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The Metabolism of Retinyl Methyl Ether in the Rat *in vivo*

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1. Retinyl methyl ether was converted into vitamin A in vitamin A-deficient rats regardless of whether administered by oral, intraperitoneal, intramuscular or subcutaneous route; intramuscular administration seemed to be the best for conversion as well as storage. 2. Significantly, unchanged retinyl methyl ether was also found in the liver after oral administration but not after administration by other routes. 3. Oral administration of 1mg of retinyl methyl ether led to a progressive increase in liver vitamin A with time reaching a value of 16% of administered dose after 24h. No retinyl methyl ether was detectable in liver at any time-interval in this experiment. 4. Conversely, oral administration of 4mg of retinyl methyl ether/day for 4 days led to the accumulation of 25% of the dose as unchanged retinyl methyl ether in the liver 1 day after the last dose; however, it was gradually but completely converted into vitamin A over a period of 18 days. 5. The significance of these findings with special reference to the fundamental metabolism of vitamin A, the site of conversion of retinyl methyl ether into vitamin A, the relative efficiency of various routes of administration and its biological activity are discussed.

Retinyl methyl ether, a synthetic derivative of retinol, is nearly as effective as retinol in promoting growth (Hanze, Conger, Wise & Weisblat, 1946; Isler *et al.* 1949). Its biological activity might be effected by the compound itself, as in the case of retinoic acid (Oroshnik, Karmas & Mebane, 1952), or might be due to retinol formed by cleavage of the ether as originally suggested by Isler (1950). The fact that retinyl methyl ether is effectively converted into retinol by the rat *in vivo* (Thompson & Pitt, 1963) supports the latter view. However, detailed studies on the metabolism and storage of this analogue of vitamin A have not previously been conducted.

In the present work the metabolism of retinyl methyl ether in the rat *in vivo* has been examined in some detail: (1) by measuring the storage of vitamin A derivatives in the liver and intestine after administration of the compound by various routes; (2) by determining the amount of retinol, retinyl palmitate and unchanged retinyl methyl ether in these organs as a function of the route of administration, time and dose.

MATERIALS AND METHODS

Chemicals. all-*trans*-Retinol was obtained from Distillation Products Industries, Rochester, N.Y., U.S.A., and retinyl palmitate from K & K Laboratories Inc., Plainview, N.Y., U.S.A. Retinyl methyl ether was a gift from Professor O. Isler, Hoffmann-La Roche and Co. Ltd., Basle, Switzerland; it was also synthesized as described by Hanze *et al.* (1946). All other reagents and chemicals were of analytical grade.

Preparation of retinyl methyl ether for metabolic studies. Retinyl methyl ether was prepared just before use at a concentration of 1mg/0.1ml of refined vegetable oil containing 0.5mg of α-tocopherol for oral, intraperitoneal and subcutaneous administrations; it was dispersed in a solution containing 6.25% Tween 40 and 1.0% bovine serum albumin at a concentration of 1mg/0.1ml for intramuscular administration.

Animals. Vitamin A-deficient rats (Wistar strain) were used while in the 'weight-plateau' stage, as described by Lakshmanan, Jungalwala & Cama (1965). Conventional symptoms of vitamin A deficiency developed in these rats about 40 days after being fed on a vitamin A-free diet.

Preparation of tissue extracts. Various tissues were extracted essentially as described by Glover, Goodwin & Morton (1948).

Saponification. Retinyl ester was saponified by refluxing with 1 vol. of aq. 60% (v/v) KOH and 5 vol. of 90% (v/v) ethanol at 60°C under N_2 for 60min.

Separation and identification of vitamin A compounds. (*a*) Adsorption chromatography. The chromatographic analyses of vitamin A compounds were carried out on 5% (v/w) water-deactivated alumina columns. Retinyl methyl ether and retinyl palmitate could not be distinctly separated from each other, irrespective of the type of adsorbent or eluent used. These two vitamin A derivatives were quantitatively eluted with 50ml of 2% (v/v)

diethyl ether in light petroleum (b.p. 40–60°C), whereas retinol was eluted with 50ml of 10% (v/v) diethyl ether in light petroleum. (*b*) T.l.c. on silica gel G plates with 6% (v/v) acetone in *n*-hexane solvent system according to John, Lakshmanan, Jungalwala & Cama (1965) was carried out for qualitative separation and identification of various vitamin A derivatives (R_F values: retinyl palmitate 0.86; retinyl methyl ether 0.70; retinol 0.10). For quantitative separation and isolation of vitamin A derivatives, t.l.c. was carried out on alumina G plates by using the same solvent system since the recoveries were more than 95%, unlike those from silica gel G plates. However, alumina G plates were employed only with saponified extracts because retinyl palmitate and retinyl methyl ether failed to separate distinctly from each other on them.

Determinations. The concentrations of retinol, retinyl palmitate and retinyl methyl ether in light petroleum (b.p.40–60°C) were determined in a Cary 15 recording spectrophotometer, by using $E^{1\%}_{1cm}$ values of 1830, 1595 (Cama, Collins & Morton, 1951) and 1626 (present investigation) respectively at 325nm in light petroleum (b.p.40–60°C). For impure extracts, the correction formula of Cama *et al.* (1951) was used.

RESULTS

Oral administration. Three vitamin A-deficient rats were given retinyl methyl ether orally (500μg/day per rat) for 10 days. Symptoms of vitamin A deficiency were alleviated in all treated animals within 2 days after receipt of the first dose. The average weight gain of the rats during this period was 41g. At 24h after the last dose the rats were killed and their livers and intestines were pooled and analysed for vitamin A derivatives.

As shown in Table 1, 11% of the administered dose was found in the liver as unchanged retinyl methyl ether and 24% was present as retinyl palmitate plus retinol. The intestine contained both vitamin A and retinyl methyl ether.

Parenteral administration. Three vitamin A-deficient rats were given retinyl methyl ether intraperitoneally (500μg/day per rat) for 10 days. The rats were relieved of all symptoms of vitamin A deficiency within 2 days and gained an average of about 35g in weight during the period. The rats were killed 24h after the last dose and the livers and intestines were pooled and analysed.

As indicated in Table 1, retinyl methyl ether was not detected in the liver after intraperitoneal administration. On the other hand, the recovery of retinyl palmitate plus retinol was 37% of the administered dose, much higher than when the retinyl methyl ether was administered orally. However, both retinyl methyl ether and retinol were detected in intestinal extracts.

Intramuscular administration. Three vitamin A-deficient rats were given retinyl methyl ether intramuscularly (500μg/day per rat) for 10 days. The rats were alleviated of vitamin A-deficiency symptoms within 1 day and gained on an average about 38g in body weight during this period. They were killed 24h after the last dose and the livers, intestines and the muscular tissue around the site of injection were pooled and analysed.

It is apparent from Table 1 that the recovery of retinyl palmitate plus retinol in the liver was the highest after intramuscular administration (45% of administered dose); significantly, administered retinyl methyl ether was not detected in the liver. Further, neither retinyl methyl ether nor vitamin A was detectable in the intestine. The lipid extract from muscles also failed to show the presence of vitamin A, although unchanged retinyl methyl ether could be found to the extent of 0.6% of the administered dose.

Subcutaneous administration. One vitamin A-deficient rat was given a single dose of 1mg of retinyl methyl ether subcutaneously. Symptoms of vitamin A deficiency disappeared in this rat within 3 days and it showed an increase in weight of 78g in 19 days. It was killed after 19 days and the liver was analysed.

Retinyl palmitate and retinol, but not retinyl methyl ether, were detected in the liver after subcutaneous administration. The recovery of retinyl palmitate and retinol from the liver was only 1% after 19 days.

Effect of time on the distribution of vitamin A

Table 1. *Recovery of vitamin A from pooled livers of three rats* 24h *after oral, intraperitoneal or intramuscular administration of retinyl methyl ether* (500μg/*day per rat*) *for* 10 *days*

Route of administration	Recovery of vitamin A derivatives: Total (μg)	Total (% of administered dose)	Retinyl methyl ether (μg)	Retinyl methyl ether (% of administered dose)	Retinol+retinyl palmitate (μg)	Retinol+retinyl palmitate (% of administered dose)
Oral	5157	35	1596	11	3597	24
Intraperitoneal	5500	37	0	0	5500	37
Intramuscular	6780	45	0	0	6780	45

Table 2. *Recovery of vitamin A derivatives from liver and intestine at various times after oral administration of* 1 mg *of retinyl methyl ether/vitamin A-deficient rat*

Each value represents the average of two rats. The variation between individual values was <6%.

	Recovery of vitamin A derivatives							
	In liver					In intestine		
Time after dose (h)	Total (μg)	Total (%)	Retinyl methyl ether (μg)	Retinyl palmitate (μg)	Retinol (μg)	Total (%)	Retinyl methyl ether (μg)	Retinol (μg)
0 (control)	3	0	0	3	—	0	0	0
½	3	0.3	0	3	—	0	0	0
1	3	0.3	0	3	—	1	10	0
3	39	4	0	27	12	1	10	<1
6	60	6	0	43	17	3	20	10
12	139	14	0	125	14	2	15	5
24	161	16	0	149	12	2	17	3

Table 3. *Recovery of vitamin A derivatives from the liver at various times after oral administration of* 4 mg *of retinyl methyl ether to vitamin A-deficient rats for* 4 *days*

Each value represents the average of two rats. The variation between individual values was <6%.

	Recovery of vitamin A derivatives						
Time after last dose (days)	Total (μg)	Retinyl methyl ether (μg)	Retinyl palmitate (μg)	Retinol (μg)	Total (% of given dose)	Retinyl methyl ether (% of total liver storage)	Retinol + retinyl palmitate (% of total liver storage)
0 (control)	3	0	3	—	—	—	—
1	5125	1342	3436	247	32	27	73
3	4180	414	4184	81	29	9	91
6	4570	362	4167	41	29	8	92
9	4775	219	4525	32	30	5	95
12	5330	183	5119	28	33	3	97
18	6120	0	6091	29	38	0	100
30	6085	0	6044	41	38	0	100

derivatives in the liver and intestine. To investigate the absorption of retinyl methyl ether and its rate of conversion into vitamin A, time-distribution studies were carried out with vitamin A-deficient rats given a single dose of 1 mg/rat. As shown in Table 2, no retinyl methyl ether was found in the liver within the experimental period. However, considerable amounts of retinyl palmitate and retinol were detected in the liver within 3 h and progressively increased up to 24 h. On the other hand, both retinyl methyl ether and vitamin A were detected in the intestine (Table 2).

To study the metabolic fate of retinyl methyl ether after it reaches the liver, larger doses (4 mg/day per rat) were administered to vitamin A-deficient rats for 4 days. The animals were killed at various time-intervals after the last dose and the livers were analysed. As shown in Table 3 about one-quarter of the total vitamin A present in the liver at 1 day after the last dose was retinyl methyl ether. Although the total amount of vitamin A present in the liver remained relatively constant during the next 30 days, the amount of retinyl methyl ether decreased and could not be detected after 18 days. Conversely the relative amount of retinol plus retinyl ester in the liver increased from 73% of the total vitamin A stored at 24 h to 92% after 2 days, and thereafter gradually increased to 100% after 18 days.

DISCUSSION

According to the current concept, vitamin A esters, regardless of the type of ester fed to the animal, are always hydrolysed to the free alcohol in the lumen of the small intestine before being

taken up by the intestinal mucosa during absorption (Ganguly, 1967). There have been instances, however, indicating that the fed ester is absorbed as such across the intestine, as reported for retinyl acetate (Shellenberger, Parrish & Sanford, 1964) and *retro*-retinyl acetate (Murray & Erdody, 1966). On the other hand, John, Lakshmanan & Cama (1967) could detect only *retro*-retinyl palmitate but not the acetate ester in the livers even after massive oral doses of *retro*-retinyl acetate to vitamin A-deficient rats, showing thereby that it has to be hydrolysed to the free alcohol. Retinyl methyl ether is also hydrolysed to the free alcohol before being absorbed across the intestine (Table 2) except when massive doses were fed (Tables 1 and 3). Hence under physiological conditions it seems reasonable that no form of vitamin A ester can be absorbed as such across the intestine except under special circumstances. Even then it is only the unnatural synthetic derivatives of vitamin A that have been reported to escape hydrolysis to the free alcohol.

The finding that the extent of conversion of retinyl methyl ether into vitamin A and subsequent storage in the liver (Table 1) varies from 45% when it is given intramuscularly to 37% when it is given intraperitoneally to 24% when it is given orally implies that the intramuscular route of administration could well be the most efficient for absorption, transport and storage of vitamin A compounds. The recovery in the liver of only 1% of a subcutaneously administered single dose after 19 days indicates that the bulk of the retinyl methyl ether is removed faster than could be accounted for by the metabolic needs of the animal. Nonetheless it is of note that a single 1mg subcutaneous dose of retinyl methyl ether can sustain the normal growth of a vitamin A-deficient rat over a period of 19 days.

The detection of retinyl methyl ether in the liver after its oral administration but not after parenteral, intramuscular or subcutaneous administration (Table 1) suggests that the liver is the major, if not the only, site for conversion of retinyl methyl ether into retinol. However, a cell-free system from rat liver has now been isolated that can catalyse the conversion of [^{3}H]retinyl methyl ether into [^{3}H]-retinol (Narindrasorasak, Lakshmanan & Olson, 1970, and unpublished work). It is probably a microsomal mono-oxygenase requiring molecular oxygen, NADPH and reduced pteridine as cofactors. Further, no form of vitamin A was detectable in the intestine after administration of retinyl methyl ether by various routes other than by oral route. All these can be explained on the basis that retinyl methyl ether is transported directly to the liver, where it is efficiently converted into vitamin A. Even when administered orally it is slowly but completely converted into vitamin A within a period of 18 days (Table 3). Failure to detect any vitamin A in the muscular tissue around the site of administration seems to rule out this tissue as another site for conversion of retinyl methyl ether into vitamin A.

The appearance of retinol in the intestine 6h after the oral administration of retinyl methyl ether (Table 2) strongly favours the intestine as another site of the latter's conversion into vitamin A. It also implies that retinyl methyl ether might first be cleaved to retinol before being esterified to retinyl palmitate and stored in the liver. However, the presence of retinyl methyl ether in the intestine even after 24h (Table 2) indicates that its conversion into vitamin A is a slow process in the intestine. Apparently rat intestine is efficient enough to prevent any unchanged retinyl methyl ether from reaching the liver when the rat is given a single oral 1mg dose of retinyl methyl ether (Table 2), but not when it is loaded with excessive doses (Tables 1 and 3).

The fact that retinyl methyl ether can be detected in the liver unchanged in appreciable amounts (Table 3) after massive oral administration implies that, although the intestine may be an important site for conversion of dietary retinyl methyl ether, it seems to have a threshold beyond which excess of retinyl methyl ether escapes cleavage and thus is absorbed as such. This accounts for 27% of the total storage of vitamin A derivatives in the liver as retinyl methyl ether at 24h (Table 3). It must be pointed out that Thompson & Pitt (1963) never detected any unchanged retinyl methyl ether in the liver after the oral administration of 800μg/day per rat over a period of 29 days. However, since dosage, time-interval and strain of the rats used in their study were different from the present study, the two results are not strictly comparable. Thus the present investigations clearly show that the efficiency with which retinyl methyl ether is converted into vitamin A in the rat irrespective of the route of its administration is commensurate with its high biological activity.

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