

## Absorption, Storage and Distribution of 3-Dehydrovitamin A in the Rat

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1. The metabolism of 3-dehydroretinal was found to be similar to that of retinal. It alleviated all the symptoms of vitamin A deficiency, and promoted the growth of vitamin A-deficient rats. 2. When administered orally, 3-dehydroretinal was reduced in the intestine of the rat and subsequently esterified and transported to the liver, where it was stored mainly as the higher fatty acid ester. 3. Intraperitoneal administration of the compound led to the accumulation of 3-dehydrovitamin A in liver and other tissues. Subcutaneous administration of the compound showed a good growth response in the rat. 4. The ratio of 3-dehydroretinyl higher fatty acid ester to 3-dehydroretinol in liver, in the post-absorptive state, was nearly 93:7. 5. There was a linear relationship between the 3-dehydroretinol concentrations of blood and liver of rats. 6. Administration of 3-dehydroretinal at a dosage of 7.5 mg./day for 3 days brought about hypervitaminosis A in the rat. 7. The maximal retention of 3-dehydrovitamin A by the kidneys was at an optimum dosage of 4.5 mg./day for 3 days.

Although much work has been done on the chemistry and biological activity of 3-dehydroretinol (Moore, 1957) as well as 3-dehydroretinal (Sundaresan & Cama, 1961), some aspects of its metabolism have not hitherto received attention. The correlation between the concentrations of blood and liver retinol is not yet clear. Steigman & Popper (1944) reported that there is a certain degree of linearity between the concentrations of unesterified vitamin A of blood plasma and liver in human subjects. Later, Glover, Goodwin & Morton (1947a) concluded that, irrespective of the amount of vitamin A given orally, there was a linear relationship between the blood vitamin A and the liver unesterified vitamin A concentrations in the rat. However, the reports of Lewis, Bodansky, Falk & McGuire (1942) and of Ganguly & Krinsky (1953) are, in a way, contradictory to the above observations. Hence it seemed worth while to study whether any similar relationship exists in the case of 3-dehydrovitamin A.

### MATERIALS AND METHODS

**Materials.** 3-Dehydroretinal was prepared from the liver oil of the Indian freshwater fish *Wallago attu* (Balasundaram, Cama, Sundaresan & Varma, 1956). The livers were homogenized in a Waring Blendor and extracted repeatedly with light petroleum (b.p. 40–60°)–ether mixture. The extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and reduced in volume. The concentrate was kept at –30° overnight and

the precipitated sterols were removed by filtration at –30°. The filtrate after reduction in volume *in vacuo* was saponified with ethanolic KOH and oxidized over MnO<sub>2</sub> (Cama *et al.* 1952a). 3-Dehydroretinal was separated by chromatography on a water-deactivated alumina column. The crystalline material obtained after four crystallizations had  $E_{1\text{cm}}^{1\%}$  1431 at 385 m $\mu$  in light petroleum.

**Oral feeding.** The compound was dissolved just before use in refined deodorized groundnut oil containing 50% (w/w) of  $\alpha$ -tocopherol.

**Preparation of tissue extracts.** The tissue extracts were prepared by the method described by Glover, Goodwin & Morton (1948). 3-Dehydrovitamin A in blood was estimated as follows. The whole blood was mixed with an equal quantity of ethanol and centrifuged for 10 min. The supernatant was mixed with an equal quantity of water and extracted three or four times with light petroleum. The combined extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, reduced in volume and taken up in a known volume of ethanol-free chloroform.

**Separation and identification.** Separation and identification of the various compounds in small quantities were done by thin-layer chromatography with 6% (v/v) acetone in light petroleum (John, Lakshmanan, Jungalwala & Cama, 1965) or by reverse-phase chromatography (Jungalwala & Cama, 1962). The quantitative separation of 3-dehydroretinyl fatty acid esters from the free alcohol was carried out by chromatography on a 10%-(v/w)-water-deactivated alumina column; the ester fraction was quantitatively eluted with light petroleum. The concentration of 3-dehydroretinol was obtained by subtracting the amount of the ester fraction from the total 3-dehydrovitamin A; this was more accurate than its quantitative separation on the column.

Table 1. Conversion of 3-dehydroretinal into 3-dehydrovitamin A in the intestine of the rat after oral administration of 2 mg. of 3-dehydroretinal

Rat no.	Time between administration and death (hr.)	3-Dehydroretinal recovered ( $\mu\text{g.}$ )			3-Dehydrovitamin A recovered ( $\mu\text{g.}$ )		Total recovery of 3-dehydroretinal+3-dehydrovitamin A (% of dose)
		Stomach	Intestinal washings	Intestine	Intestine	Liver	
1 (control)	—	0	0	0	0	0	0
2 (control)	—	0	0	0	0	0	0
3	0.5	1080.0	14.41	39.38	8.9	0	57.1
4	0.5	769.3	18.18	33.22	5.1	0	41.3
5	1	63.0	24.87	9.09	437.0	3.55	26.9
6	1	1094.0	3.15	13.46	110.6	0	61.1
7	3	70.0	8.57	20.55	211.55	467.7	38.9
8	3	335.7	3.2	6.89	106.4	318.0	38.5
9	6	495.0	1.15	6.78	18.93	398.8	46.0
10	6	334.0	1.47	7.13	21.13	493.7	42.9
11	12	137.1	0	0	Trace	512.2	32.5
12	12	25.87	0	0	Trace	529.1	27.8
13	24	0	0	0	0	503.4	25.2
14	24	141.0	0	0	0	544.6	34.3

*Estimation.* 3-Dehydroretinal was estimated by the thiobarbituric acid method (Futtermann & Saslaw, 1961) in a Beckman model DU spectrophotometer, 550 m $\mu$  being taken as the absorption maximum. 3-Dehydrovitamin A was estimated spectrophotometrically at its maximum absorption at 350 m $\mu$  and also by the SbCl<sub>3</sub> colour test, 693 m $\mu$  being taken as the absorption maximum (Cama & Morton, 1953).

Male rats of this Institute strain were used when they reached the plateau stage of vitamin A deficiency as described by Lakshmanan, Jungalwala & Cama (1965). For the studies on 3-dehydroretinal in blood and liver, 32 plateau-stage vitamin A-deficient rats were each given 5  $\mu\text{g.}$  of 3-dehydroretinal/day for another 2-3 weeks, until they reached a body weight of 130-170 g., so that they were in a healthy condition to give more blood. They were then divided into eight groups of four rats, and each rat was given dosages of 3-dehydroretinal (Table 3). Each dosage was given during three successive days and the rats were killed 96 hr. after the last dose, the animals being starved for the last 24 hr. Blood was drawn from the heart of each rat under light ether anaesthesia with the aid of an all-glass syringe rinsed with sodium oxalate. Blood, livers and kidneys from animals in each group were pooled for analyses.

## RESULTS

*Oral administration.* To study the mode of absorption of 3-dehydrovitamin A, time-distribution studies were carried out with vitamin A-deficient rats. The results (Table 1) show that 3-dehydrovitamin A reached the liver as early as 1 hr. after administration and there was a progressive increase of 3-dehydrovitamin A in liver during the next 11 hr.

*Parenteral administration.* Three vitamin A-

Table 2. Recovery of 3-dehydrovitamin A from various tissues of three vitamin A-deficient rats after intraperitoneal administration of 3-dehydroretinal for 8 days at a dose rate of 1.0 mg./rat/day

Tissue	Recovery of 3-dehydrovitamin A (% of dose)
Control	—
Experimental	
Liver	
Total	12.5
3-Dehydroretinyl ester	11.84
3-Dehydroretinol	0.66
Intestinal wall	2.29
Kidneys	0.005
Lungs	0.11
Spleen	0.18
Blood	0.003

deficient rats were given 3-dehydroretinal (1 mg./rat/day) intraperitoneally for 8 days. The rats were killed 24 hr. after the last dose. During this period the rats gained, on average, 27 g. in weight. The recovery of 3-dehydrovitamin A from the various tissues is shown in Table 2.

*Enterectomy.* The experiment was performed as described by Lakshmanan *et al.* (1965). A 2 mg. sample of 3-dehydroretinal was administered intraperitoneally and the rat was returned to the cage. The rat was killed 7 hr. after the administration of the compound. The lipid extract from liver on thin-layer chromatography revealed two spots, corresponding to 3-dehydroretinyl higher fatty acid

Table 3. Relationship between blood 3-dehydrovitamin A and 3-dehydroretinol concentrations in liver in rats given different oral dosages of 3-dehydroretinal

Group no.	Total 3-dehydroretinal administered to four rats (mg.)	Total 3-dehydrovitamin A in liver ( $\mu\text{g./g.}$ )	3-Dehydroretinol in liver ( $\mu\text{g./g.}$ )	3-Dehydrovitamin A in blood ( $\mu\text{g./100 ml.}$ )	3-Dehydrovitamin A in kidney ( $\mu\text{g./g.}$ )
1	12	181.4	17.0	2.67	1.24
2	18	337.0	45.0	3.67	1.55
3	24	446.1	68.92	5.35	1.75
4	36	683.2	60.64	4.83	4.77
5	54	1014.0	97.2	6.47	9.77
6	72	1059.0	89.0	6.03	2.71
7	90	1090.0	108.6	7.52	1.63

ester and 3-dehydroretinol. The extracts from kidney and spleen also showed the presence of 3-dehydroretinol.

*Subcutaneous administration.* Two vitamin A deficient rats were each given 2mg. of 3-dehydroretinal subcutaneously. The rats were relieved of all the symptoms of vitamin A deficiency in 1-2 days and gained, on average, 82g. in weight in 4 weeks, after which they were killed. The liver storage of 3-dehydrovitamin A was 9.6% of the administered dose. The analysis of the subcutaneous tissue at the site of injection showed no form of 3-dehydrovitamin A.

*Relationship between 3-dehydroretinol concentrations of blood and liver.* Different dosages of 3-dehydroretinal (Table 3) were administered and the blood and kidney 3-dehydrovitamin A as well as liver unesterified 3-dehydrovitamin A were determined. The plot of 3-dehydrovitamin A concentration in blood ( $\mu\text{g./100 ml.}$ ) against 3-dehydroretinol concentration in liver ( $\mu\text{g./g.}$ ) showed a linear relationship (Fig. 1).

Table 3 also shows the relationship between 3-dehydrovitamin A concentration in kidney ( $\mu\text{g./g.}$ ) and dose of 3-dehydroretinal administered.

#### DISCUSSION

Cama, Dalvi, Morton & Salah (1952b) have shown that 3-dehydroretinal is reduced to 3-dehydroretinol in the small intestine of the rat and subsequently transported to liver for storage. The present investigations have confirmed this observation (Table 1).

Intraperitoneal or subcutaneous administration of 3-dehydroretinal induces a better growth response in rat than oral feeding. The compound is stored in the liver mainly as the higher fatty acid ester. After intraperitoneal administration, appreciable amounts of 3-dehydrovitamin A were detected in the intestinal wall (Table 2). In contrast with studies on the metabolism of 5,6-mono-

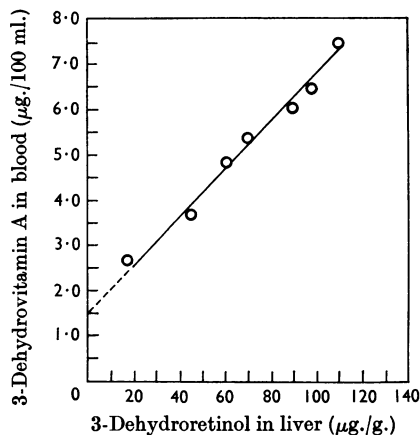


Fig. 1. Relationship between concentrations of total 3-dehydrovitamin A in blood and 3-dehydroretinol in liver.

epoxyretinal (Lakshmanan *et al.* 1965), 3-dehydroretinol could be detected in the liver, spleen and kidney of the rat after the administration of 3-dehydroretinal intraperitoneally after enterectomy.

The ratio of 3-dehydroretinyl higher fatty acid ester to 3-dehydroretinol in liver increased progressively up to 3 days, after which the ratio remained fairly constant at 93:7. The liver 3-dehydroretinyl higher fatty acid ester behaves as a single spot on thin-layer and reverse-phase chromatography.

The small intestine has been shown to be the main site of conversion of  $\beta$ -carotene into retinol (Glover, Goodwin & Morton, 1947b; Sexton, Mehl & Deuel, 1946; Thompson, Ganguly & Kon, 1947). Retinal is postulated to be an intermediate in this conversion. It has been demonstrated (Budowski & Gross, 1965) that the mouse intestine can convert 3-dehydro- $\beta$ -carotene into 3-dehydroretinol. Since the present investigations show that

3-dehydroretinal is rapidly reduced by the rat intestine, it is reasonable to assume that 3-dehydro- $\beta$ -carotene is converted into 3-dehydroretinol via 3-dehydroretinal.

According to Glover *et al.* (1947a), when the liver retinol concentration is varied between 25 and 820 i.u./g. there is a concomitant variation in plasma retinol concentration between 35 and 183 i.u./100ml. However, according to Ganguly & Krinsky (1953), despite the variation in the liver retinol concentration from 0.1 to 169  $\mu$ g./g. the plasma retinol concentration remains fairly stable between 17.4 and 27.0  $\mu$ g./100ml.

A direct proportionality between the 3-dehydrovitamin A concentration in blood and the unesterified 3-dehydrovitamin A concentration in liver has now been demonstrated (Fig. 1), as has been reported by Glover *et al.* (1947a) for vitamin A. Further, our results show a high coefficient of correlation (0.995). Most of the 3-dehydrovitamin A present in blood was found to be in the alcohol form. With vitamin A, the discrepancy existing between the two groups of workers with regard to the relationship between the concentrations of blood vitamin A and unesterified vitamin A in liver seems to be due, at least in part, to differences in the experimental designs employed by the two groups. The rats used by the former group were 6-9 months old, whereas the latter group used mostly 3-5-month-old rats maintained on different diets. However, all the experimental animals used in the present investigations received the same diet, and other previous treatments were uniform so as to reduce the variables as much as possible. At the same time, the present investigations are partly in accordance with the observations of Ganguly & Krinsky (1953) in that the blood 3-dehydrovitamin A concentration varies only between 2.5 and 7.5  $\mu$ g./100ml. when the liver concentration is varied between 20 and 110  $\mu$ g./g. Thus it is likely that, although the blood vitamin A concentration may not vary between wide limits at various concentrations of liver unesterified vitamin A, a close proportionality between the blood and liver unesterified vitamin A does exist.

Fig. 1 shows that the curve does not pass through the origin, but cuts the ordinate at a point representing a value of 1.52  $\mu$ g./100ml. which is the blood concentration of 3-dehydroretinol at zero liver storage. There is no strict proportionality between the 3-dehydroretinol concentration in liver and the 3-dehydroretinal dose administered. Similar observations have been made by Glover *et al.* (1947a) in their experiments with retinal.

Irrespective of the dosage and the time-interval after the administration of 3-dehydroretinal, the percentage of 3-dehydrovitamin A stored in the liver is lower than that with retinal (Glover *et al.*

1948; Ganguly & Krinsky, 1953). This implies that retinal is metabolized by the rat much more efficiently than 3-dehydroretinal. Lakshmanan, Vaidyanathan & Cama (1964), in their studies on liver aldehyde oxidase and vitamin A metabolism, have demonstrated that the rat may not be able to metabolize 3-dehydroretinal and related compounds as efficiently as retinal and related compounds.

Although at the high dosages of 7.5 and 9.0 mg./day the rat is unable to metabolize efficiently or tolerate 3-dehydroretinal for more than 3 days, rats receiving retinyl acetate at similar dosages show no detectable symptoms of hypervitaminosis A within this period.

*3-Dehydrovitamin A in kidneys.* The percentage of 3-dehydrovitamin A stored in kidneys when 3-dehydroretinal is administered intraperitoneally is much less than after oral administration. Even in the enterectomy experiment, 3-dehydroretinol could be detected in kidneys. The concentrations of 3-dehydrovitamin A present in kidneys at various oral dosages of 3-dehydroretinal are shown in Table 3. The optimum dosage at which the maximum concentration of 3-dehydrovitamin A is retained in the kidneys was 4.5 mg./day for 3 days (Table 3). Most of the 3-dehydrovitamin A present in kidneys was in the alcohol form, as shown by its behaviour on thin-layer and reverse-phase chromatography, but traces of the ester form could also be detected.

These observations indicate a degree of threshold exhibited by the kidneys towards the retention of 3-dehydrovitamin A, and a similar phenomenon may also occur with vitamin A.

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