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Further Studies on the Absorption of Vitamin A

BY S. MAHADEVAN AND J. GANGULY
Department of Biochemistry, Indian Institute of Science, Bangalore 12, India

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Irrespective of whether vitamin A is fed to rats as the free alcohol or as its esters, 75 % of it is found in the esterified form in the mucosae and muscles of the small intestine, whereas only the ester is transported exclusively through the lymphatic system, so that in the post-absorptive blood the ester shows a sharp increase (Ganguly, 1960). Gray & Cawley (1942) concluded from the molecular-distillation data on the oil obtained from rat liver that vitamin A was probably stored in the liver as its palmitate. Later work of Kaiser & Kagan (1956) showed that the vitamin A ester of blood and liver of rats was composed of higher fatty acids, and Mahadevan, Krishnamurthy & Ganguly (1959) showed that the lower esters of vitamin A were not absorbed from the intestine.

Gray, Hickman & Brown (1940) suggested that when rats are fed with different types of esters of vitamin A the liver, in time, converts them into one type of ester, probably the palmitate. We have recently demonstrated that an enzyme preparation from the intestinal mucosae of rats can rapidly esterify vitamin A with almost equal efficiency with saturated or unsaturated fatty acids containing more than 10 carbon atoms. However, it fails to esterify the vitamin with acids containing fewer carbon atoms (Murthy, Mahadevan, Seshadri Sastry & Ganguly, 1961). It might therefore be assumed that different types of esters (depending upon the availability of fatty acids of either endogenous or exogenous source) would be present in the mucosae and muscles of the small intestine, as well as in the blood and liver of rats, after a dose of the vitamin. It is demonstrated here that contrary to this expectation, it is the palmitate of vitamin A that always predominates in the mucosae, muscles and blood, and only the palmitate is deposited in the liver of rats.

MATERIALS AND METHODS

Methanol (Merck; Pro Analyst grade) and butanol (British Drug Houses Ltd.; Laboratory Reagent grade) were redistilled before use. Tweens 20, 40 and 60 (lauric, palmitic and stearic acid esters respectively of polyoxyethylene sorbitol) were obtained from Atlas Powder Co., Wilmington, Del., U.S.A. Refined coconut oil was obtained from the local market. Groundnut, sesame (*Sesamum indicum*) and safflower (*Carthamus tinctorius*) oils were expressed in an expeller from the respective oil seeds commercially available and were used without further purification. Silicone fluid F 110/300 was from Imperial Chemical Industries Ltd., London. The rest of the materials have been described in previous papers (Mahadevan *et al.* 1959; Ganguly, Krishnamurthy & Mahadevan, 1959).

Synthetic vitamin A alcohol, purified by chromatography on alumina columns (Ganguly, Krinsky, Mehl & Deuel, 1952), was dissolved in light petroleum (b.p. 40–60°) together with the Tween or the oil so as to obtain a final concentration of 6 mg. of the vitamin/ml. of the carrier. The solvent was evaporated under vacuum at 40–50°, the last traces being removed with a gentle stream of N_2. Colloidal dispersions were prepared according to Mahadevan *et al.* (1959).

Rats of either sex of this Institute strain were maintained after weaning on a vitamin-A-low diet composed of (%): starch, 65; solvent-extracted casein, 20; groundnut oil, 10; salt mixture, 5; with daily supplements of adequate amounts of the other vitamins. The animals were used after they had been on the diet for 5–6 weeks; at this stage their livers were essentially free from vitamin A.

After starvation for 24 hr. the animals were given an oral dose of 0·5 ml. of the carrier containing 3 mg. of vitamin A alcohol and were killed 2–3 hr. later by drawing as much blood as possible from the heart, while under light ether anaesthesia. Separation of the contents, mucosae and muscles of the small intestine, and extraction of these and of blood and liver were carried out as described earlier (Ganguly *et al.* 1959; Mahadevan *et al.* 1959). The extracts were chromatographed through alumina columns and the ester fractions were collected (Ganguly *et al.* 1952) for further analysis. Since the esterified vitamin A alone is transported from the intestine, this work was concerned with the study of the esters only; the alcohol fraction was not taken into account.

Separation of the higher fatty acid esters of vitamin A by reverse-phase circular chromatography on silicone-impregnated papers

Paper-chromatographic techniques were used for the separation of vitamin A alcohol, acetate and palmitate (Brown, 1953), and of the acetate and the higher esters (Kaiser & Kagan, 1956; Mahadevan *et al.* 1959). These techniques cannot resolve the higher esters of vitamin A, whereas our present work required a successful resolution of these esters. We obtained the best separation in a system where the filter paper is impregnated with silicone fluid and is irrigated with a mixture of methanol–butanol–water (85:10:5, by vol.).

The esters used as reference materials were prepared enzymically. A water extract (1 ml.) of 100 mg. of acetone-dried powder of rat pancreas and 1 ml. of ethanol containing 2 mg. of pure vitamin A alcohol plus 6 mg. of the required fatty acid were incubated at 37° for 1 hr. in 8 ml. of 0·01 M-phosphate buffer (Na_2HPO_4–NaH_2PO_4) at pH 6·6. At the end of the incubation the vitamin A was twice extracted with light petroleum after the addition of 10 ml. of ethanol. The esters formed were then separated from the unchanged vitamin A alcohol by chromatography on alumina columns (Ganguly *et al.* 1952).

Whatman no. 3 or no. 1 filter paper (45 cm. × 45 cm.) was dipped into a 7·5% (v/v) solution of silicone fluid in light petroleum for 30 sec. and allowed to dry at room temperature for 24 hr. The impregnated paper keeps for a considerable length of time without any change in its properties.

Usually the circular chromatogram was developed in the dark in large wooden chambers in an atmosphere of N_2 after spotting 10–15 μl. of light petroleum solutions containing 5–10 μg. of the ester at each point on the circumference of a circle drawn in the centre of the paper. Use of methanol alone for irrigation gave satisfactory separation, but addition of butanol cut down the length of the run, and the presence of water gave very sharp bands. With the solvent system of methanol–butanol–water (85:10:5) the separation was usually complete within 10–12 hr. The chromatogram was inspected under ultraviolet light and the fluorescent bands were marked. The rest of the procedure for the quantitative estimation of the esters was as described before (Mahadevan *et al.* 1959). Mixtures of known esters were chromatographed together with the extracts. It was extremely important to keep the chromatograms in the dark and not expose them to ultraviolet light while the irrigation was going on, as this led to the appearance of several additional fluorescent bands.

The recovery of the esters with this procedure was 90–95% and the R_F values were as follows: stearate, 0·29; palmitate, 0·32; myristate, 0·36; laurate, 0·4; oleate, 0·32; linoleate, 0·36; linolenate, 0·4. Complete separation of the saturated acid esters or of the unsaturated esters was possible but the 'critical pairs' (e.g. palmitate and oleate etc.) could not be separated.

For convenience, throughout this paper these bands will be referred to as if the saturated acids alone were present (e.g. stearate, palmitate, myristate and laurate). It is quite possible that the bands, especially those from the contents and mucosae may contain considerable amounts of the unsaturated esters of identical R_F values. This does not affect the objects and conclusions of this work to any extent, because we are demonstrating here that only one of these critical pairs, the 'palmitate', is actively transported and deposited. Also, as will be seen later on, we have been able to prove that this fraction of the intestinal muscles, blood and liver is composed almost entirely, if not wholly, of the palmitic acid ester.

RESULTS

Effect of carriers on the fatty acid composition of the vitamin A esters in the rat

Representative results of experiments, where different types of carriers were used, are summarized in Table 1. It is most striking that in the absence of exogenous palmitic or oleic acid an almost constant amount of the 'palmitate' was always present in the contents of the small intestine, as will be seen from the experiments with colloidal vitamin A alcohol, and with Tween 20 and Tween 60 dispersions of vitamin A alcohol. This same amount of the 'palmitate' was probably common in all other cases also, so that their enhanced 'palmitate' values are obviously due to the palmitic or oleic acid contributed by the carrier. The increase in the other esters is clearly due to the exogenous fatty acids. Thus, with Tween 20 (a laurate) and Tween 60 (a stearate), the 'laurate' and 'stearate' esters respectively rose sharply, whereas Tween 40 (a palmitate) gave rise to an extra amount of the 'palmitate'. Coconut oil, containing about 48% of lauric acid, yielded considerable quantities of vitamin A 'laurate', and, since this particular oil contains practically no linolenic acid, the ester should represent the lauric acid ester. The 'myristate' band was prominent with the other three oils, which have practically no myristic acid but are rich in linoleic acid, so that the materials of this band should be mostly vitamin A linoleate. It is thus obvious that the patterns of the vitamin A esters of the intestinal contents reflect the fatty acid composition of the vehicle used.

In the mucosae the ester composition in every case was very similar to that of the contents with the exception that the 'palmitate', as compared with the other esters, was more prominent. Where colloidal vitamin A was given, the 'palmitate' con-

centration was very high, and was practically the only ester.

The picture of the esters in the intestinal muscles was radically different from that in the mucosae. Whereas the esters of the mucosae largely reflected the fatty acid composition of the vehicle, only the concentration of the 'palmitate' was of any real significance in the muscles, because in all cases it was present to the extent of 79–93 % of the total esters. However, smaller quantities of the other esters representing the fatty acid composition of the carrier persisted. Thus when Tween 20 and coconut oil were used, there were small amounts of the 'laurate', whereas Tween 60 gave some 'stearate' and with the other three oils some of the 'myristate' was present. The ester composition of the blood appears to resemble that of the intestinal muscles: the 'palmitate' predominated in all the samples.

The liver, on the other hand, presents a rather unique picture since only the 'palmitate' was stored in spite of the presence of other esters in the blood.

Identification of the fatty acid of the vitamin A ester of intestinal muscles, blood and liver

These results show that only one of the critical pairs, i.e. the 'palmitate', is admitted inside the animal body to any significant extent. But, as pointed out earlier, the chromatographic procedure adopted here cannot resolve the critical pairs. Attempts were therefore made to obtain more definite information about the nature of the fatty acid of the 'palmitate' band of the muscles, blood and liver. These tissues from 10 rats, dosed with similar amounts of vitamin A alcohol dissolved in groundnut oil and killed 2–3 hr. later, were extracted according to Ganguly *et al.* (1959). The tissue extracts in light petroleum were shaken in separatory funnels five or six times with equal volumes of fresh aq. 85 % (v/v) ethanol each time. The ethanol layers were discarded: the light petroleum layers were twice chromatographed on alumina columns when the ester fractions were eluted with 1 % (v/v) of acetone in light petroleum. The esters were now chromatographed in batches on silicone-impregnated filter papers; the other smaller bands were discarded and only the 'palmitate' band was recovered. The paper-chromatographic procedure was repeated once more, after which the eluate was finally passed through an alumina column. The esters, thus purified, were saponified by heating with alcoholic potassium hydroxide (20 %, w/v) on a water bath at 60–80°; the fatty acids were then extracted with light petroleum after acidification with hydrochloric acid.

Duplicate samples of the liberated fatty acids were now separately subjected to reverse-phase chromatography on paraffin-impregnated papers with two different solvent systems. One of the chromatograms was developed with 90 % acetic acid and the other with acetic acid–formic acid–20 % hydrogen peroxide (6:1:1, by vol.). The latter system is known to destroy all the unsaturated acids and to carry the products to the solvent front

Table 1. *Vitamin A ester composition of the contents, mucosae and muscles of the small intestine, and of the blood and liver, of rats 2 to 3 hr. after feeding with vitamin A alcohol in different carriers*

Each rat was given orally 3·0 mg. of vitamin A alcohol in 0·5 ml. of the Tween or oil or in 0·5 ml. of water containing 1 % of sodium taurocholate. The results are averages of three separate experiments with individual rats. Values are expressed as μg. of vitamin A alcohol present as ester per whole organ in contents, mucosae, muscles and liver, and per 10 ml. of blood. S, 'Stearate'; P, 'palmitate'; M, 'myristate'; L, 'laurate'.

Carrier used	Main fatty acid(s) present	Intestine: Contents				Intestine: Mucosae				Intestine: Muscles				Blood				Liver			
		S	P	M	L	S	P	M	L	S	P	M	L	S	P	M	L	S	P	M	L
Colloidal dispersion in water	None	8	27	0	0	6	143	2	4	2	78	1	1	0	30	0	0	0	153	0	0
Tween 20	Lauric	11	26	3	70	15	107	19	67	3	133	3	12	2	39	2	8	0	116	0	0
Tween 40	Palmitic	14	92	0	0	19	219	9	13	2	207	3	5	0	45	0	0	0	179	0	0
Tween 60	Stearic	62	23	3	0	53	99	8	7	14	115	1	1	3	37	0	0	0	115	0	0
Coconut oil	Lauric	10	48	12	48	28	104	12	86	6	126	2	17	1	29	1	3	0	136	0	0
Groundnut oil	Oleic, linoleic	4	60	30	7	23	126	58	9	5	128	15	2	1	28	3	1	0	159	0	0
Sesame oil	Oleic, linoleic	2	47	43	0	9	117	93	7	3	135	22	2	2	23	6	1	0	145	0	0
Safflower oil	Linoleic, oleic	2	42	52	3	6	113	67	3	2	83	19	1	1	18	4	1	0	127	0	0

leaving the saturated ones unaffected (Buchanan, 1959). The positions of the fatty acids were located by treating the papers with cupric acetate solution followed by potassium ferrocyanide (Wagner, Abisch & Bernhard, 1955). In both chromatograms of samples from intestinal muscles, blood and liver only one band, corresponding to palmitic acid, was present, and by visual comparison no difference in the intensity of the colour between the peroxide-treated and untreated chromatograms could be observed.

Since the liver contained larger amounts of the ester, we were able to use other methods to prove that it is a palmitate. Representative samples of the liver ester were purified as described above. From a portion of the purified ester fraction the exact amount of vitamin A present was accurately determined, expressed as vitamin A alcohol (1·36 mg.). From this vitamin A alcohol value, the theoretical amount of palmitic acid, that should combine with it, was calculated (1·22 mg.). The rest of the sample was saponified and the fatty acids were chromatographed on paper with and without H_2O_2 as described above. The paper was treated with 0·1% mercuric acetate solution, washed thoroughly with running tap water, dried and sprayed with a solution of 0·2% diphenylcarbazide reagent. The coloured bands thus formed were eluted with toluene and methanol, and the quantity of fatty acids present was determined colorimetrically (Viswanathan & Meera Bai, 1961). There was no difference in the values between the peroxide-treated and untreated samples. Also, the amount of palmitic acid estimated (1·27 mg.) agreed closely with the theoretical value as calculated above. Finally, the iodine vapour test of another chromatogram was negative. Thus although oleic acid is more abundant than palmitic acid in groundnut oil, there was practically no vitamin A oleate in the intestinal muscles, blood or liver of rats.

Changes in the ester composition of vitamin A in the rat with time

Gray *et al.* (1940) indicated that several esters of vitamin A might reach the liver to be eventually converted into one type, probably the palmitate. The above experiments have demonstrated that, irrespective of the ester composition of the contents and mucosae, the liver can store only the palmitate. These experiments, however, were carried out within a very short time interval (2–3 hr.) after dosing and hence it remained a possibility that given longer time intervals the liver might store the other esters in addition to the palmitate. Rats were therefore fed with vitamin A alcohol in coconut oil and killed at the stated time intervals. The relative appearances of the different esters with respect to time in the various tissues of these rats are tabulated in Table 2. Up to 6 hr. the ester patterns in all the samples were similar to those obtained with the same oil in the experiments of Table 1. However, after 10 hr. the palmitate became the most prominent constituent, and after 24 hr. it was the sole component. Here again, despite the fact that lauric acid is the major acid of coconut oil, at no time did the liver show any traces of any other ester but the palmitate, even though the total ester stored per liver increased from 125 μg. at 2 hr. to 1200 μg. after 24 hr.

Fatty acid specificity for the esterification of vitamin A by the enzymes of the intestinal muscles

The ester patterns in the contents and mucosae are probably due to the rather non-specific nature of esterification of vitamin A by the enzymes of the pancreas and mucosae. The predominance of the palmitate in the intestinal muscles, on the other hand, might well be due to a specific esterification of vitamin A brought about by the muscle enzyme with palmitic acid. The esterifying activity of the acetone-dried powder of the muscles with different

Table 2. *Vitamin A ester composition of the contents, mucosae and muscles of the small intestine, and of the blood and liver of rats, at different time intervals after feeding with vitamin A alcohol in coconut oil*

Each rat was given orally 3·0 mg. of vitamin A alcohol in 0·5 ml. of coconut oil. The results are averages of three separate experiments with individual rats. Values are expressed as μg. of vitamin A alcohol present as ester per whole organ in contents, mucosae, muscles and liver, and per 10 ml. of blood. S, 'Stearate'; P, 'palmitate'; M, 'myristate'; L, 'laurate'.

	Time after dosing																							
	2 hr.				3 hr.				4 hr.				6 hr.				10 hr.				24 hr.			
Tissue	S	P	M	L	S	P	M	L	S	P	M	L	S	P	M	L	S	P	M	L	S	P	M	L
Intestine																								
Contents	9	37	8	56	8	53	7	58	4	42	3	37	4	39	4	26	5	23	3	15	2	12	0	0
Mucosae	6	59	6	38	14	128	5	92	13	132	5	89	10	78	4	47	1	39	1	5	0	5	0	0
Muscles	4	67	4	18	6	132	5	15	6	138	4	19	4	84	3	13	0	48	1	4	0	8	0	0
Blood	1	14	1	3	1	23	1	4	1	32	1	8	1	27	1	5	0	18	0	2	0	9	0	0
Liver	0	125	0	0	0	170	0	0	0	376	0	0	0	729	0	0	0	820	0	0	0	1200	0	0

fatty acids was therefore tested as described by Murthy *et al.* (1961). The relative esterification with the different acids, as shown in Table 3, was similar to that obtained with the mucosal and pancreatic enzymes, inasmuch as no preference for any particular acid was exhibited. In a separate experiment, where an ethanolic solution of 1 mg. of vitamin A alcohol was similarly incubated with a mixture containing 1 mg. each of lauric, myristic, palmitic and stearic acids, no significant preference for esterification with palmitic acid was noticed.

It is also possible that blood and liver might bring about the formation of larger amounts of the palmitate by means of a mechanism of transesterification with other lipid materials, without going through the processes of hydrolysis and re-esterification [these two latter processes have been shown to be absent from these tissues (Mahadevan, Murthy, Krishnamurthy & Ganguly, 1961)]. Experiments were therefore carried out in which 0·5 ml. of an ethanolic solution of the esters (laurate, myristate, palmitate and stearate) containing 250 μg. of each was separately incubated in 4 ml. of phosphate buffer (pH 6·6; 0·1 M) with 1 mg. of Tween 40 and 1 ml. of rat-liver homogenate (equivalent to 500 mg. of wet liver) or 1 ml. of plasma for 1 hr. at 37°. No formation of any additional palmitate was observed.

Nature of vitamin A esters in the livers of other species

Samples of vitamin A ester fractions from the livers of chickens, sheep, shark and rats, when chromatographed in our system, revealed that shark and rat liver display only one band, corresponding to the 'palmitate', whereas the chicken-liver sample resolved itself into two distinct bands representing about 70 % of 'palmitate' and 30 % of 'stearate'. In sheep liver the 'palmitate' was prominent, accounting for about 90 % of the total esters.

Table 3. *Fatty acid specificity for the esterification of vitamin A by the enzymes of rat intestinal muscles*

Conditions and procedures were as described by Murthy, Mahadevan, Seshadri Sastry & Ganguly (1961) except that the enzyme extract added contained 20 mg. of protein.

Fatty acid	Vitamin A alcohol esterified (μg.)
Lauric	64·2
Myristic	54·9
Palmitic	53·7
Stearic	51·4
Oleic	57·5
Linoleic	58·3
Linolenic	54·6

DISCUSSION

The techniques employed in the earlier work of Kaiser & Kagan (1956) and of Mahadevan *et al.* (1959) were not suitable for the resolution of the higher esters of vitamin A, so that it was possible to arrive only at the broad conclusion that the higher esters are admitted into the rat. With the successful separation of these esters (or pairs of esters), we have been able to conclude in the present report that this ester in the rat is a palmitate. In no case have we been able to detect in the tissues of the rat vitamin A esters with acids shorter than lauric acid, fully confirming the earlier findings of Kaiser & Kagan (1956), Mahadevan *et al.* (1959) and of Bruggemann & Tiews (1960). However, Loran & Althausen (1959) have claimed that in an isolated intestine *in vitro* the long-chain esters of vitamin A are hydrolysed on the lumen side and are re-esterified inside the mucosae with acetic acid. This is in sharp disagreement with the information given in our earlier experiments (Mahadevan *et al.* 1959) and in the present paper. We have already demonstrated that the intestinal enzymes preferentially esterify vitamin A with long-chain acids, whereas no esterification could be obtained with acetic acid. On the other hand, as compared with the higher esters, vitamin A acetate is more rapidly hydrolysed by the enzymes of this tissue (Mahadevan *et al.* 1961).

The fatty acid composition of the carrier employed for vitamin A is reflected by the patterns of the esters in the lumen and mucosae of the small intestine of rats. This can readily be explained, because the pancreas and mucosae of rats esterify vitamin A non-specifically with long-chain acids. But 'palmitate' is almost the sole constituent in the absence of exogenous fatty acids, e.g. after feeding with colloidal vitamin A. It appears preferentially in the intestinal muscles, in spite of the presence of considerable amounts of other esters in the mucosae, as found when Tween 20 or 60 or one of the four oils is used as the carrier. This cannot be explained on the basis of the assumption of any preferential esterification of the vitamin in this tissue with palmitic acid alone, because the muscle enzyme, like the mucosal enzyme, seems to be rather non-specific towards the fatty acid for this process. Nor can this be explained by assuming ready availability of palmitic acid in the intestine, because according to Coniglio & Cate (1958) this acid is not the only major fatty acid in the normal intestinal tissue of rats. The failure to demonstrate any transesterification mechanism in the blood and liver rules out the possibility of such reactions accounting for the presence of 'palmitate' only in these tissues.

One possible explanation seems to be that a certain amount of specificity for the vitamin A

palmitate exists in the mechanism of its transport from the mucosae onwards and in its final storage in the liver. Indeed Krinsky, Cornwell & Oncley (1958) have demonstrated the specificity of lipoproteins in their binding with vitamin A ester and vitamin A alcohol in post-absorptive human blood. In an earlier paper we also have discussed and suggested a similar possibility in the transport and storage of vitamin A in the rat (Ganguly *et al.* 1959), and this aspect has been more exhaustively reviewed by Ganguly (1960). Krinsky *et al.* (1958) are of the view that the specific lipoprotein, responsible for the transport of vitamin A ester in blood, occurs in the Kupffer cells of the liver. It is possible that this lipoprotein exists in the Kupffer cells, in blood and in the central lacteals of the intestinal villi. The 'palmitate', even in the mucosae, could then be in actual combination with this lipoprotein and probably inside the central lacteals. The picture in the intestinal muscles would then appear to represent that phase of absorption where the ester, after entering the lymphatic system in the lipoprotein-bound state, has travelled far away from the actual site of esterification (the mucosae) on its way to the systemic blood, so that mostly the palmitate is found. Since the Kupffer cells of the liver contain the same lipoprotein, it preferentially removes only the palmitate from the systemic blood and totally rejects the others.

McGillivery, Thompson & Worker (1957) have demonstrated that, on intravenous injection of emulsions of vitamin A alcohol or palmitate in rats and goats, the palmitate is more efficiently taken up by the liver, whereas more of the administered alcohol keeps on circulating in the blood. Preliminary experiments being carried out by us also indicate that, after intravenous injection of vitamin A stearate dispersed in water with Tween 40, no stearate could be detected in rat liver. These observations would lend further support to the hypothesis that rat liver selects only the palmitic acid ester of vitamin A.

There were, however, traces of the other esters in the intestinal muscles and blood, but not in the liver. Since the liver has no acceptors for them, they tend to accumulate in the circulating blood either to be detoxicated or to be hydrolysed and re-esterified with palmitic acid in the intestine or pancreas during their rapid circulation, so as to be deposited ultimately in the liver as the palmitate.

To summarize, it would thus appear possible that the lipoprotein exerts its selective binding probably at the mucosal cells for the palmitate only, the bound palmitate then travels through the lymphatic system and finally finds its way to the Kupffer cells, where it is deposited and the free lipoprotein comes back to the intestine to carry more palmitate.

SUMMARY

1. A circular paper-chromatographic procedure for the separation of higher fatty acid esters of vitamin A, with silicone-impregnated paper and a solvent system of methanol–butanol–water (85:10:5, by vol.), is described.

2. Rats raised on a vitamin-A-low diet and starved for 24 hr. were dosed with vitamin A alcohol in the carriers Tweens 20, 40 and 60, groundnut oil, sesame oil, coconut oil or safflower oil; the vitamin A ester fractions of the contents, mucosae and muscles of the small intestine, and of blood and liver, were analysed by the above method 2–3 hr. after the dose.

3. After the feeding of colloidal vitamin A, mostly vitamin A palmitate was found in the contents, mucosae and muscles of the small intestine, and in the blood and liver, of rats.

4. The types of the esters of the contents and mucosae in all other cases were governed by the fatty acids of the carrier. No such relationship was found for the ester composition of intestinal muscles and blood, which contained almost entirely the palmitic acid ester. The liver, under all conditions studied, stored exclusively the palmitate.

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