Preparation, Properties and Metabolism of *retro*-3-Dehydroretinyl Acetate

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1. Treatment of 3-dehydroretinyl acetate with aqueous hydrobromic acid resulted in the formation of *retro*-3-dehydroretinyl acetate, which, on alkaline hydrolysis, gave the corresponding alcohol. 2. *retro*-3-Dehydroretinyl acetate was isomerized to 3-dehydrovitamin A when fed to vitamin A-deficient rats. 3. When *retro*-3-dehydroretinyl acetate was administered orally, it was hydrolysed to *retro*-3-dehydroretinol in the rat intestine, isomerized to 3-dehydroretinol and esterified before being transported to the liver for storage. 4. When administered intraperitoneally, both 3-dehydrovitamin A and *retro*-3-dehydrovitamin A were accumulated in liver and other tissues, whereas after enterectomy 3-dehydrovitamin A was not detected anywhere in the body. 5. The small intestine was shown to be the major site of conversion of *retro*-3-dehydrovitamin A into 3-dehydrovitamin A. 6. The extent of conversion of *retro*-3-dehydroretinyl acetate into 3-dehydrovitamin A was much smaller than that of the conversion of *retro*-retinyl acetate into vitamin A. 7. The biological potency of *retro*-3-dehydroretinyl acetate, determined by the rat-growth assay, was 2.6% of that of all-*trans*-retinyl acetate, when given orally.

Retinol and 3-dehydroretinol are converted into the corresponding anhydrovitamin A compounds on treatment with ethanolic hydrogen chloride (Shantz, Cawley & Embree, 1943; Shantz, 1948; Cama, Dalvi, Morton & Salah, 1952a). During this conversion, there is a shift of all the conjugated double bonds, along with dehydration at the end of the side chain. In the absence of the alcoholic group in the molecule, as in retinyl acetate, acid treatment results in the formation of *retro*-retinyl acetate, in which only the double bonds are shifted (Beutel, Hinkley & Pollack, 1955). Such a conversion is indicated by the shift of the absorption maximum to longer wavelengths and by the appearance of fine structure in the spectrum.

Vitamin A compounds possessing the *retro* system of conjugated double bonds have been encountered in biological samples (Balasundaram, Bamji, Cama, Sundaresan & Varma, 1958; Varma, Erdody & Murray, 1965a). The structure of the naturally occurring anhydrovitamin A₂ was shown to be 3-hydroxyanhydrovitamin A₁ (Barua & Nayar, 1966), whereas the chemically synthesized anhydrovitamin A₂ was 3-ethoxyanhydrovitamin A₁ (Henbest, Jones, Owen & Thaller, 1955a). When chemically synthesized anhydrovitamin A₂ was fed to vitamin A-deficient rats, it gave rise to 'rehydrovitamin A₂' (Balasundaram *et al.* 1958; Bamji, Cama & Sundaresan, 1962).

retro-Retinyl acetate was shown to be isomerized to vitamin A in the rat (Varma & Murray, 1963; Murray & Erdody, 1966; John, Lakshmanan & Cama, 1967) and was distinctly different from 'rehydrovitamin A₁' isolated from liver after anhydrovitamin A₁ had been fed to vitamin A-deficient rats (Shantz, 1950; Varma, Erdody & Murray, 1965b).

retro-3-Dehydrovitamin A and 'rehydrovitamin A₂' have not been hitherto studied in detail. The present paper describes the preparation, properties and metabolism of *retro*-3-dehydroretinyl acetate.

EXPERIMENTAL

Preparation of 3-dehydroretinyl acetate. 3-Dehydroretinyl acetate was prepared from 3-dehydroretinal by reduction and subsequent acetylation of 3-dehydroretinol. 3-Dehydroretinal was prepared from the liver oil of the Indian freshwater fish *Wallago attu*, which is rich in 3-dehydrovitamin A (Balasundaram, Cama, Sundaresan & Varma, 1956). The livers were homogenized in a Waring Blender, and extracted repeatedly with light petroleum (b.p. 40–60°)–diethyl ether mixtures. The extract was dried over anhydrous Na₂SO₄, filtered and decreased in volume. The concentrated oil was kept at –30° overnight, and the precipitated sterols were removed by filtration at –30°. The filtrate after decrease in volume *in vacuo* was saponified with ethanolic KOH, and the 3-dehydroretinol fraction was oxidized over MnO₂ (Cama *et al.* 1952b). 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 385m μ in light petroleum (b.p. 40–60°). Alternatively, 3-dehydroretinal was prepared from retinal by the method of Henbest, Jones & Owen (1955b) by brominating retinal with *N*-bromosuccinimide and then dehydrobrominating the product with *N*-phenylmorpholine. 3-Dehydroretinal was reduced with LiAlH_4 to 3-dehydroretinol, which was then acetylated with acetyl chloride to 3-dehydroretinyl acetate.

Preparation of retro-3-dehydrovitamin A compounds. *retro*-3-Dehydroretinyl acetate was prepared as follows. 3-Dehydroretinyl acetate (250mg.) ($E_{1\text{cm}}^{1\%}$ 1270 at 350m μ) was dissolved in 10ml. of methylene chloride and cooled to 0°. The solution was mixed with 5ml. of ice-cold aq. HBr and shaken at 0° for 5min. The conversion of 3-dehydroretinyl acetate into the corresponding *retro*-vitamin A compound was indicated by the deep-greenish coloration of the reaction mixture. If the reaction was continued beyond 5min., the reaction mixture became brownish, probably as a result of decomposition. The reaction was accordingly stopped after 5min. by adding aq. 5% NaHCO_3 . The organic layer was washed with water till free of alkali, dried over anhydrous Na_2SO_4 and filtered. The solvent was removed *in vacuo*. The resulting red-yellow oil had an absorption spectrum with λ_{max} 295, 350, 370, 390 and 415m μ and an inflexion at 330m μ . A solution of this oil in light petroleum (b.p. 40–60°) was chromatographed over 6% (v/w) water-deactivated neutral alumina (Jungalwala & Cama, 1965). On elution with diethyl ether-light petroleum (b.p. 40–60°) (1:49, v/v), a single band was obtained. All attempts to crystallize the compound after repeated chromatography were unsuccessful. The $E_{1\text{cm}}^{1\%}$ value was not increased by chromatography. The compound was entirely homogeneous; it moved as a single spot on thin-layer chromatography with acetone-cyclohexane (1:99, v/v) as solvent system (John, Lakshmanan, Jungalwala & Cama, 1965).

retro-3-Dehydroretinol was prepared by alkaline hydrolysis of the acetate with ethanolic 10% KOH. It was chromatographed over 8% (v/w) water-deactivated neutral alumina; *retro*-3-dehydroretinol was eluted with diethyl ether-light petroleum (b.p. 40–60°) (2:23, v/v). The spectroscopic properties of the alcohol were identical with those of the acetate.

retro-3-Dehydroretinyl palmitate was prepared from 3-dehydroretinyl palmitate by the same procedure used for the preparation of *retro*-3-dehydroretinyl acetate.

Oral administration. For metabolic studies, the compound was dissolved just before use in refined groundnut oil containing 50% (w/w) of α -tocopherol. For bioassay experiments, *retro*-3-dehydroretinyl acetate was freshly prepared every 3 days and administered daily in concentrations of 10 and 15 $\mu\text{g.}/0.1\text{ ml.}$ of refined deodorized groundnut oil containing 0.5% (w/w) of α -tocopherol. all-*trans*-Retinyl acetate was given in concentrations of 0.5 and 1.0 $\mu\text{g.}/0.1\text{ ml.}$ of diluent oil.

Preparation of tissues for experiments in vitro. For experiments with everted intestinal sacs, normal rats weighing 120–150g. were starved for 24hr. and killed by a blow on the head. The middle one-third of the intestine of each rat was washed twice with ice-cold 0.9% NaCl, everted as described by Wilson & Wiseman (1954) and filled with 5.0ml. of Krebs-Ringer phosphate buffer, pH 7.4.

The everted sac was put in 20ml. of the same buffer containing 5mg. of sodium taurocholate and 500 $\mu\text{g.}$ of GSH. The compound under study was dissolved in ethanol and dispersed in sodium phosphate buffer by using Tween 20.

Preparation of tissue extracts. The procedure followed for the extraction of tissues was essentially the same as that described by Glover, Goodwin & Morton (1948).

Separation of retro-3-dehydroretinol and retro-3-dehydroretinyl palmitate from the corresponding 3-dehydrovitamin A compounds by thin-layer chromatography on Kieselgel (John et al. 1965). *retro*-3-Dehydroretinol and 3-dehydroretinol were separated by using acetone-light petroleum (b.p. 40–60°) (2:23, v/v) as solvent system; the R_F values were: *retro*-3-dehydroretinol, 0.31; 3-dehydroretinol, 0.26. *retro*-3-Dehydroretinyl palmitate, *retro*-3-dehydroretinyl acetate and 3-dehydroretinyl palmitate were separated in the same way by using acetone-cyclohexane (1:99, v/v) as solvent system; the R_F values were: *retro*-3-dehydroretinyl acetate, 0.47; *retro*-3-dehydroretinyl palmitate, 0.71; 3-dehydroretinyl palmitate, 0.79. In the above system 3-dehydroretinyl palmitate moved faster than *retro*-3-dehydroretinyl palmitate, unlike 3-dehydroretinol, which moved slower than *retro*-3-dehydroretinol.

Estimations. All the quantitative data relating to *retro*-3-dehydrovitamin A were calculated on the basis that the purest *retro* compound obtained was the acetate. When 3-dehydrovitamin A or *retro*-3-dehydrovitamin A was present as a single component in tissue extracts, it was determined by the SbCl_3 colour-test method; the $E_{1\text{cm}}^{1\%}$ value for 3-dehydrovitamin A was 3870 at 693m μ (Cama & Morton, 1953), and that for *retro*-3-dehydrovitamin A was 1420 at 670m μ . However, when these two compounds were present together, the procedure employed for the determination of the individual components from a mixture of the two was similar to that used by Varma & Murray (1963) and John et al. (1967) for vitamin A and *retro*-vitamin A. The ratio E_{390}/E_{350} for various mixtures containing known amounts of 3-dehydrovitamin A and *retro*-3-dehydrovitamin A was linearly related to the percentage of *retro*-3-dehydrovitamin A. From the E_{390}/E_{350} ratio of the mixture, the percentages of the components were calculated by using the standard graph. From the total extinction at 390m μ of the mixture in light petroleum (b.p. 40–60°) and the known $E_{1\text{cm}}^{1\%}$ values of *retro*-3-dehydrovitamin A (734) and 3-dehydrovitamin A (528), the actual amounts present were calculated. This method was found to be fairly good only with chromatographed samples of the mixtures.

For bioassay and metabolic studies, male albino rats of this Institute's strain, made deficient in vitamin A, were used (Lakshmanan, Jungalwala & Cama, 1965). The biological assays were carried out essentially as described by Bliss & György (1951) according to U.S.P. XIV specifications (1950).

Spectroscopic measurements. The ultraviolet- and visible-absorption spectra of the compounds were measured with a Beckman model DU spectrophotometer. Infrared-absorption spectra of the compounds dissolved in CCl_4 [1% (w/v) solutions] were determined in 0.1cm.-light-path NaCl cells with a Perkin-Elmer Infracord double-beam spectrometer. The nuclear-magnetic-resonance spectrum was recorded on a Varian A-60 spectrometer with tetramethylsilane as the reference standard, at the National Chemical Laboratory, Poona, India.

Table 1. *Spectroscopic properties of retro-3-dehydrovitamin A compounds*

Compound	Light petroleum (b.p. 40–60°)		Cyclohexane		Ethanol		Chloroform		SbCl ₃ colour test ($E_{1\text{cm.}}^{1\%}$)
	$\lambda_{\text{max.}}$ (m μ)	$E_{1\text{cm.}}^{1\%}$	$\lambda_{\text{max.}}$ (m μ)	$E_{1\text{cm.}}^{1\%}$	$\lambda_{\text{max.}}$ (m μ)	$E_{1\text{cm.}}^{1\%}$	$\lambda_{\text{max.}}$ (m μ)	$E_{1\text{cm.}}^{1\%}$	
<i>retro</i> -3-Dehydro- retinyl acetate	295	406	295	423	295	432	297	312	1240 at 670m μ
	333	356	335	325	350	410	355	363	
	350	415	352	381	370	510	375	451	
	370	519	372	471	390	614	397	552	
	390	640	393	577	415	515	420	454	
<i>retro</i> -3-Dehydro- retinol	415	523	417	483					
	295	410	295	427	295	435	297	315	1350 at 670m μ
	333	360	335	329	350	413	355	365	
	350	420	352	392	370	512	375	453	
	370	522	372	480	390	620	397	560	
	390	650	393	585	415	517	420	455	
3-Dehydroretinol*	415	530	417	490					
	348	1390	352	1320	351	1410	357	1280	3870 at 693m μ
	286	750	287	733	286	698	292	704	

* Results from Cama & Morton (1953).

RESULTS

The $E_{1\text{cm.}}^{1\%}$ values of *retro*-3-dehydroretinyl acetate and the alcohol in different solvents are lower than those of 3-dehydroretinol (Table 1). Typical ultraviolet- and visible-absorption spectra of *retro*-3-dehydroretinyl acetate in light petroleum (b.p. 40–60°) and in cyclohexane are shown in Fig. 1. Compared with those of 3-dehydroretinyl acetate, the absorption maxima of *retro*-3-dehydroretinyl acetate are displaced towards longer wavelengths and the extent of the fine structure is increased.

In the infrared spectrum of *retro*-3-dehydroretinyl acetate, there is a strong band at 1742cm.⁻¹, which is due to the C=O stretching of the acetate group. The strong band at 1238cm.⁻¹ arises from the C–O–C asymmetric stretching of the acetate group. A doublet at 1377 and 1361cm.⁻¹ is due to –CH₃ symmetric deformation of the *gem*-dimethyl group. C=C stretching of conjugated double bonds is shown by the presence of a band at 1634cm.⁻¹.

Metabolic experiments

Oral administration. Four vitamin A-deficient rats were given *retro*-3-dehydroretinyl acetate orally for 7 days (1.0mg./rat/day). The average weight gain of the rats during this period was 9g. They were anaesthetized and killed 24hr. after the last dose, and the various tissues were pooled and analysed. All the tissues showed the presence of 3-dehydrovitamin A (Table 2), whereas *retro*-3-dehydrovitamin A was found only in the intestine and the liver. The lipid extract from liver was chromatographed over 5% (v/w) water-deactivated neutral alumina. On elution with light petroleum

Fig. 1. Ultraviolet-absorption spectra of *retro*-3-dehydroretinyl acetate in light petroleum (○) and in cyclohexane (●).

(b.p. 40–60°), a fraction could be obtained that showed the characteristic properties of 3-dehydrovitamin A. The identification was confirmed by saponification, conversion of the alcohol into anhydrovitamin A₂ by ethanolic hydrogen chloride and oxidation of the alcohol with manganese dioxide to 3-dehydroretinal.

To investigate the active absorption of *retro*-3-dehydroretinyl acetate and its probable site of conversion into 3-dehydrovitamin A, time-distribution studies were carried out with vitamin A-deficient rats. 3-Dehydrovitamin A was detected in the liver from 3hr. after administration. Both 3-dehydrovitamin A and *retro*-3-dehydrovitamin A were found in the liver for up to 24hr. (Table 3).

Intra-intestinal administration. This experiment was performed as described by Lakshmanan *et al.*

Table 2. *Recovery of 3-dehydrovitamin A and retro-3-dehydrovitamin A from various tissues of rats given retro-3-dehydroretinyl acetate (1.0mg./rat/day) for 7 days orally or intraperitoneally*

Tissue	Recovery (% of administered dose)			
	Oral		Intraperitoneal	
	retro-3-Dehydro-vitamin A	3-Dehydro-vitamin A	retro-3-Dehydro-vitamin A	3-Dehydro-vitamin A
Intestine	0.037	0.037	2.737	0.147
Liver	0.481	3.093	2.699	0.376
Blood	—	0.003	—	0.005
Kidneys	—	Traces	Traces	Traces
Spleen	—	Traces	Traces	Traces
Lung	—	Traces	Traces	Traces

Table 3. *Conversion of retro-3-dehydroretinyl acetate into 3-dehydrovitamin A in the rat after oral feeding of 2.0mg. of retro-3-dehydroretinyl acetate*

Rat no.	Time between administration and killing (hr.)	retro-3-Dehydrovitamin A recovered (μg.)						3-Dehydrovitamin A recovered (μg.)				Total recovery of 3-dehydrovitamin A and retro-3-dehydrovitamin A (% of dose)
		Stomach	Intestinal washings	Intestine		Liver		Intestine		Liver		
				Ester	Alcohol	Ester	Alcohol	Ester	Alcohol	Ester	Alcohol	
1	Control	—	—	—	—	—	—	—	—	—	—	—
2	1	654.2	—	—	—	—	—	—	—	—	—	32.7
3	1	719.4	176.2	113.8	—	—	—	—	—	—	—	50.5
4	3	Traces	107.8	84.4	84.0	31.2	12.1	—	—	—	8.4	16.4
5	6	—	10.6	11.5	9.8	8.6	2.6	6.5	4.6	30.6	17.3	5.1
6	12	—	—	—	—	18.2	—	—	—	17.9	Traces	1.8
7	24	—	—	—	—	85.2	—	—	—	64.3	Traces	7.5

(1965). *retro*-3-Dehydroretinyl acetate (2.0mg.) was administered to a vitamin A-deficient rat, and the animal was killed 5hr. after the administration of the compound. The lipid extracts from both the liver and the intestine showed the presence of 3-dehydrovitamin A.

Intraperitoneal administration. Three vitamin A-deficient rats were given *retro*-3-dehydroretinyl acetate intraperitoneally (1.0mg./rat/day) for 7 days; the average weight-gain of these rats during the period was 10g. They were anaesthetized and killed 24hr. after the last dose and the various tissues pooled and analysed. All the tissues showed the presence of 3-dehydrovitamin A and *retro*-3-dehydrovitamin A (Table 2). *retro*-3-Dehydrovitamin A and 3-dehydrovitamin A in trace amounts were identified by their thin-layer-chromatographic behaviour and also by the antimony trichloride colour on the chromatogram.

Enterectomy. A vitamin A-deficient rat was kept under ether anaesthesia and the intestine was removed as described by Lakshmanan *et al.* (1965). *retro*-3-Dehydroretinyl acetate (2.0mg.) was injected intraperitoneally and the rat was returned to the cage. It was killed 8hr. after the administration of the compound. The lipid extract from liver

contained *retro*-3-dehydrovitamin A (46 μ g.); however, 3-dehydrovitamin A was not detectable. Traces of *retro*-3-dehydrovitamin A were present in lungs and kidneys, but no 3-dehydrovitamin A was detectable anywhere in the body.

Subcutaneous administration. Two vitamin A-deficient rats were each given 3.0mg. of *retro*-3-dehydroretinyl acetate subcutaneously. The rats were relieved of all symptoms of vitamin A deficiency within 3 days and gained an average of about 45g. in weight in 3 weeks, after which they were anaesthetized and killed. Analyses of the livers and the subcutaneous tissue at the site of injection failed to show the presence of 3-dehydrovitamin A or *retro*-3-dehydrovitamin A.

Conversion of retro-3-dehydrovitamin A into 3-dehydrovitamin A in vitro

Experiment with everted intestinal sacs. This experiment was conducted as described in the Experimental section. *retro*-3-Dehydroretinyl acetate (100 μ g.) was added to the buffer medium containing one everted intestinal sac, and this was kept in a metabolic shaker at 37° for 2hr. The sacs from four reaction flasks were washed with ethanol,

and the serosal fluid as well as the intestinal wall was analysed. The lipid extract from serosal fluid showed the characteristic fluorescence of 3-dehydrovitamin A. It gave two spots on thin-layer chromatography corresponding to 3-dehydroretinol and 3-dehydroretinyl higher fatty acid ester. Another spot corresponding to unchanged *retro*-3-dehydroretinyl acetate was also observed. The lipid extract from the intestinal wall showed the presence of both 3-dehydrovitamin A and *retro*-3-dehydrovitamin A. The extent of conversion of *retro*-3-dehydrovitamin A into 3-dehydrovitamin A was very small (0.5–1.0%).

Biological potency of retro - 3 - dehydroretinyl acetate. The growth rate (g./week) over 4 weeks of individual litter-materats receiving *retro*-3-dehydroretinyl acetate and retinyl acetate orally was measured. All the negative controls lost considerable weight and died before the end of the assay. The mean weekly gain in weight (6 and 8g. for the daily dosages of 10 and 15 μ g. of *retro*-3-dehydroretinyl acetate and 9.6 and 13.0g. for the daily dosages of 0.5 and 1.0 μ g. of all-*trans*-retinyl acetate) plotted against the logarithm of dose gave lines parallel to each other for the standard and the test doses. The biological potency of *retro*-3-dehydroretinyl acetate, as compared with that of all-*trans*-retinyl acetate, was 2.6%.

DISCUSSION

Beutel *et al.* (1955) transformed retinyl acetate into *retro*-retinyl acetate in good yield by treatment with aqueous hydrobromic acid. The present work demonstrates that treatment of 3-dehydroretinyl acetate with aqueous hydrobromic acid gives rise to a major fraction with $\lambda_{\max}$. 295, 350, 370, 390 and 410 $m\mu$ (Table 1). Transition from normal to *retro* structures gives rise to conspicuous spectroscopic changes in the visible region, the near-ultraviolet region or both (Oroshnik, Karmas & Mebane, 1952). When vitamin A acquires *retro* structure, the absorption maxima are displaced towards longer wavelengths and the extent of the fine structure increases (Beutel *et al.* 1955). Double-bond shifts

in 3-dehydroretinyl acetate due to acid treatment can result in the formation of structure (II) or structure (III). A double bond exocyclic to a six-membered ring, as in structure (III), is less stable than the corresponding endocyclic double bond as in structure (II) (Woodward, 1942). Structure (II) is assigned to the hydrobromic acid reaction product of 3-dehydroretinyl acetate on the basis of ultraviolet, infrared and nuclear-magnetic-resonance spectra.

By the reaction of anhydrovitamin A₁ with *N*-bromosuccinimide and subsequent dehydrobromination, Henbest *et al.* (1955a) isolated a compound (compound IV) exhibiting a main absorption maximum at 408 $m\mu$ with subsidiary maxima at 387 and 433 $m\mu$. The absorption maxima of the hydrobromic acid reaction product of

Table 4. *retro*-Ionylidene compounds

No. of conjugated double bonds in <i>retro</i> structure	$\lambda_{\max}$. (m μ)	Compound	Reference
4	— 317, 331	—	Oroshnik <i>et al.</i> (1952)
5	332.5, 348, 367	<i>retro</i> -Retinyl methyl ether	Oroshnik <i>et al.</i> (1952)
6	351, 371, 392	Anhydrovitamin A ₁	Shantz (1950)
7	374, 393, 415	Anhydroretinylidene propan-2-ol	Hawkins & Hunter (1944)
8	388, 409, 436	Anhydro- β -apo-12'-carotenol	Rüegg <i>et al.</i> (1959)
6	370, 390, 415	<i>retro</i> -3-Dehydroretinyl acetate (compound II)	Present work
7	390, 410, 435	Compound (IV)	Henbest <i>et al.</i> (1955a)

3-dehydroretinyl acetate clearly indicate that it possesses one double bond fewer than compound (IV).

Several *retro*-ionylidene compounds possessing four to eight double bonds in conjugation are known (Table 4). Compounds containing homoannular diene units absorb in the ultraviolet at higher wavelengths than similar compounds containing heteroannular diene units (Scott, 1964). Anhydrovitamin A₁ has only six conjugated double bonds and absorbs maximally at 371 mμ. Anhydroretinylidene propan-2-ol has an additional double bond in conjugation to give a total of seven conjugated double bonds, which causes a bathochromic shift to 393 mμ. *retro*-3-Dehydroretinyl acetate, on the other hand, although it contains only six double bonds in conjugation, has a homoannular diene unit, which is responsible for shifting the λ_{max.} to a higher wavelength (390 mμ). Although both compound (IV) and anhydroretinylidene propan-2-ol possess seven conjugated double bonds, the absorption maximum of the former is at 408 mμ and that of the latter is at 393 mμ. The factor responsible for the higher λ_{max.} of compound (IV) is also evidently the homoannular diene system present.

The infrared-absorption spectrum of *retro*-3-dehydroretinyl acetate is very similar to that of *retro*-retinyl acetate (compound I). In the nuclear-magnetic-resonance spectrum of *retro*-3-dehydroretinyl acetate, there is a clear triplet centred at 4.13 p.p.m. due to methylene protons at C-15. This signal is split into a triplet because of spin-spin coupling with the adjacent C-14 methylene protons. In the lower field, 5.7–5 p.p.m., eight olefinic protons are available. The methyl protons (3H) of the acetoxy group appear as a sharp signal at 1.95 p.p.m. These are consistent with the proposed structure of *retro*-3-dehydroretinyl acetate (structure II). The proposed structure (II) for *retro*-3-dehydroretinyl acetate is further supported by the isomerization of the compound in the rat to 3-dehydrovitamin A, which is closely analogous to the isomerization of the chemically synthesized *retro*-retinyl acetate to vitamin A (Varma & Murray, 1963; Murray & Erdody, 1966; John *et al.* 1967).

Balasundaram *et al.* (1958) and Bamji *et al.* (1962) isolated 'rehydrovitamin A₂' from the liver of vitamin A-deficient rats given anhydrovitamin A₂. However, the spectroscopic properties and $\epsilon_{1\text{cm.}}^{1\%}$ values of rehydrovitamin A₂ and *retro*-3-dehydrovitamin A are very different. Moreover, there is no report that rehydrovitamin A₂ gives rise to 3-dehydrovitamin A in the rat, whereas *retro*-3-dehydrovitamin A does give rise to 3-dehydrovitamin A in the rat. Thus rehydrovitamin A₂ and *retro*-3-dehydrovitamin A are not the same, but different entities.

When administered orally to vitamin A-deficient

rats at 1.0 mg./rat/day (Table 2), *retro*-3-dehydroretinyl acetate was stored in the liver mainly in the form of 3-dehydrovitamin A; an appreciable amount of *retro*-3-dehydrovitamin A was also present. At the same time, all the rats were alleviated of their vitamin A-deficiency symptoms and grew well. All these findings imply that, unlike retinoic acid or the epoxides of vitamin A, which exert their biological activities as such and not through vitamin A (Van Dorp & Arens, 1946; Lakshmanan *et al.* 1965), *retro*-3-dehydrovitamin A exerts its biological activity after its conversion into 3-dehydrovitamin A. John *et al.* (1967) were not able to detect *retro*-vitamin A in the liver when *retro*-retinyl acetate was administered orally to vitamin A-deficient rats at 1.0 mg./rat/day. The presence of appreciable amounts of *retro*-3-dehydrovitamin A in the liver implies that the extent of conversion of *retro*-3-dehydrovitamin A into 3-dehydrovitamin A, unlike that of *retro*-vitamin A into vitamin A, is small, and *retro*-3-dehydrovitamin A is not metabolized by the rat as efficiently as *retro*-vitamin A.

When *retro*-3-dehydroretinyl acetate was administered peritoneally, it was taken up directly by various tissues, and then probably underwent isomerization in the intestine (Table 2). *retro*-3-Dehydroretinol was detected in the intestine, which implies that *retro*-3-dehydroretinyl acetate is hydrolysed in the intestine to give the alcohol, which is further isomerized, esterified and transported to the liver for storage. When the compound was administered intraperitoneally after enterectomy, no 3-dehydrovitamin A was detected anywhere in the body, in spite of the fact that *retro*-3-dehydrovitamin A was taken up by all the tissues examined. The inefficiency of the rat in isomerizing *retro*-3-dehydrovitamin A after its intestine was removed clearly shows that the intestine is the major, if not the only, site of isomerization.

John *et al.* (1967) detected vitamin A in the liver and intestine of vitamin A-deficient rats as early as 1 hr. after administration of *retro*-retinyl acetate. With *retro*-3-dehydroretinyl acetate, 3-dehydrovitamin A was detected in the liver only from 3 hr. after administration of the compound, and was present up to 24 hr. (Table 3). This indicates that the absorption of *retro*-3-dehydrovitamin A and its isomerization to 3-dehydrovitamin A are slower processes than with *retro*-vitamin A. These findings suggest that 3-dehydrovitamin A compounds are not metabolized by the rat as efficiently as vitamin A compounds.

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