under varying experimental circumstances, are questions that are being examined.

The adrenal response is analogous to that of the kidney. Increased salt ingestion causes greater suppression of the adrenal glomerulosa, and it is even possible that the high level of carbohydrate decreases the demand for glucocorticoid, thus contributing to reduced adrenal weight. On the other hand the stress imposed by hypertensive vascular disease favors adrenal enlargement. The usual result of these operations is that the average group adrenal weight is depressed(5,6,11,12), but not always to a significant degree, partly because of glandular enlargement in some severely hypertensive rats, and also because the glomerulosa, to which the atrophy is principally confined, contributes relatively little to adrenal mass.

Summary. The quantity of 1% saline consumed and the development of salt hypertension in rats were comparably enhanced by addition of either glucose or sucrose in 5% concentration to the drinking solution. Cardiac hypertrophy and lesions were equivalently increased. A curious, and as yet unexplained finding, is that whereas kidney enlargement accompanied consumption of either saline or sucrose-saline solution, such was not the case with glucose-saline. Here the kidney weight did not exceed that of controls, and renovascular lesions although present were less pronounced than when other saline solutions were drunk.

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Intestinal Metabolism of Cholesterol: Evidence Against Side-Chain Oxidation by Mammalian Intestinal Mucosa.* (31492)

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The intestinal mucosa carries out several functions which are critical to the sterol economy of the mammal. Not only does this tissue absorb both dietary sterol and biliary sterol undergoing enterohepatic circulation (1), but also it delivers sterol to the intestinal lumen for fecal excretion(2). Moreover, the mucosa synthesizes sterols(3,4), and recent studies have shown that cholesterol of intestinal origin may account for a significant portion of the total circulating cholesterol(5).

It is apparent from the above that the sterol content of the intestinal mucosa itself represents a balance between additions, including sterol absorbed or synthesized, and losses, including sterol delivered to the gut lumen or to the peripheral circulation. It has not been established, however, that these known routes of loss are adequate to account for the disposition of the absorbed and locally synthesized sterol. Therefore, the possibility exists that there are additional pathways by

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which intestinal sterol may be metabolized, and by which the intestine may further participate in the regulation of the total body sterol economy.

One possible pathway involves the oxidation of the side-chain. This pathway is known to operate in other tissues in the formation of bile acids and steroid hormones and has been shown to be quantitatively the most important mechanism for cholesterol disposition by the intact animal(6). In 1952, Meier *et al*(7), showed that slices of liver, adrenal, gonad, and kidney were capable of converting ^{14}C in the 26-position of cholesterol to $^{14}CO_2$, but studies of intestinal tissue have apparently not been reported.

The present studies, therefore, were designed to explore the possibility that some cholesterol undergoes side-chain oxidation in the intestinal mucosal cell. The formation of $^{14}CO_2$ from cholesterol-26-^{14}C by mucosal tissue preparations under a variety of conditions was assayed.

Materials and methods. Female albino rats and guinea pigs† were maintained on standard laboratory diets until sacrifice by decapitation. The small intestine was immediately rinsed *in situ* with 100 ml ice-cold isotonic saline, removed, and rinsed again. When appropriate, the intestine was divided transversely into thirds, designated "proximal", "middle", and "distal". Mucosal scrapings (intact cells) were obtained for incubation by opening the intestinal segments on a chilled surface, blotting, and scraping with a blunt spatula. Intestinal mucosa was obtained for homogenization by scraping everted segments with a glass slide. In a few experiments, 15-20 mm lengths of everted gut with mucosa intact were incubated. Liver and intestinal mitochondria, and the heat-stable "supernatant factor" were prepared according to the method of Lee and Whitehouse(8). Liver was homogenized in 2.5 volumes of 0.25 M sucrose, but larger volumes were required for successful homogenization of intestinal mucosa (10 volumes for rat, 15 volumes for guinea pig) in order to remove the abundant mucus. Alternatively, homogenization of intestinal mucosa was carried out in 0.15 M KCl or in Tris-mannitol buffer, pH 7.0(9). Liver slices approximately 1 mm in thickness were prepared with a Stadie-Riggs microtome.

Preparation of Bile-Fistula Rats: Rats were anesthetized with ether, and a polyethylene catheter (PE-10, O.D. 0.11″, Clay-Adams) was inserted into the common bile duct. The catheter was led subcutaneously to the back, and the animals were placed in a restraining cage for 48 hours and allowed free access to a solution of 2.5% glucose in 0.45% NaCl.

Preparation of Substrates: Cholesterol-26-^{14}C was obtained from New England Nuclear Corp. Aqueous solutions were prepared with Tween-20 (Sigma Chemical Co.) by dissolving the appropriate amount of radioactive cholesterol, with or without added unlabeled cholesterol (Eastman Organic Chemicals, recrystallized from methanol), and Tween-20 in methanol, evaporating the methanol under a stream of nitrogen at 50°, and adding the required amount of water or buffer. Three solutions were thus prepared: 1) 2.0 mg cholesterol and 40 mg Tween-20 per ml H_2O; 2) 80 μg cholesterol and 2 mg Tween-20 per ml H_2O; and 3) 4 μg cholesterol and 40 μg Tween-20 per ml Tris-hydrochloride buffer, pH 8.5. A similar method was used to prepare a solution of 80 μg cholesterol and 20 μl propylene glycol per ml H_2O, used in a few experiments.

Incubations: Preparations of mucosal scrapings, everted intestinal segments, and liver slices were incubated with substrate in Krebs-Ringer bicarbonate medium containing 5.5 mM glucose and one-half the usual concentration of calcium ion, in an atmosphere of 95% O_2 and 5% CO_2. Homogenates were incubated in Tris-HCl buffer, pH 8.5, with the additions used by Lee and Whitehouse(8) including citric acid, disodium-ATP (Sigma), NAD (Sigma), $MgCl_2$, and reduced glutathione (Schwarz BioResearch, Inc.). Incubations were carried out in 25 ml flasks fitted with center wells in a shaking incubator at 37° for the specified time. Control flasks contained at least 2.4×10^6 dpm of cholesterol-26-^{14}C, but no tissue, in a volume of 3.0 ml.

Isolation and Assay of Products: The

† Charles River Laboratories.

TABLE I. Conversion of Cholesterol-26-^{14}C to $^{14}CO_2$ by Liver and Intestinal Mucosal Homogenates.

Animal	Tissue	"Supernatant factor"	Time (hr)	$^{14}CO_2$ evolved (dpm)
Rat*	Liver			
	Mitochondria	Liver (fresh)	3	432
	"	" "	5	329
	"	" (frozen)	"	438
Rat*	Intestine			
	600 g supernatant	—	3	<10
	Mitochondria	Intestine	"	"
	"	Liver	"	"
Guinea pig†	Intestine			
	950 g supernatant	—	3	<10
	Mitochondria	Liver	"	"
	"	Intestine	"	"

* System of Lee & Whitehouse(8).

† Three variations of this experiment were carried out, in which tissue was homogenized in 0.25M sucrose, 0.15M KCl, or Tris-mannitol, pH 7.0. The 3 incubations from each of the 3 experiments (total 9 incubations) gave identical results.

All incubations contained tissue, 0.4 μg cholesterol-26-^{14}C (4.13×10^4 dpm), 4 μg Tween-20, and the appropriate cofactors (see text).

Mitochondrial suspensions, per incubation, were equivalent to 1/5 of one rat liver, or 1/3 of one total intestinal mucosal harvest.

reaction was terminated by addition of 0.5 ml 6N H_2SO_4 to the mixture. The evolved CO_2 was collected in 0.4 ml Hydroxide of Hyamine (Packard Instruments Co.), injected into the center well immediately before addition of the acid, while the flask was agitated for an additional 45 minutes at 37°. The hyamine solution was then assayed for radioactivity in a solution of 0.4% diphenyloxazole and 0.05% 1,4-bis-2-(5-phenyloxazolyl)-benzene in toluene. Assays for ^{14}C were carried out in glass vials, using a Nuclear-Chicago Corp. Model 723 Liquid Scintillation System. Radioactivity in disintegrations per minute was calculated by the channels ratio method.

Results. Control Incubations: In each of 3 four-hour incubations, 10 or fewer dpm above background were measured as "$^{14}CO_2$". Thus, in subsequent experiments, the actual value for dpm is given only when it exceeded 10.

Conversion of Cholesterol-26-^{14}C to $^{14}CO_2$ by Homogenates of Liver and Intestinal Mucosa: Rat liver mitochondria and rat or guinea pig intestinal mucosal homogenate or mitochondria were incubated with "supernatant factor" prepared from either intenstine or rat liver (Table I). Rat liver mitochondria converted approximately 1% of the substrate to $^{14}CO_2$ in 3 hours; a 5-hour incubation resulted in no additional conversion, and "supernatant factor" which had been frozen and thawed was as active as that which had been freshly prepared. Preparations from intestinal mucosa were found to be completely inactive, however, including rat mucosal 600 g supernatant and mitochondria, and guinea pig 950 g supernatant and mitochondria, homogenized in a variety of media and incubated with "supernatant factor" prepared from either intenstine or liver.

Conversion of Cholesterol-26-^{14}C to $^{14}CO_2$ by Mucosal Scrapings from Normal Rats and Guinea Pigs: To test the ability of the intact cell to oxidize cholesterol, mucosal scrapings from proximal, middle, and distal thirds of both guinea pig and rat small intestine were employed. No tissue from either species produced significant $^{14}CO_2$ (Table II).

Conversion of Cholesterol-26-$^{14}CO_2$ by Intact Intestinal Mucosa and Liver Slices from Bile-Fistula Rats: Because it has been shown that bile salts inhibit both the oxidation of the cholesterol side-chain by liver homogenates(8) and the biosynthesis of sterols by intestinal mucosa(10), and that intestinal sterol biosynthesis is augmented in bile-fistula rats(3), the possibility was considered that a latent ability of the intestinal mucosa to

TABLE II. Conversion of Cholesterol-26-^{14}C to $^{14}CO_2$ by Mucosal Scrapings.

Animal	Region of intestine	Wet tissue (g)	$^{14}CO_2$ evolved (dpm)
Guinea pig	Proximal	.50	<10
	Middle	.47	"
	Distal	.19	"
Rat	Proximal	.57	<10
	Middle	.46	"
	Distal	.31	"

Incubations were for 2 hr, and contained tissue, 0.5 mg cholesterol-26-^{14}C (1.11×10^6 dpm), and 10 mg Tween-20 in 3.0 ml modified Krebs-Ringer bicarbonate medium.

oxidize the cholesterol side-chain is ordinarily inhibited by the enterohepatic circulation of endogenous bile salts. To test this possibility, intestinal mucosal scrapings or everted segments of small intestine from bile-fistula rats were compared with liver slices from the same animals (Table III). Although liver slices under these conditions showed considerable activity, formation of $^{14}CO_2$ by intestinal tissue was negligible. Doubling of the concentration of substrate (animal C) did not appreciably increase the radioactivity in CO_2. The use of propylene glycol instead of Tween-20 to dissolve the substrate did not affect the results (animals D, E).

TABLE III. Conversion of Cholesterol-26-^{14}C to $^{14}CO_2$ by Liver and Intestinal Tissue from Bile-Fistula Rats.

Animal	Tissue preparation	Region of intestine	Wet tissue (g)	$^{14}CO_2$ evolved (dpm)
A	Liver slices	—	.47	2086
	Everted segments	Proximal	.88	20
		Middle	.77	16
		Distal	.82	16
B	Mucosal scrapings	Proximal	.21	<10
		Middle	.37	"
		Distal	.29	"
C	Everted segments	Proximal	.89	18
		Middle	1.08	22
		Distal	.99	27
D	Liver slices	—	.56	1588
	Everted segments	Proximal	.79	16
		Middle	.83	13
		Distal	.80	16
E	Mucosal scrapings	Proximal	.34	<10
		Middle	.47	"
		Distal	.16	"

Incubations were carried out for 4 hr in 3.0 ml modified Krebs-Ringer bicarbonate medium, containing, in addition to tissue:
Animals A, B: 20 μg cholesterol-26-^{14}C (2.53×10^6 dpm) and 0.5 mg Tween-20.
Animal C: 40 μg cholesterol-26-^{14}C (5.06×10^6 dpm) and 1.0 mg Tween-20.
Animals D, E: 20 μg cholesterol-26-^{14}C (2.35×10^6 dpm) and 5.0 μl propylene glycol.

Discussion. In the present experiments, mammalian intestinal mucosal tissue was found to be incapable of significant oxidation of the side-chain of cholesterol. Even under conditions which presumably would favor maximum oxidation, i.e., the decreased intestinal bile salt levels resulting from prior bile-fistula, appreciable activity was not observed. It is possible that exogenous sterol does not reach the mitochondrial site of sterol oxidation under the present conditions; were this the case, failure to demonstrate oxidation of added sterol would not necessarily exclude oxidation of sterol synthesized in the cell itself. While there is no direct evidence bearing on this question of access, Glover and Green(11) showed that sterol administered orally to rats was distributed among all subcellular fractions of the intestinal mucosal cell, including mitochondria, within 5 hours. Furthermore, since the final steps of sterol synthesis are microsomal, even sterol synthesized in the cell itself gains access to mitochondria only by traversing the cytoplasm, where rapid mixing of endogenous and exogenous sterol is presumed to occur(11).

It is therefore concluded that side-chain oxidation in the intestinal mucosal cell is not a significant metabolic fate of cholesterol. The possibliity that other sterols, such as 7-dehydrocholesterol and lathosterol (Δ7-cholesten-3β-ol), do undergo side-chain oxidation is not excluded. Because all known metabolic pathways for degradation of cholesterol in the mammal involve oxidation of the side-chain (as in bile acid and steroid hormone synthesis), the failure to demonstrate oxidation of the 26-carbon by intestinal mucosa indicates that cholesterol is converted neither to bile acids nor to other steroid metabolites in that tissue. Thus, the contribution of the intestinal mucosa to mammalian cholesterol economy appears to depend exclusively on its function in absorption, synthesis, and excretion, and not degradation.

Summary. Preparations of intestinal mu-

cosal scrapings, homogenates, and mitochondria from normal rats and guinea pigs, and mucosal scrapings and everted intestinal segments from bile-fistula rats, failed to oxidize cholesterol-26-^{14}C to $^{14}CO_2$. It is concluded that oxidation of side-chain in the intestinal mucosal cell is not a quantitatively significant metabolic fate of cholesterol.

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Alterations in Serine Metabolism in Rats Fed Liquid Synthetic Diets.* (31493)

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The liquid synthetic diet proposed by Greenstein *et al,* has been found to support good growth rates, reproduction and lactation in rats(1,2). Therefore, this diet has been considered nutritionally complete and the content of essential plus non-essential amino acids considered optimal. This diet has recently been used as a control diet in studies of hepatic serine biosynthesis in rats. Prompt alterations in the concentration of several enzymes important in serine metabolism were noted. This observation suggests that synthetic diets supporting normal or near normal growth rates may nevertheless require significant compensatory changes in enzyme levels to provide required metabolites. The importance of this observation to the study of alterations caused by specific dietary deficiencies is apparent. These studies also demonstrate the importance of dietary amino acids in the regulation of enzyme levels in serine metabolism.

Methods. Male Osborne-Mendel rats (40-80 g) were used in these studies. Liquid synthetic diets, based on the diet of Greenstein *et al*(2), (Diet A) were prepared by General Biochemicals, Inc. (Diet No. 116). Identical diets were also prepared omitting serine and glycine (Diet B) or serine, glycine and cysteine (Diet C). Rats were pair fed these liquid diets from Richter feeding tubes. Control rats were fed, *ad libitum,* either Purina Lab Chow or a solid 25% casein diet(3). The rats were fed the diets for 7 days, killed and enzyme preparations made from liver as described previously(3). Assays for 3-P-glycerate† dehydrogenase, using 3-P-glycerate as substrate, serine dehydratase and D-glycerate dehydrogenase have been described(3). One unit of enzyme activity is defined as a change

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† 3-P-glycerate = 3-phosphoglycerate.