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ABSTRACT

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ABSTRACT

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1. Studies on the metabolism of exogenous cholesterol in the intact rat, using cholesterol labeled with carbon-14 and hydrogen-3, are described.

2. In the case of both cholesterol-C^{14} and cholesterol-H^3 feeding, radioactivity was present in the urine and in the fatty acid and non-saponifiable portions of the feces, carcass, adrenals and liver.

3. In the case of the rats fed cholesterol-C^{14}, activity was also present in the breath, liver phospholipid and liver glycogen.

4. In the case of one pregnant animal, 20% of the administered activity was present in the foeti.

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* This work was sponsored by the U.S. Atomic Energy Commission.

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These experiments were carried out as part of a study concerning the fate of ingested cholesterol in the intact animal. With the availability of cholesterol labeled with radioactive isotopes it is now possible to trace the metabolic pathway of all or part of this steroid. In our studies, both cholesterol-C^{14} and cholesterol-H^3 were fed and the distribution of radioactivity was determined following the feeding of each labeled compound. The steroid was administered in oil, via stomach tube, and after four days there was label present in the feces, urine and fatty acid and non-saponifiable fractions of the liver, adrenals and carcass. Where cholesterol-C^{14} was fed, radioactivity was also present in the liver glycogen, liver phospholipid and breath. The data definitely indicate the metabolic utilization of all or part of the cholesterol molecule to give fatty acids, glycogen, phospholipid and, presumably, other steroids.

The carbon-labeled cholesterol was biologically synthesized from sodium acetate-1-C^{14} (1,2); the tritium-labeled material was prepared from cholesterol by platinum catalyzed exchange with tritium oxide (3).

Experimental Procedure: All rats were of the Curtis-Dunning strain and weighed between 185-220 grams. Rats A and B were males and rats C,D,E and F were females;

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rat C was pregnant. The rats were starved for 48 hours prior to feeding of the labeled cholesterol, which was dissolved in 1 cc. of Wesson oil and administered by stomach tube. The tube was rinsed with an additional 0.5 cc. of oil after each feeding. Rats A and B each received 0.94 μc. (20.5 mg., 4.6×10^{-2} μc/mg.) of cholesterol-C^{14}; rats C and D each received 0.59 μc. (13.1 mg., 4.47×10^{-2} μc/mg.) of cholesterol-C^{14}; and rats E and F each received 20 μc. (20 mg., 1 μc/mg.) of cholesterol-H^3. Following the feeding of the labeled compounds, the rats were placed in individual metabolism cages and allowed to take food and water freely. Urine and feces were collected daily. In the case of rats A and B, breath samples were collected at half-hour intervals for the first 2-1/2 hours; then samples were collected after 3-1/2, 5, 24, 29, 48, 53, 72, 77 and 95 hours. After 96 hours the animals were sacrificed, the liver and adrenals removed, and the carcass allowed to dissolve in 40% aqueous potassium hydroxide. In the case of rat C, the foeti were removed and treated separately.

Except where indicated, all radioactive carbon determinations were carried out by direct plating of samples and assay in a windowless counter. The tritium-containing samples were combusted to water from which radioactive hydrogen was generated. The tritium gas was assayed in an ionization chamber (4).

Breath: The expired carbon dioxide was collected in sodium hydroxide, precipitated as barium carbonate, and the resultant precipitate assayed for radioactivity in an ionization chamber (5).*** The first 30 minute sample showed a small but detectable amount of radioactivity.

Urine: In the case of rats A, B, C and D, the combined urine fractions were acidified and extracted continuously with ether for 24 hours. The ether was dis-

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*** We are indebted to Judith Yafet, Evelyn Morgan and Roberta Molsberry for these determinations.

tilled and the residues plated directly. In the experiments where cholesterol-H^3 was fed, each daily sample was lyophilized, the water assayed for tritium activity, and the combined residues treated as described above. None of the eight water samples showed any detectable tritium activity.

Feces: The feces were extracted with ether for eight hours in a Soxhlet extractor. After assay for total activity the extracts were saponified and the activities of the non-saponifiable and fatty acid fractions determined. In every case the bulk of the radioactivity was present in the first day feces.

Carcass: The basic solution resulting from the drastic treatment with potassium hydroxide was acidified and continuously extracted with ether for 24 hours. The ether solution separated into fatty acid and non-saponifiable fractions by passing it over a column of Amberlite IRA-400 ion exchange resin on which the acids are adsorbed (6). The acids were eluted with strong hydrochloric acid.

In the case of rat A, a sample of crystalline cholesterol, m.p. 145°, was isolated. Conversion to barium carbonate by the Van-Slyke Folch wet combustion procedure (7) and assay of the barium carbonate showed the samples to have a specific activity of 2.16×10^{-4} μc/mg. This represents a two hundred fold dilution of the original sample.

In the case of rat C, the pregnant animal, the eight foeti were removed, pooled and dissolved in hot, concentrated potassium hydroxide solution. Separation into fatty acid and non-saponifiable fractions was carried out as described above.

A sample of pure cholesterol, m.p. 146°, was isolated from the non-saponifiable fraction. The specific activity of this material was found to be 3.30×10^{-4} μc/mg, representing a hundred and thirty fold dilution of the original material.

Adrenals: The adrenals were dissolved in 30% aqueous potassium hydroxide and the basic solution extracted with ether to yield the non-saponifiable material.

Acidification of the basic solution remaining yielded the fatty acid fraction.

Liver: In the cholesterol-C^{14} experiments the livers were homogenized and lyophilized and the dry residue was extracted with acetone for 24 hours in a Soxhlet extractor. This sample was designated as lipid material. The residue was ether extracted for 12 hours to give phospholipids. The phospholipids were reprecipitated prior to assay for radioactivity. The liver residue was dissolved in 30% aqueous potassium hydroxide and the glycogen precipitated with ethanol and sodium sulfate. The glycogen was also reprecipitated prior to radioactivity measurements. The initial acetone extract was saponified by eight hour reflux with 10% alcoholic potassium hydroxide and separated into non-saponifiable and fatty acid fractions.

In experiments involving cholesterol-H^3 the livers were dissolved in hot, concentrated base and only the fatty acid and non-saponifiable fractions were obtained.

DISCUSSION

These experiments demonstrate the metabolic utilization of cholesterol for the synthesis of fatty acids and glycogen. The conversion of ring-labeled cholesterol to steroid hormones (8) has been demonstrated and this suggests that the side chain of cholesterol may be the major contributor towards the radioactivity found in the fatty acids. It also points up the possibility that not all the non-saponifiable activity is due to cholesterol.

Recent experiments have shown that following the feeding of cholesterol-26-C^{14} to rats a considerable portion of the radioactivity may be recovered in the breath in a relatively short time (9). The small amount of radioactivity found in the breath samples obtained from rats A and B would suggest that the findings of Little and Bloch (10) that cholesterol biologically synthesized from carbonyl-

Table I. Distribution of Radioactivity Following Cholesterol-C^{14} Feeding

Origin	Rat A (0.94 μc.)		Rat B (0.94 μc.)		Rat C (0.59 μc.)		Rat D (0.59 μc.)	
	μc	%	μc	%	μc	%	μc	%
Breath	0.012	1.28	0.006	0.64	———	———	———	———
Urine	.077	8.19	.138	14.68	0.005	0.85	0.329	55.73
Feces NS*	.324	34.46	.276	29.36	.328	55.56	.227	38.45
Feces FA*	.045	4.79	.063	6.70	.011	1.86	.016	2.71
Carcass NS	.023	2.45	.064	6.81	.038	6.44	.010	1.73
Carcass FA	.083	8.83	.306	32.55	.020	3.39	.008	1.35
Adrenal NS	.030	3.19	.023	2.45	.053	8.89	.047	7.96
Liver NS	.394	41.91	.017	1.81	.005	0.85	.003	0.51
Liver FA	.044	4.68	.066	7.03	.004	0.68	.0003	0.05
Liver PL*	.014	1.49	.071	7.55	.0002	0.03	.0005	0.08
Liver G*	.006	0.64	.008	0.85	.004	0.68	.005	0.80
Foeti NS	———	———	———	———	.095	16.09	———	———
Foeti FA	———	———	———	———	.028	4.74	———	———
TOTAL	1.069	113.72	1.055	112.24	0.656	111.16	0.660	111.74

* NS = Non-saponifiable; FA = fatty acids; PL = phospholipids; G = glycogen

Table II. Distribution of Radioactivity Following Cholesterol-H^3 Feeding

Origin	Rat E (20 μc.)		Rat F (20 μc.)	
	μc	%	μc	%
Urine	0.18	0.90	0.15	0.75
Feces	11.90	59.50	13.50	67.50
Carcass NS	3.94	19.70	3.62	18.10
Carcass FA	0.53	2.65	0.27	1.35
Adrenal NS	0.05	0.25	0.07	0.35
Adrenal FA	0.39	1.95	0.005	0.03
Liver NS	0.11	0.55	0.08	0.40
Liver FA	0.02	0.10	0.03	0.15
TOTAL	17.12	85.65	17.725	88.63

labeled acetate contains no label in carbon atoms 26 and 27, may hold true generally. The breath activity during the course of the experiment may be due to oxidation of the fatty acids formed as well as from cholesterol breakdown, but the activity present in the first 30 minute sample must surely represent breakdown of a small portion of the cholesterol to carbon dioxide.

Within the error of the experiment, the major portion of the activity was accounted for in all animals which suggests that the differences in the origin of radioactivity are due to individual metabolic differences in the animals involved. In all but one case, about half of the administered activity was excreted. In the four rats fed carbon-labeled cholesterol, there seemed tobe one major source of activity, in addition to the feces, in every case. In rat A this source was the liver non-saponifiable which could mean storage at that site. The great conversion of cholesterol to fatty acids in rat B is mirrored not only in the high activity of the carcass fatty acid, but also in the higher activity of the liver fatty acid and phospholipid fractions. In the pregnant rat, rat C, there were two high radioactivity pools, the adrenals and the foeti, both of which contained about 20% of the administered activity. The presence of a relatively high concentration of radioactivity in the foeti indicates ready transport of cholesterol across the placental membrane. Rat D excreted 97% of the administered activity in the urine and feces.

Although the qualitative aspects of the metabolic utilization of cholesterol were the same in all cases, there was a large quantitative variation from animal to animal. While the female rats (C and D) converted much more of the fed radioactivity to adrenal radioactivity, the data do not warrant assumption of a sex difference. It must be pointed out that many similar experiments will have to be performed before any conclusions concerning the percentage converted into each constituent may be assessed.

In the case of the tritium-labeled cholesterol feedings, the finding of activity in various fatty acid fractions would implicate the terminal isopropyl group of the cholesterol side chain in these syntheses, since it has been shown (11) that in cholesterol labeled with heavy hydrogen, the activity is found in the areas of carbon atoms 3-6 (46%) or in carbon atoms 25,26 and 27 (54%). The more comparable data obtained from rats E and F might also suggest that the differences found in the first four animals may be due to different breakdowns of the cholesterol molecule following loss of the terminal isopropyl group.

Saponification of the extracts obtained from feces E and F gives the following breakdown of activities: Feces E - non-sap., 6.58 μc. (32.9%); fatty acids, 0.24 μc. (1.2%). Feces F - non sap., 11.0 μc. (55%); fatty acids, 0.41 μc. (2.05%). The loss of activity may be attributed to the loss of labeled enolizable hydrogen during saponification. This suggests the loss of some activity from the other fractions for which no data was obtained prior to saponification. Although it has been shown that cholesterol-H^3 is a suitable tracer in experiments involving the entire molecule (12), these data again indicate that this compound is not a suitable tracer for metabolic degradation experiments.

SUMMARY

1. Studies on the metabolism of exogenous cholesterol in the intact rat, using cholesterol labeled with carbon-14 and hydrogen-3, are described.

2. In the case of both cholesterol-C^{14} and cholesterol-H^3 feeding, radioactivity was present in the urine and in the fatty acid and non-saponifiable portions of the feces, carcass, adrenals and liver.

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