TABLE III. *In Vitro* Inhibition of Wheat Germ Lipase by Suspensions of Quinidine Sulfate or Quinine Sulfate.*

Inhibitor	No. of exp.	K_s (moles triacetin/l) ± SEM	K_i (± SEM)
Quinidine sulfate	5	$2.3 \pm .2 \times 10^{-1}$	$1.0 \pm .5 \times 10^{-3}$
Quinine sulfate	3	$1.9 \pm .3 \times 10^{-1}$	$5.7 \pm .5 \times 10^{-4}$

* Performed at 37°C under N_2 with a 10 min. preincubation.

quinidine sulfate. To the extent tested, the same relationship holds for wheat germ lipase using triacetin as substrate. In the wheat germ lipase system the K_i for quinidine sulfate $= 1 \times 10^{-3}$, and the K_i for quinine sulfate $= 5.7 \times 10^{-4}$. Heparin inhibits the pancreatic TUA in presence or absence of quinidine. Metals, such as Cu^{+2} and Mg^{+2} inhibit this system and Cu^{+2} increases quinidine inhibition but Mg^{+2} does not. Fe^{+3} does not inhibit the system or interfere with quinidine inhibition. EDTA inhibits this system by 70% at a level of 10^{-3} *M*. This inhibition is partially reversed by Ca^{+2} ions which also markedly stimulate TUA activity. EDTA does not greatly influence quinidine inhibition. Cu^{+2} inhibition is partially reversed by EDTA in spite of the fact that EDTA is an inhibitor. EDTA reduces the stimulatory effect of Ca^{+2}. Quinidine inhibition is only slightly lessened by Ca^{+2} so that it appears that the site of action of quinidine may not involve a Ca^{+2} ion stabilizer.

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Oxidation of Ergosterol by Rat and Mouse Liver Mitochondria.*† (26448)

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That plant sterols may be absorbed by mammals was first suggested by Ellis and Gardner(1), who carried out experiments involving feeding of ordinary or ether-extracted feedstuffs to rabbits and found the liver sterol content of the group fed the untreated diet to be the greater. Since then, other workers, using more direct means, have demonstrated absorption of phytosterols such as sitosterol (2,3) and ergosterol(4,5) in a number of experimental animals. The metabolic pathway of plant sterols is of interest since they comprise a significant proportion of the sterol

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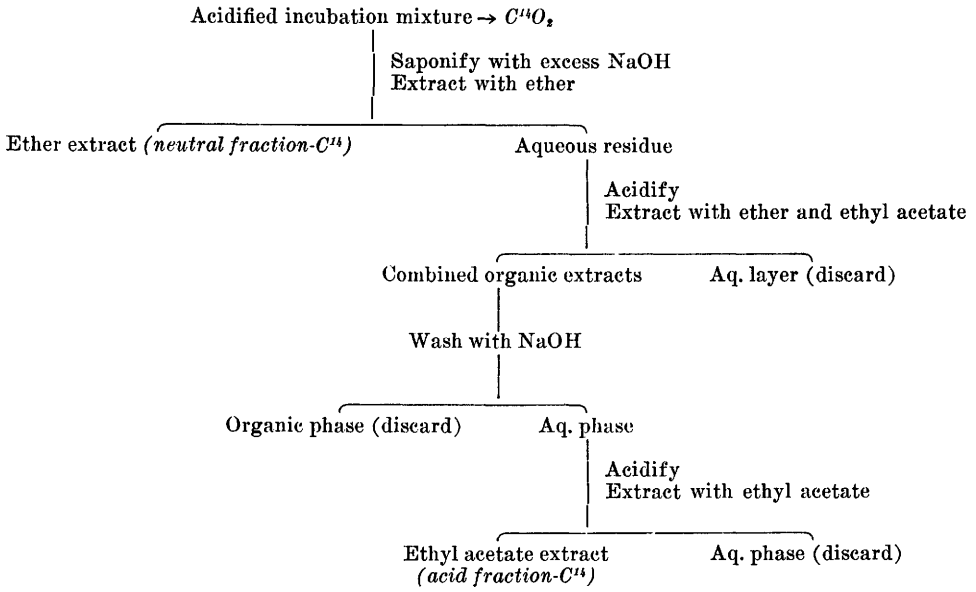

FIG. 1. Isolation of radioactive products from incubation of ergosterol-C^{14} with liver mitochondria.

content of the average human diet. The experiments described here have demonstrated that ergosterol is readily oxidized by enzymes present in rat and mouse liver mitochondria.

Methods. Ergosterol labelled with C^{14} was prepared biosynthetically from yeast grown in presence of formic acid-C^{14}, yielding ergosterol-28-C^{14}(6,7) or sodium acetate-1-C^{14}, yielding ergosterol-U-C^{14}(8) containing radioactivity at carbon atom 25 *inter locos* (8,9). Cholesterol-26-C^{14} was synthesized according to the procedure of Dauben and Bradlow(10). Cholesterol-4-C^{14} was purchased from the New England Nuclear Corp., Boston, Mass. The 3*a*, 7*a*, 12*a*-trihydroxycoprostan-26-oic acid was isolated from alligator bile(11).

Mitochondria were isolated from the livers of male Wistar rats and Swiss Hygienic mice of both sexes. The procedure has been described in detail(12,13). The liver supernatant fraction (SF) was prepared by boiling the solution remaining after removal of all particles from a liver homogenate by centrifugation at 100,000 × g for 25 minutes.

Incubations were carried out at 37°C in stoppered 125 ml Erlenmeyer flasks with center wells containing 2.0 ml of 2.5N sodium hydroxide. Each flask contained washed mitochondrial preparation (1 ml), a sterol emulsion in 0.25 M tris (hydroxymethyl) aminomethane HCl, pH 8.5 (5 ml) and one ml of a solution containing the following: diphosphopyridine nucleotide (5 mg), adenosine-5′-monophosphate (8 mg), adenosine triphosphate (25 mg), glutathione (15 mg), trisodium citrate monohydrate (30 mg), magnesium chloride (10 mg), potassium penicillin G (2000 U), and streptomycin sulfate (1 mg). In addition 5 ml of SF or of 10% (w/v) aqueous sucrose, was added to all incubations.

At the end of incubation period sulfuric acid was added and the flasks re-stoppered and shaken for 3 hours to displace carbon dioxide from the medium. The carbon dioxide trapped in the alkali present in the center well was precipitated as $BaC^{14}O_3$ with barium chloride. Radioactivity of the barium carbonate, suspended in a thixotropic gel(12,13), was assayed in a Packard Tri Carb liquid scintillation counter.

Neutral and acidic radioactive products were isolated from the incubation mixtures according to the scheme given in Fig. 1. The acidic material was chromatographed on paper according to the method of Beyreder and Rettenbacher-Daubner(14) using the

TABLE I. Oxidation of Ergosterol-28-C^{14} and Ergosterol-U-C^{14} by Rat and Mouse Liver Mitochondria.

Substrate	Mitochondria	% oxidation* Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
Ergosterol-28-C^{14}	Rat	3.6	2.4			
	Mouse	1.3	1.8			
Ergosterol-U-C^{14}	Rat	.7	2.2	.9	.6	2.7
	" †	(10.5)	(33.0)	(13.5)	(9.0)	(40.5)
	Mouse	.9	.9	.9		
	" †	(13.5)	(13.5)	(13.5)		

* Computed as $BaC^{14}O_3$/substrate-C^{14}.
† Recalculated assuming $C^{14}O_2$ arises only from C_{25}.

upper phase of the mixture glacial acetic acid:toluene:water—40:40:8 v/v as the solvent. For determination of R_f values each chromatogram was cut into 1 cm strips which were counted directly, in the presence of scintillation medium, in a liquid scintillation counter.

Results and discussion. Both ergosterol-28-C^{14} and ergosterol-U-C^{14} were oxidized by rat and mouse liver mitochondria yielding radioactive carbon dioxide (Table I). Parallel incubations without mitochondria or with boiled mitochondria gave no radioactive carbon dioxide. Inclusion of penicillin and streptomycin in all incubations strengthens the conclusion that ergosterol oxidation was due to the liver mitochondria and not to any contaminating bacteria.

The experimental conditions required for *in vitro* oxidation of ergosterol are similar to those under which the cholesterol side chain is oxidized(12,15). Especially noteworthy is the requirement for a thermostable factor (SF), present in the supernatant fraction of the liver homogenate, for optimal oxidation of both ergosterol and cholesterol (Table II). This factor is apparently not identical with TPNH, nor with any other known enzyme for steroid metabolism(15).

TABLE II. Influence of Supernatant Fraction (SF) on Sterol Oxidation by Rat and Mouse Liver Mitochondria.

Substrate	% oxidation* Rat -SF	Rat +SF	Mouse -SF	Mouse +SF
Ergosterol-28-C^{14}	.2	7.5	.7	3.3
Ergosterol-U-C^{14}	1.8	5.9	.4	4.9
Cholesterol-26-C^{14}	.1	7.9	.1	5.9

* Computed as $BaC^{14}O_3$/substrate-C^{14}.

Comparison of the relative yields of radioactive carbon dioxide at different time intervals from ergosterol-28-C^{14} and from ergosterol-U-C^{14} (labelled at C_{25}) suggests that C_{28} may be cleaved before C_{25} and the terminal isopropyl group (Table III). Since the C^{14}-labelled steroids used in these experiments were of different specific activities, no useful comparison can be made between absolute yields of radioactive carbon dioxide formed from the different substrates.

TABLE III. Relative Rates of Sterol Oxidation by Rat Liver Mitochondria.

Substrate	% oxidation* 3 hr	6 hr	18 hr
Ergosterol-28-C^{14}	5.8	6.1	11.8
Ergosterol-U-C^{14}	.6	1.3	1.7
(Ergosterol-25-C^{14})†	(9.0)	(19.5)	(25.5)
Cholesterol-26-C^{14}	1.7	5.3	17.1

* Computed as $BaC^{14}O_3$/substrate-C^{14}.
† Recalculated assuming $C^{14}O_2$ from ergosterol-U-C^{14} arises only from C_{25}.

In an effort to obtain an indication of the type of acidic products formed, from both ergosterol-C^{14} substrates, all incubations were worked up according to the scheme given in Fig. 1. Whereas an appreciable amount of radioactivity was recovered from all incubations where ergosterol-U-C^{14} was the substrate, radioactivity was recovered in the acidic fraction of only one of the experiments with ergosterol-28-C^{14}. The acidic fractions obtained were combined and chromatographed on paper. These results were compared with those obtained on chromatography of the acidic products isolated after incubations of cholesterol-4-C^{14} and cholesterol-26-C^{14} with rat and mouse liver mitochondria. Comparisons were also made with

TABLE IV. R_f Values of Radioactive Acidic Products Formed by Mitochondrial Oxidation of Sterols.*

Substrate	Mitochondria	R_f				
Cholesterol-26-C^{14}	Rat		.15			.94
	Mouse		.11			.93
Cholesterol-4-C^{14}	Rat			.80	.87	
	Mouse		.11			.93
Ergosterol-U-C^{14}	Rat	.03			.88	
	Mouse	.03			.88	
Cholic acid	(Reference Cpd)			.82		
Trihydroxycoprostanic acid	(" ")				.89	

* Paper chromatography according to Beyreder and Rettenbacher-Daubner(14).

chromatograms of cholic acid and trihydroxycoprostanic acid (THCA). The results are presented in Table IV.

In vivo experiments have shown that rat liver is able to oxidize several 27-carbon sterols to products resembling, but not identical with, the normal bile acids formed from cholesterol(16,17,18). Conversion of C_{28} and C_{29} sterols such as ergosterol and sitosterol to compounds resembling cholesterol has been adduced from experiments with beetles (19) and flies(20). That insects can metabolically alter the side chain and degree of unsaturation of sterols has been directly demonstrated in the conversion of ergosterol to 22-dehydrocholesterol by cockroaches(21). Hanahan(4) and Glover(5) have found that ergosterol is converted to acidic material, "pseudo" bile acid in nature, in the rat and guinea pig, respectively. Werbin *et al.*(22, 23) have presented evidence that, in the guinea pig, β-sitosterol is converted to cortisol, acidic products and, probably, cholesterol.

In animals, the principal pathway of cholesterol catabolism is through formation of bile acids by the liver(16). Our earlier experiments have shown that liver mitochondria readily oxidize the terminal carbon atoms of cholesterol. The experiments reported here indicate that ergosterol is degraded in a similar fashion with removal of the "extra" methyl group (C_{28}) and at least C_{25} of the side chain, presumably as a consequence of the oxidation of the terminal isopropyl group. The many similarities between the oxidation of cholesterol and of ergosterol *in vitro* suggest that the enzyme systems concerned are closely related to each other, if not identical.

Paper chromatography of the radioactive acidic products also indicates the similarity of the metabolites of cholesterol and ergosterol. Oxidation of cholesterol-4-C^{14} by rat liver mitochondria yields 2 radioactive compounds whose R_f values are similar to those of cholic and trihydroxycoprostanic acids. When incubated with mouse liver mitochondria, cholesterol-4-C^{14} yields 2 radioactive compounds, one slightly less polar and one much more polar than trihydroxycoprostanic acid. When the substrate is cholesterol-26-C^{14} both rat and mouse liver mitochondria yield radioactive compounds whose R_f values resemble the 2 obtained from oxidation of cholesterol-4-C^{14} by mouse liver mitochondria. When cholesterol-26-C^{14} is the substrate, no radioactive compound resembling cholic acid (in R_f value) is obtained, as would be expected, although such a compound could be detected by spray reagents (13). Oxidation of ergosterol-U-C^{14} by either rat or mouse liver mitochondria yields the same 2 radioactive acidic compounds. One is more polar than the most polar substance observed when cholesterol-C^{14} is the substrate; the other has an R_f value akin to that of trihydroxycoprostanic acid. Hoshita (24,25) has recently demonstrated that the acid isolated from toad bile by Shimizu and Oda(26) (trihydroxybufosterocholenic acid) is 3α, 7α, 12α-trihydroxy-24-methyl-Δ^{22}-coprostenic acid. If this bile acid may be regarded as a metabolite of ergosterol it is one in which ring saturation and hydroxylation similar to those observed in the initial stages

of cholesterol degradation have taken place. Such a compound could conceivably be that encountered by us in our chromatographic experiments.

The physiological significance of our observations is that plant sterols, absorbed from the intestine, can be degraded and presumably excreted *via* the bile in the form of water soluble acids.

Summary. Oxidation of ergosterol-28-C^{14} and of ergosterol-U-C^{14} by mitochondrial preparations from rat or mouse livers has been investigated. Either substrate yielded $C^{14}O_2$. Ergosterol-U-C^{14} yielded radioactive neutral and acidic products, while ergosterol-28-C^{14} gave only neutral radioactive material. Among the acidic products obtained from ergosterol-U-C^{14} was a radioactive acid with R_f close to that of trihydroxycoprostanic acid. Similarities between the oxidation of cholesterol and ergosterol by liver mitochondria *in vitro* suggest that enzyme systems involved are closely related to each other, if not identical.

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Endocrine Studies of Chlordiazepoxide.* (26449)

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Several reports in the literature indicate that psychotropic drugs of the phenothiazine and reserpine type have endocrine effects, particularly on the pituitary-gonadal axis (1-6). We therefore decided to study the endocrine effects of chlordiazepoxide, a member of a new chemical class of compounds possessing potent pharmacological activity in animals(7-8) and psychotropic effects in man(9).

* Librium®, Hoffmann-La Roche.