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Formation of Cholesta-3:5-dien-7-one in Rats

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Addition of cholesterol to the diet of rats is well known to result in fatty infiltration of the liver (Chanutin & Ludewig, 1933; Sperry & Stoyanoff, 1934), but the process is not fully understood.

Dehydrogenation of cholesterol to provitamin D₃ can occur in the intestinal mucosa (Glover, Glover & Morton, 1952) on a significant though small scale.

Cholesta-3:5-dien-7-one is a potentially significant metabolite and we have here studied its formation in rats.

EXPERIMENTAL

Animals and diets. Ten healthy adult male hooded rats very similar in age and weight were divided into groups of five; one group was fed on crushed cubes (15 g./rat/day) to which 5% (w/w) arachis oil had been added; the other group was given a diet made up of 98% of the above (control) diet and 2% purified cholesterol. At the end of 60 days, the animals were killed. The cholesterol was given in the food in the ordinary way because previous trials had shown that rats refused an aqueous dispersion of cholesterol in the

detergent Tween 80. In the 8th week, the cholesterol-fed rats became inactive and dull compared with the control animals.

Examination. The rats were killed (CHCl₃), and the abdomen laid open for inspection of the organs *in situ*. The organs were removed and the livers were tested for vitamin A and other lipid constituents, after portions had been removed for preparing sections. Unsaponifiable fractions of lipids were obtained and subjected to chromatography on alumina, followed by spectrophotometric study of the fractions obtained.

RESULTS

Tables 1 and 2 summarize the results. Cholesterol feeding reduced the total vitamin A in each rat liver by about one-third, or (expressed as vitamin/g. liver) by half. A trace of cholesta-3:5-dien-7-one was found in the normal livers (<0.06 mg./rat), whereas 0.6 mg./rat was found in the cholesterol-fed animals. 7-Dehydrocholesterol was not detectable in the fractions from the chromatography of the normal liver unsaponifiable matter, but approximately 1.6 mg. was present in the appropriate fraction from the cholesterol-fed animals.

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Table 1. *Comparison of normally fed rats with similar animals given 2% cholesterol as a dietary addition*

	Normal rats	Cholesterol-fed rats
Abdomen	Normal	Appeared larger, and fuller
Intestines	Normal	Apparently normal
Liver, wet wt. (five livers)	45.3 g.	60 g.
Liver appearance	Normal size and colour, edges well defined	Enlarged, inelastic yellow spots, edges rounded (one lobe pale yellow, very soft)
	Normal histological picture	Sections stained with Sudan red, Sudan black and osmic acid
		Some cells showed heavy fatty infiltration.
		Much deposition of cholesterol
Aorta	Normal	No thickening, no fatty degeneration observed
Liver unsaponifiable fraction	0.275 g. (0.605% on liver wt.)	0.395 g. (0.66% on liver wt.)
$E_{1\text{cm}}^{1\%}$ (325 mμ.)	71 gross (53.1 corr.)*	30 gross (24.4 corr.)
Vitamin A/rat	5500 i.u.	3662 i.u.
Vitamin A/g. liver	607 i.u.	305 i.u.

* Allowance made for irrelevant absorption see Cama, Collins & Morton (1952).

Table 2. *Chromatographic examination of liver unsaponifiable matter from livers of normally fed rats and rats receiving 2% dietary cholesterol*

Adsorbent: Spence (Grade 0) alumina weakened by addition of 5% (w/w) water. Solvent, light petroleum except where stated.

		Normal rats			Cholesterol-fed rats			
Dienone in 5 livers ...		<0.3 mg. Spectrum			3.0 mg. Spectrum			
Fraction	Eluent: ether (%) in light petroleum	Wt. of eluate (mg.)	$\lambda_{\max}$ (m μ .)	$E_{1\%}^{1\text{cm.}}$ (m μ .)	Wt. of eluate (mg.)	$\lambda_{\max}$ (m μ .)	$E_{1\%}^{1\text{cm.}}$ (m μ .)	Type of absorbing substance
1	2	2	270	92.5	15	268	137.5	3:5-Dien-7-one
			325	37.5	—	325	37.5	Vitamin A (?).
2	10	83	325	80.5	47.4	325	19.3	Vitamin A
3	20	132	General absorption only		302	270	1.51	5:7-Dien-3 β -ol
						281		
						293.5		
4	100	13	Slight inflexion 290		23	235 (ethanol)		Unsaturated ketone?
						285		

The 2% ether-light petroleum eluate (Table 2) which showed $\lambda_{\max}$ 268 m μ . in light petroleum was reduced to dryness and taken up in ethanol when $\lambda_{\max}$ occurred at 278 m μ . ($E_{1\%}^{1\text{cm}}$ 127). In conc. H_2SO_4 the fraction showed $\lambda_{\max}$ 315 m μ . (due to a congener) with a marked inflexion at 355 m μ . These observations indicate that the selectively absorbing fraction contains cholesta-3:5-dien-7-one. The ether eluate for the cholesterol-fed rats (Table 2) contained a trace of unsaturated ketone (probably cholest-4-en-3-one).

The unsaponifiable matter (0.11 g.) from the small intestine of five cholesterol-fed rats showed $\lambda_{\max}$ 270, 281 ($E_{1\%}^{1\text{cm}}$ 3.6) and 295 m μ . presumably due to 7-dehydrocholesterol.

DISCUSSION

The control group gave a small fraction (2 mg.) exhibiting a 270 m μ . peak, and a weaker band near 325–330 m μ . The corresponding fraction from the cholesterol-fed rats weighed 15 mg. and showed $\lambda_{\max}$ 268 m μ . with a weaker band at 325–330 m μ . The displacement of $\lambda_{\max}$ from 268 to 278 m μ . recorded when cyclohexane as solvent was replaced by ethanol, is characteristic of cholesta-3:5-dien-7-one. The cholesterol-fed rats thus showed a tenfold increase in selective absorption at 268 m μ . compared with the controls. This increase is predominantly due to the dienone since the spectrum of the fraction dissolved in conc. H_2SO_4 is characterized by a marked inflexion at 355 m μ . There is, however, also a peak in H_2SO_4 at 315 m μ . which cannot be attributed to cholesterol, but is rather due to a congener of the dienone yet to be identified.

The tenfold increase in selective absorption cannot be attributed to an artifact, because the liver lipids from the control animals and the cholesterol-fed animals were treated in exactly the

same manner. The increased intensity of 7-dehydrosteroid(cholesta-5:7-dien-3-ol) absorption resulting from cholesterol feeding is outside experimental uncertainty. This is supported by clear-cut evidence of the presence of similar selective absorption in the intestinal lipid.

The fall in liver vitamin A reserves is real, because parallel tests on five adult male rats taken about the same time from the colony, showed an average of 610 i.u./g. liver (633, 582, 711, 515, 608) in good agreement with the results on the control animals.

The evidence points to the need for further study of the reciprocal relationship between vitamin A stored and cholesterol ingested. Finally, if cholesta-5:7-dien-3-ol and cholesta-3:5-dien-7-one are members of a sequence of cholesterol metabolites, the smallness of the amounts actually detected is not necessarily surprising.

SUMMARY

1. Adult male rats given a diet containing 2% cholesterol for 60 days have been compared with similar rats on the same diet minus cholesterol.

2. Cholesterol feeding substantially reduced the liver store of vitamin A.

3. Control animals showed a trace (<0.06 mg./rat) of cholesta-3:5-dien-7-one in the liver, whereas cholesterol-fed animals had 0.6 mg./rat. 7-Dehydrocholesterol was present (a) to a greater extent in the livers of the cholesterol-fed animals than in the controls and (b) in the intestinal tissue after cholesterol feeding.

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