vitamin A alcohol was adsorbed from a light petroleum solution while the esters passed through unabsorbed. Vitamin A alcohol was then eluted with 20% acetone in light petroleum. For further details of this method see Eden (1950).

Estimation of vitamin A. The intensity of the blue colour in the $SbCl_5$ reaction for the estimation of vitamin A was measured in a single photocell absorptiometer (Eden & Sellers, 1949).

Table 1. *Concentrations of Vitamin A alcohol and ester in the intestinal contents of calves*

Calf no.	Duodenum (D) or small intestine (S)	Vitamin A (i.u./ml.)			Percentage of total vitamin A esterified
		Alcohol fraction	Ester fraction	Total	
		Vitamin A ester (5000 i.u./kg. of body wt.) given 4 hr. before slaughter			
55	D and S	3.3	21.0	24.3	86
56	D and S	5.0	2.39	7.39	32
57	D	0.84	0.94	1.78	53
43*	D	3.9	0.07	3.97	3
43	S	3.1	0.16	3.17	5
46*	D	28.8	21.9	50.70	42
46	S	10.3	64.2	74.50	86
47*	D	5.85	40.0	45.85	88
47	S	14.85	39.3	54.15	72
48*	D	6.08	15.1	21.18	71
48	S	0.67	0.8	1.45	55
Average					54
Vitamin A alcohol (5000 i.u./kg. of body wt.) given 4 hr. before slaughter					
49	D	3.6	0.25	3.85	6
50	D and S	32.2	2.2	34.4	6
Untreated animals					
52	D and S		0.03		
53	D and S		0.03		

* Newborn calves.

Table 2. *Concentrations of vitamin A alcohol and ester in the intestinal contents of sheep*

Sheep no.	Duodenum (D) or small intestine (S)	Vitamin A (i.u./ml.)			Percentage of total vitamin A esterified
		Alcohol fraction	Ester fraction	Total	
		Vitamin A ester (5000 i.u./kg. of body wt.) given 4 hr. before slaughter			
14	D and S	1.87	0.3	1.90	15
15	D and S	1.8	0.1	1.9	5
20	D	9.0	11.0	20.0	55
20	S	12.0	13.0	25.0	52
21	D	21.0	7.0	28.0	25
21	S	20.0	5.0	25.0	20
22	D	8.9	51.7	60.6	85
22	S	3.37	6.2	9.57	64
23	D	74.0	66.0	140.0	47
23	S	30.0	74.6	104.6	71
30	D and S	8.1	3.6	11.7	31
Average					43

RESULTS

Since it was impracticable in the present experiments to collect and examine the whole intestines and their contents, samples were taken from representative portions of the small intestine. Previous work showed this to be the site of the maximum absorption of vitamin A. The amount of food present in the intestinal tract differed from animal to animal, and the concentrations of vitamin A found in different animals varied. In two undosed animals, the amounts

of vitamin A in the intestinal contents, wall and lymph were found to be negligible. Tables 1, 3 and 5 show the results obtained for calves, and Tables 2, 4 and 6 those for sheep.

Intestinal contents. Nearly complete hydrolysis was found to have occurred in the intestines of animals nos. 14, 15 and 43 4 hr. after administration of vitamin A esters, whereas in others the hydrolysis was only partial (Tables 1 and 2). When vitamin A alcohol was administered, the dose remained in the intestine in the free unesterified form (Table 1).

Table 3. *Concentrations of vitamin A alcohol and ester in the intestinal mucosa of calves*

Calf no.	Duodenum (D) or small intestine (S)	Vitamin A (i.u./g.)			Percentage of total vitamin A esterified
		Alcohol fraction	Ester fraction	Total	
Vitamin A ester (5000 i.u./kg. body wt.) given 4 hr. before slaughter					
55	D and S	7.9	12.6	20.5	61
56	D and S	1.1	3.9	5.0	78
43*	D	1.11	3.6	4.71	76
43	S	1.11	5.8	6.91	84
46*	D	2.12	18.0	20.12	89
46	S	2.7	6.0	8.7	69
47*	D	0.7	2.0	2.7	74
47	S	2.6	2.8	5.4	52
48*	D	1.2	5.5	6.7	82
48	S	0.6	1.3	1.9	68
Average					73
Vitamin A alcohol (5000 i.u./kg. body wt.) given 4 hr. before slaughter					
49	D	1.06	3.3	4.36	76
50	D	14.7	103.0	117.7	87
50	S	17.8	83.0	100.8	82
Average					82
Undosed animals					
52	D and S		0.12		
53	D and S		0.20		

* Newborn calves.

Table 4. *Concentrations of vitamin A alcohol and ester in the intestinal mucosa of sheep*

Sheep no.	Duodenum (D) or small intestine (S)	Vitamin A (i.u./g.)			Percentage of total vitamin A esterified
		Alcohol fraction	Ester fraction	Total	
	Vitamin A ester (5000 i.u./kg. body wt.) given 4 hr. before slaughter				
14	D and S	0.56	0.86	1.46	60
15	D and S	0.32	0.56	0.88	64
20	D	3.64	7.26	10.9	67
20	S	1.84	1.96	3.80	52
21	D	5.9	1.71	7.61	22
21	S	6.3	2.96	9.26	32
22	D	3.57	4.60	8.17	56
22	S	1.41	4.83	6.24	77
23	D	3.29	3.79	7.08	53
23	S	8.5	38.6	47.1	82
30	D and S	0.6	0.51	1.11	46
				Average	56
	Vitamin A alcohol (5000 i.u./kg. body wt.) given 4 hr. before slaughter				
25	D	1.8	4.56	6.36	72
26	D	0.66	4.7	5.36	88
26	S	1.5	6.8	8.3	82
27	D	2.25	4.48	6.73	67
				Average	77

Intestinal wall. In general, after administration of vitamin A esters, most of the vitamin in the mucosa existed as ester, the percentage of the total vitamin A varying from 52 to 89 %, and from 22 to 82 %, in calves (Table 3) and in sheep (Table 4) respectively. When vitamin A alcohol was fed similar results were obtained, thus the percentage of the esterified form ranged from 76 to 87 % for calves (Table 3) and from 67 to 88 % for sheep (Table 4) respectively, showing that esterification had taken place.

Lymph and blood. After administration of either form of vitamin A, the lymph content was mostly (76–98 %) in the esterified form (Tables 5 and 6). In plasma from adult sheep, the average concentration of vitamin A alcohol before dosing was 98 i.u./100 ml., but only about 5 i.u./100 ml. were esterified (Table 7). After dosing, the rise was mainly in the

ester fraction, but was only temporary. On the other hand, in newborn calves which usually have a low vitamin A alcohol level, a rise occurred after dosing in the vitamin A alcohol fraction as well (Table 7). The concentration of vitamin A alcohol in this instance increased from 20 to 55 i.u./100 ml. Preliminary experiments suggest that the increase was only temporary, as 24 hr. later it fell almost to the original levels.

DISCUSSION

Hydrolysis of vitamin A esters

Gray *et al.* (1940) found that vitamin A esters were hydrolysed in the intestines of adult rats. In the present work a similar observation has been made on ruminants. It has been suggested by Parrish, Wise & Hughes (1948) that the mechanism for the ab-

Table 5. *Concentrations of vitamin A alcohol and ester in the intestinal lymph of calves*

Calf no.	Vitamin A (i.u./ml.)			Percentage of total vitamin A esterified
	Alcohol fraction	Ester fraction	Total	
Vitamin A ester (5000 i.u./kg. of body wt.) given 4 hr. before slaughter				
55	7.0	26.0	33.0	78
56	1.2	3.8	5.0	76
43*	4.14	15.4	19.54	79
46*	1.60	24.4	26.0	94
47*	0.90	8.0	8.9	90
48*	1.10	14.3	15.4	93
			Average	85
Vitamin A alcohol (5000 i.u./kg. of body wt.) given 4 hr. before slaughter				
49	0.87	5.98	6.85	87
50	5.30	72.7	78.0	93
			Average	90
Undosed animals				
52		0.18		
53		0.02		

* Newborn calves.

Table 6. *Concentrations of vitamin A alcohol and ester in the intestinal lymph of sheep*

Sheep no.	Vitamin A (i.u./ml.)			Percentage of total vitamin A esterified
	Alcohol fraction	Ester fraction	Total	
Vitamin A ester (5000 i.u./kg. of body wt.) given 4 hr. before slaughter				
14	1.82	8.35	10.17	82
20	2.23	116.0	118.23	98
21	2.79	19.3	22.09	87
22	4.56	44.0	48.56	90
23	8.60	98.0	106.60	91
30	1.09	4.34	5.43	80
			Average	88
Vitamin A alcohol (5000 i.u./kg. of body wt.) given 4 hr. before slaughter				
25	2.9	38.1	41.0	93
26	1.68	14.9	16.58	40
27	1.42	39.8	41.22	97
			Average	93

Table 7. *Vitamin A alcohol and ester content in plasma before and after dosing*

(Dose administered: 5000 i.u./kg. of body wt.)

Animal no.	Vitamin A in plasma (i.u./100 ml.)					
	Ester fraction			Alcohol fraction		
	Before dosing	4 hr. after	Rise	Before dosing	4 hr. after	Rise
Adult animals						
22	5	44	39	96	95	-1
23	5	49	44	132	139	7
27	7	48	41	120	127	7
1	2	109	107	45	42	-3
Average	5	63	58	98	101	3
Young animals						
43	8	117	109	26	85	59
46	2	163	161	21	61	40
47	5	28	23	13	24	8
48	2	56	54	13	40	27
49	1	70	69	24	60	36
51	5	145	140	25	62	37
Average	4	97	93	20	55	35

sorption of vitamin A in newborn calves might differ from that occurring in adult animals. Recalling the claim of Howe (1924), that calves during the first few days of life may absorb unhydrolysed proteins, Parrish *et al.* (1948) discussed the possibility that the vitamin A esters of colostrum might also be absorbed without hydrolysis. In the present work, however, the absorption of vitamin A when mixed with 'reconstituted' milk (p. 261) was found to be essentially the same in newborn calves as in older animals (Tables 1, 3 and 5). Hydrolysis of vitamin A esters derived from colostrum was also observed to occur in the intestinal tract.

The possibility of the absorption of vitamin A esters

Although we have confirmed that considerable hydrolysis of vitamin A esters occurs in the intestinal tract, it does not follow that all the vitamin A esters have to be hydrolysed prior to absorption. Frazer (1946) has shown that complete hydrolysis of glycerol esters prior to absorption is not essential, and that a part of the ingested fat is absorbed as finely emulsified particles. If, in the present experiment, any vitamin A esters were absorbed, the percentage of esterified vitamin A in the intestinal mucosa should have been larger after administration of esters than after administration of vitamin A alcohol. This difference was not observed (Tables 3 and 4), and, so far, other workers have likewise been unable to obtain any evidence that esterified vitamin A is absorbed in its unaltered form.

Re-esterification of vitamin A

As most of the vitamin in the intestinal mucosa, after the feeding of vitamin A alcohol, was in the ester form, esterification must have taken place.

Glover & Morton (1948) have suggested that vitamin A alcohol in the intestinal wall is esterified by the lipase concerned in the hydrolysis and resynthesis of fats, some of the fatty acids already present in the gut wall being utilized for this purpose. This theory is supported by the work of Gray, Hickmann & Brown (1940), who found that, when vitamin A esters containing unnatural fatty acids such as caproic acid were administered, the vitamin A in the liver was re-esterified with the fatty acids of natural glycerides. Thus the absence of vitamin A caproate in the liver gave further indirect evidence that vitamin A esters are hydrolysed before absorption.

The different roles of vitamin A alcohol and ester in the body

The results of the present work confirm our preliminary findings (Eden & Sellers, 1948) that, after the ingestion of vitamin A, most of it is found in the intestinal lymph in the esterified form. Goodwin & Gregory (1948) have also reported that under similar conditions 75% of the total vitamin A in the lymph was esterified. Thus it seems that after the re-esterification of vitamin A in the intestinal mucosa, the esters pass into the lymph, where they are probably incorporated into the chylomicrons and enter the general circulation as part of these fat particles. The hypothesis that the vitamin reaches the blood in esterified form is supported by the present experiments and by previous work on other species (Chevalier & Choron, 1938; Clausen, McCoord, Ryden & Breese, 1942; Hoch & Hoch, 1946). Thus, before dosing, the vitamin A in the blood is found mainly in the form of the alcohol, whereas after absorption, all the surplus of vitamin A in the blood is found in the form of the ester. This excess vitamin

A is removed generally within 24 hr. leaving the vitamin A content of the blood at the same level as before dosing.

In the present experiments, it was not possible to raise the level of vitamin A alcohol in the blood of adult animals by giving large doses of the vitamin. This suggests that each animal has a characteristic blood threshold of vitamin A alcohol. When the vitamin A content of the blood is at this level all the excess incoming vitamin A would remain esterified, whereas if the vitamin A content of the blood were below the threshold, some of the esters would be hydrolysed to raise the vitamin A alcohol content to the characteristic level. To test this hypothesis, we have carried out some preliminary experiments on the changes found in the blood of young calves after administering the vitamin. In calves the vitamin A content of the blood is usually much lower than in adults. A dose of the vitamin resulted in a temporary rise in the vitamin A alcohol of the blood, although some of the excess vitamin A was in the ester form. Such a rise of vitamin A alcohol following dosage has also been obtained by Thompson, Braude, Cowie, Ganguly & Kon (1949).

These studies on the relative distribution of the free (alcohol) and esterified form of vitamin A in the body show that, although the circulating vitamin A is mainly in the free form, the excess vitamin A in the body is esterified. As noted above, this is the form in which the excess vitamin A is present in the lymph and blood after absorption, and it is also the form in which it is stored in the liver (Gray, Hickmann & Brown, 1940; Gray & Cawley, 1942; Glover, Goodwin & Morton, 1947). All these tissues and body fluids, however, contain a small amount of free vitamin A, and the proportion increases as the total amount of vitamin A in the tissues decreases (Glover *et al.* 1947). Vitamin A alcohol may not be

the form in which the vitamin exerts its physiological function in the body, but probably is a step nearer to this functional form than vitamin A esters, or the provitamins, such as carotene.

Note added 19 November 1949

Thompson, Ganguly & Kon (1949) have recently reported similar results on the absorption of vitamin A by rats and pigs.

SUMMARY

1. Calves and sheep were dosed with vitamin A alcohol or ester (5000 i.u./kg. of body weight) and slaughtered 4 hr. after dosing. The concentrations of vitamin A alcohol and of ester were determined in the blood before and after dosing, and, on slaughter, in the intestinal contents and wall and in the lymph.

2. Four hours after the administration of vitamin A esters, almost complete hydrolysis had occurred in the intestinal lumen of some animals, whereas in others hydrolysis was only partial.

3. Determinations made on the mucosa after dosing with vitamin A ester showed that the ester fraction contained 73 and 56 % of the total vitamin A in calves and sheep respectively. The values obtained after dosing with vitamin A alcohol were 82 and 77 % for the two species, showing that esterification had taken place.

4. After passing across the intestinal wall the absorbed vitamin A apparently remained esterified, as the rise in the vitamin content of the intestinal lymph following administration of either form was nearly all in the ester fraction. In the normal adult blood also the increase after administration of vitamin A was mainly in the ester form.

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