2. Inhibition of true cholinesterase in rats brought about by the injection of Nu-1250 elicits symptoms indicative of acetylcholine accumulation, in spite of the undiminished activity of pseudo-cholinesterase. Thus, pseudo-cholinesterase is not essential to the hydrolysis of acetylcholine *in vivo*, as previous experiments have shown, nor is it capable of assuming even an auxiliary role in this process when the activity of the true cholinesterase is impaired.

We are indebted to Dr J. A. Aeschlimann and Hoffmann-La Roche Inc., Nutley, N.J. for making available to us the Nu-1250 used in these experiments.

REFERENCES

Adams, D. H. & Thompson, R. H. S. (1948). *Biochem. J.* **42**, 170.
Aeschlimann, J. A. & Stempel, A. (1946). *Jubilee Volume Emil Barrell*, p. 306. Basle, Switzerland: Hoffmann-La Roche.
Gordon, J. J. (1948). *Nature, Lond.*, **162**, 146.
Gunter, J. M. & Mendel, B. (1945). *Canad. Chem. Proc. Ind.* **29**, 136.
Harris, M. M. & Harris, R. (1944). *Proc. Soc. Exp. Biol., N.Y.*, **56**, 223.
Hawkins, R. D. & Gunter, J. M. (1946). *Biochem. J.* **40**, 192.
Hawkins, R. D. & Mendel, B. (1946). *J. cell. comp. Physiol.* **27**, 69.
Hawkins, R. D. & Mendel, B. (1947). *Brit. J. Pharmacol.* **2**, 173.
Mendel, B., Mundell, D. B. & Rudney, H. (1943). *Biochem. J.* **37**, 473.
Mendel, B. & Rudney, H. (1943*a*). *Biochem. J.* **37**, 59.
Mendel, B. & Rudney, H. (1943*b*). *Science*, **98**, 201.
Mendel, B. & Rudney, H. (1944). *Science*, **100**, 499.
Sawyer, C. H. & Everett, J. W. (1946). *Endocrinology*, **39**, 307.
Warburg, O. (1925). *Biochem. Z.* **164**, 481.
Zeller, E. A. (1942). *Helv. chim. Acta*, **25**, 1099.
Zeller, E. A. & Bissegger, A. (1943). *Helv. chim. Acta*, **26**, 1619.

The Absorption of Vitamin A in Ruminants and Rats

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(*Received* 20 *August* 1948)

Drummond, Bell & Palmer (1935) and McCoord, Breese & Baum (1943) demonstrated an increased concentration of vitamin A in the lymph collected from the thoracic duct, after oral administration of the vitamin. Popper & Volk (1944) observed a fluorescence typical of vitamin A in the lacteals of the rat following dosage. Radice & Herraiz (1947) confirmed the results of Popper and claimed that they had observed a similar fluorescence in portal blood. These findings suggested that vitamin A may be absorbed by two different routes, as has been described for fats by Frazer (1946).

In the present study, both the portal blood and the lymph were examined as possible pathways of absorption, in order to ascertain the relative importance of the two routes. The experiments were performed on three species, oxen, sheep and rats, by dosing them with vitamin A and estimating the vitamin in systemic and portal blood, and in lymph glands from various regions of the body. The samples were collected as soon as possible after slaughter of the animals.

EXPERIMENTAL

Treatment of animals

The animals were given vitamin A (5000 i.u./kg. body wt.) in the form of halibut liver oil, by mouth. Doses were prepared for bullocks and sheep by emulsifying the halibut liver oil with reconstituted separated milk by means of a Waring Blendor. Rats, fasted for 12 hr., were dosed from a precision pipettè with undiluted oil.

Bullocks. Fourteen Ayrshire and two Friesian bullocks weighing 150–250 kg. each were dosed at different times, ranging from 2 to 24 hr., before slaughter. The total bulk of the dose was 750 ml. Before dosing, samples of jugular blood were taken into oxalate, and after dosing, samples of jugular and portal blood were collected, usually within 2 and not exceeding 5 min. after slaughter. All blood

samples were oxalated to prevent clotting by mixing the blood at collection with a 10% oxalate solution to give a final dilution of 0·1%. Lymph glands from various intestinal regions and other parts of the body were removed, sliced and allowed to drain; vitamin A estimations were performed within 24 hr. on the lymph thus obtained.

Sheep. Nineteen adult sheep, weighing 60–90 kg. each, were used; the dose of halibut liver oil was made up in a bulk of 400 ml. and given 4–7 hr. before slaughter. The procedure closely followed that for the bullocks, but no attempt was made to separate the mesenteric lymph glands according to their connexions with the various parts of the intestine.

Rats. It was difficult to obtain from single rats enough material for accurate estimations, and therefore the experiments were performed by pooling the material from five rats. Albino and piebald rats weighing 200–250 g. each were used and a group was killed at times varying from 1 to 8 hr. after dosing. Systemic and portal blood (0·5 ml.) was collected from each rat and the five samples pooled. It was impossible to collect the fluid from the lymph glands, and hence vitamin A estimations were performed on the whole of the mesenteric lymph tissue.

Chemical methods

Methods of extraction. Plasma and lymph were treated according to the method of Yudkin (1941) which consists in precipitating the proteins with ethanol and extracting the vitamin with light petroleum. For estimations 2 ml. of plasma or lymph were generally taken, although it was not always possible to obtain this amount from the lymph glands of the body. The method described by Glover, Goodwin & Morton (1947) for liver tissue was used for lymph tissues. They were ground with sand, dehydrated with anhydrous Na_2SO_4 and extracted with hot ether.

Estimations of carotene and vitamin A. Colour intensities were measured in a single photocell absorptiometer, similar to that described by Evelyn (1939). Small Ogal cells (Tintometer Ltd.) of 1 cm. depth and 1·7 ml. capacity were used for the solutions. Carotene, when present, was estimated by measuring the yellow colour of the light petroleum extracts of the samples, using Wratten filter no. 47. Crystalline β-carotene was used for calibration. The method of estimating vitamin A was similar to that described by Eden (1948), except for the following modifications. After adjusting the galvanometer to full-scale deflexion with chloroform as the blank, the cell containing 0·1 ml. of the extract was reinserted into the absorptiometer. As the colour of the $SbCl_3$ reaction mixture fades rapidly, it was necessary to obtain a quick reading. Hence 0·4 ml. of $SbCl_3$ reagent was blown into the cell so as to ensure rapid mixing and the reading was taken within 10 sec. A concentrate of vitamin A ester (Distillation Products, Inc.) containing 700,000 U.S.P. units/g. was used for the standard reference curve. One U.S.P. unit was regarded as equivalent to one i.u.

Correction for carotenoids. In the estimation of vitamin A in bullocks' plasma, which contains sufficient carotenoids to interfere with the Carr-Price $SbCl_3$ colour reaction, a correction was made by deducting one quarter of the carotene values expressed as i.u. from the original total vitamin A figures. Other materials examined contained only traces of carotenoids, and hence no correction was applied.

RESULTS

The results for bullocks, sheep and rats are presented in Tables 1, 2 and 3 respectively.

Blood. After dosing, the vitamin A concentration of both the systemic and portal blood rose in all three species approximately 80% above the levels before dosing. In general, the vitamin A values of systemic blood were higher than those of portal blood. The actual figures for portal and systemic blood after dosing were 147 and 162 for bullocks, 187 and 220 for sheep and 224 and 234 i.u./100 ml. for rats, respectively.

Lymph. The vitamin A content of the lymph draining the small intestine of the dosed animals was on the average about ten times that of the undosed animals. There was a rise from 225 to 1500 i.u./100 ml. in bullocks, from 100 to 4830 i.u./100 ml. in sheep and from 0·4 to 3·2 i.u./rat. On the other hand, the differences between the body lymph of dosed and undosed animals were within the experimental error of the method, which was greatly increased by the small amounts available for estimations.

An attempt was made to find out which part of the intestine was mainly responsible for the absorption of the vitamin A, by analyzing lymph from various parts of the gut. It was found that with one exception lymph obtained from the glands draining the duodenum had a higher vitamin A content than the lymph from jejunum or ileum (Table 1). On the other hand, the lymph draining the large intestine showed no rise above that of the body lymph (169 and 175 i.u. of vitamin A/100 ml. respectively) indicating that no marked absorption of the vitamin had occurred from this region of the gut; similarly, no absorption could be observed from the stomach.

DISCUSSION

Our experiments have shown that in ruminants and rats vitamin A is absorbed through the intestinal lymph. This agrees with results obtained on other animals by Drummond *et al.* (1935), McCoord *et al.* (1943), Popper & Volk (1944) and Radice & Herraiz (1947).

From our experiments it seems that most of the absorption occurred from the upper part of the intestine (Table 1). Popper & Volk (1944) also contend that the upper two thirds of the intestine is the most effective region of absorption.

We have been unable to confirm the work of Radice & Herraiz (1947) on portal absorption. After dosing, the average figures in our experiments for portal blood showed no marked difference from those for systemic samples. It is possible that the period of absorption through the portal blood may

Table 1. *Concentration of vitamin A in plasma and lymph of bullocks after administration of 5000 i.u./kg. of body weight*

Animal no.	Time killed (hr.) after dose	Vitamin A (i.u./100 ml.) Plasma: Before dosing	Plasma: At slaughter: Systemic	Plasma: At slaughter: Portal	Lymph (at slaughter): Non-intestinal	Lymph (at slaughter): Intestinal: Duodenum	Jejunum	Ileum
			Dosed animals					
1	2	64	146	143	67	1130	305	—
2	5	84	212	225	245	1250	2870	1430
3	8	91	204	209	115	2900	1600	405
4	12	81	145	140	237	2030	1330	300
5	12	59	100	101	—	581	305	150
6	16	69	139	79	144	1480	658	215
7	16	85	166	100	95	1270	1020	170
8	24	66	145	115	—	2550	1660	—
9	24	57	170	176	132	202	192	—
10	24	61	189	183	185	1680	560	363
	Average	**72**	**162**	**147**	**175**	**1500**	**1030**	**460**
			Undosed animals					
11	0	65	65	72	146	178	178	146
12	0	108	100	109	114	320	190	115
	Average	**87**	**83**	**90**	**130**	**225**	**185**	**130**

Table 2. *Concentration of vitamin A in plasma and lymph of sheep after administration of 5000 i.u./kg. of body weight*

Animal no.	Time killed (hr.) after dose	Vitamin A (i.u./100 ml.) Plasma: Before dosing	Plasma: At slaughter: Systemic	Plasma: At slaughter: Portal	Lymph (at slaughter): Non-intestinal	Lymph (at slaughter): Intestinal
			Dosed animals			
1	4	138	344	170	104	1,020
2	4	112	410	355	75	9,640
3	4	103	328	156	21	4,140
4	4	138	159	188	15	8,880
5	4	172	182	175	—	14,000
6	4	170	143	120	—	2,800
7	5	108	149	140	39	610
8	5	128	267	172	9	1,300
9	5	82	197	118	95	3,500
10	5	107	182	160	57	7,500
11	5	107	215	170		
12	7	86	126	350	49	1,020
13	7	122	182	143		
	Average	**121**	**220**	**187**	**52**	**4,830**
			Undosed animals			
14	0	170	143	120	—	314
15	0	86	71	52		
16	0	86	74	47	22	47
17	0	77	84	54		
18	0	107	116	48	55	71
19	0	107	77	57		
	Average	**105**	**94**	**63**	**35**	**100**

Table 3. *Concentration of vitamin A in plasma and lymph of rats after administration of* 5000 *i.u./kg. of body weight*

Group no.	Time killed (hr.) after dose	Vitamin A: i.u./100 ml. plasma: Systemic	Vitamin A: i.u./100 ml. plasma: Portal	Vitamin A: i.u./rat lymph tissue (intestinal)
		Dosed animals		
1	2	156	250	—
2	2	240	255	3·0
3	2	215	162	3·0
4	2	638	470	3·7
5	3	243	220	2·3
6	5	145	147	—
7	5	133	114	3·7
8	5	225	245	2·5
9	8	113	153	3·3
	Average	**234**	**224**	**3·2**
		Undosed animals		
10	0	104	96	—
11	0	104	117	0·4
12	0	104	104	0·4
	Average	**104**	**105**	**0·4**

be short, and that in these experiments any rise was missed. This is, however, considered unlikely as various periods between 1 and 24 hr. were allowed for absorption. Another possibility is that the absorption rate of vitamin A through the portal blood is low, causing a rise too small to be detected by the present methods of estimation. As the portal circulation during digestion is rapid, the total amounts of vitamin A thus absorbed may be quite considerable. The difference between the results obtained by Radice & Herraiz (1947) and those in this experiment may also be influenced by the fact that they gave a dose forty times larger.

A possible criticism of the present investigation is that the amounts of vitamin A administered were considerably larger than those normally taken in food. However, in experiments not yet published, we obtained similar results when newborn calves were fed artificial colostrum, fortified by amounts of vitamin A within the range occurring in natural colostrum.

From our results we have been unable to obtain any conclusive evidence that vitamin A is carried from the intestine by the portal blood. On the other hand, the lymph draining the small intestine seems to be the main pathway by which the vitamin A reaches the general circulation.

SUMMARY

1. Bullocks, sheep and rats were dosed with 5000 i.u. of vitamin A/kg. body weight and slaughtered 1–24 hr. after dosing. On slaughter, vitamin A was estimated in systemic and portal blood, and also in the lymph or lymphatic tissues obtained from glands of the intestinal and other regions of the body.

2. In all three species, the lymph or lymph glands of the intestine contained considerably more vitamin A in dosed than in undosed animals. The average values for sheep and bullocks after dosing were 4830 and 1500 i.u. of vitamin A/100 ml. and 180 and 225 i.u./100 ml. for animals not dosed. This marked rise was not found in the body lymph after dosing, the concentrations being only 52 and 175 i.u./100 ml. respectively. The major part of this absorption seems to take place in the upper part of the intestine.

3. The vitamin A content of portal blood and of systemic blood rose after dosing, but the average figures for the portal blood were if anything slightly lower than those for systemic.

Our thanks are due to Dr L. J. Harris, Dr T. Moore and Mr F. Blakemore for their valuable criticism and one of us (E.E.) is grateful to the Agricultural Research Council for financial support.

REFERENCES

Drummond, J. C., Bell, M. E. & Palmer, E. T. (1935). *Brit. med. J.* i, 1208.
Eden, E. (1948). *Brit. J. Nutrit.* **2**, 42.
Evelyn, V. (1939). *J. biol. Chem.* **115**, 63.
Frazer, A. C. (1946). *Physiol. Rev.* **26**, 103.
Glover, J., Goodwin, T. W. & Morton, R. A. (1947). *Biochem. J.* **41**, 97.
McCoord, A. B., Breese, B. B. & Baum, W. S. (1943). Unpublished. Quoted by Clausen, S. W. (1943), *Harvey Lect.* **37**, 219.
Popper, H. & Volk, B. W. (1944). *Arch. Path. Lab. Med.* **38**, 71.
Radice, J. C. & Herraiz, M. L. (1947). *Rev. Asoc. méd. argent.* **61**, 287.
Yudkin, S. (1941). *Biochem. J.* **35**, 551.