mg protein per ml; (c) with poly-U to a concentration of 0.4 mg poly-U per ml at which point there was a slight inhibition.

Summary. A cell-free system which was active with respect to amino acid-^{14}C incorporation into hot TCA insoluble material was derived from *Cellvibrio gilvus* and characterized. This amino-acid incorporation was typical in its requirement for ribosomes, pH 5 fraction, ATP, and magnesium ions, and in its inhibition by ribonuclease and puromycin. Deoxyribonuclease and actinomycin D had little effect, whereas chloramphenicol caused 65% inhibition. The optimal condition for incorporation was 30 min incubation at 30° and at pH 8.0. The addition of an exogenous source of a polyamine (spermidine, spermine, or putrescine) had little effect on this system. Poly-U dependent phenylalanine incorporation was observed with this system and found to be stimulated by an exogenous source of tRNA, although tRNA had no stimulatory effect on leucine incorporation.

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Inhibition of Intestinal Cholesterogenesis *in Vitro** (32636)

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Most mammalian tissues are capable of synthesizing cholesterol *in situ* (1) and it is possible that this cholesterogenic capacity is a general property of growing cells (2). The liver has long been regarded as the principal endogenous source of the circulating cholesterol pool. Recently (3), it has been found that, in man, a significant portion of endogenous cholesterol arises in extrahepatic tissues. While it has been known for some time that the intestine is highly active in synthesizing cholesterol (4–7), its contribution to the pool of circulating cholesterol in man has been established only recently (8). Subsequently, several studies have been reported on the localization and control of intestinal cholesterogenesis in the rat (9, 10).

It is believed that intestinal synthesis of cholesterol follows a pathway similar to that found in the liver. Such a similarity depends upon the presence of similar enzyme systems in both organs. A convenient method of establishing the presence of these enzymes is based on the use of agents known to inhibit enzymatic reactions involved in the biosynthesis of cholesterol (*e.g.*, 11). In the present report, the effect was studied of several inhibitors of hepatic cholesterogenesis on the incorporation of acetate-[2-^{14}C] and DL-mevalonate-[^{3}H] into cholesterol by sections of everted rat ilea. The agents used were AY-9944 (12), 22,25-diazacholestanol[1] (13),

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triparanol[2] (14), SKF 525-A (15), and clofibrate (16).[3]

Materials and Methods. Male white albino rats (150–180 gm) were allowed food and water until decapitation. Intestines were removed and immersed in ice-cold physiological saline. The ilea (distal 20 cm) were cut away and flushed with 100 ml Krebs–Ringer bicarbonate buffer, pH 7.4 (containing 2 mg/ml glucose) which had been previously gassed with 95% O_2/5% CO_2. The ilea were everted[4] (17) with the aid of a glass rod and cut into sections of approximately 5 mm in length. For any given experiment, different parts of individual ilea were distributed equally among the incubation flasks. The combined sections for each incubation were blotted and weighed (800–1000 mg per incubation). Incubations were carried out in 5 ml buffer containing 5 μC (0.2 μmole) acetate-[2-^{14}C] with or without 1 μC (0.01 μmole) mevalonate-[^{3}H] (Radiochemical Centre, Amersham) for 1 hour at 37°C in an atmosphere of 95% O_2 and 5% CO_2. The inhibitors were added at concentrations known to inhibit cholesterol synthesis in rat liver homogenates. Enzymatic activity was terminated with 12 pellets of KOH. Ethanol (4 ml), water (2 ml), and carrier cholesterol (50 mg) were added, the suspension was heated at 75–80°C for 1 hour, and the neutral lipids were extracted with petroleum ether (bp 30–50°C). Cholesterol was isolated, purified and counted as 5,6-dibromocholestan-3β-ol (18). Samples were dissolved in 15 ml Liquifluor solution [4 gm/liter 2,5-diphenyloxazole and 50 mg/liter 1,4-*bis*(5-phenyloxazolyl)benzene] and the radioactivity was measured in a Nuclear Chicago Liquid Scintillation System, model 720.

Results. In the first experiment, AY-9944 was used at different concentrations in order to establish the sensitivity of the incubation procedure (Table I). Inhibition values were similar to those obtained with rat liver homogenates (19).

TABLE I. Effect of AY-9944 on the Incorporation of Acetate-[2-^{14}C] into Cholesterol by Rat Intestinal Sections.

	dpm/gm tissue[a]	
	Neutral lipids	Cholesterol[b]
Controls	278,500	22,500
AY-9944, 1×10^{-6} *M*[c]	256,100	226 (99)[d]
1×10^{-6} *M*	252,400	2940 (87)[d]
1×10^{-7} *M*	270,600	13,630 (39)[d]

[a] All values are averages of duplicate incubations. Each flask contained 5 μC (0.2 μmole) acetate-[2-^{14}C].

[b] Isolated, purified, and counted as 5,6-dibromocholestan-3β-ol.

[c] Final concentration.

[d] Percentage inhibition.

The effect of the other inhibitors on the incorporation of acetate-[2-^{14}C] and mevalonate-[^{3}H] into cholesterol is presented in Table II. The results of several experiments are reported; in each experiment, AY-9944 (1×10^{-5} *M*) was included as reference standard in order to establish the cholesterogenic activity of the intestinal preparation. At the concentrations used, triparanol, SKF 525-A and 22,25-diazacholestanol inhibited the biosynthesis of cholesterol from both precursors. No change took place in the presence of clofibrate; this finding corresponds with the lack of inhibition observed when clofibrate was added to liver slices and homogenates prepared from normal rats (20).

Discussion. The incorporation of acetate-[2-^{14}C] into neutral lipids (2–3%) by rat intestine was considerably higher than into cholesterol (0.2–0.4%). With rat liver homogenates, it has been routinely found in our laboratory that 0.7–1.0% of acetate is incorporated into neutral lipids and 0.3–0.6% into cholesterol, while the incorporation into cholesterol of radioactivity originally associated with mevalonate has routinely approxi-

[2] Kindly provided by Dr. C. A. Bunde, Wm. S. Merrell Co.

[3] AY-9944 = *trans*-1,4-bis(2-chlorobenzylaminomethyl)cyclohexane dihydrochloride; triparanol = 1-[4(diethylaminoethoxy)phenyl]-1-(*p*-tolyl)-2-(*p*-chlorophenyl)ethanol; SKF 525-A = β-diethylaminoethyl diphenylpropylacetate hydrochloride; clofibrate = ethyl-α-*p*-chlorophenoxyisobutyrate.

[4] The incorporation of acetate-[2-^{14}C] into neutral lipids was lower in sections prepared without eversion.

TABLE II. Effect of Several Inhibitors of Hepatic Cholesterogenesis on the Incorporation of Acetate-[2-^{14}C] and DL-Mevalonate-[^{3}H] into Cholesterol by Rat Intestinal Sections.

	dpm/gm tissue[a]			
	Neutral lipids		Cholesterol[b]	
	Acetate	Mevalonate	Acetate	Mevalonate
Control	238,900	28,230	8790	915
SKF 525-A (5×10^{-4} *M*)[c]	183,300	22,300	287 (97)[d]	72 (92)[d]
Control	221,800	27,000	8220	915
Triparanol (5×10^{-4} *M*)	248,900	27,700	376 (95)	96 (89)
Control	256,100	29,500	9650	915
22,25-Diazacholestanol (1×10^{-4} *M*)	257,100	28,900	454 (95)	70 (92)
Control	137,100	—	4930	—
Clofibrate (1×10^{-3} *M*)	176,800	—	4920	—

[a] All values are averages of triplicate incubations. Each flask contained 5 μC (0.2 μmole) acetate-[2-^{14}C] and 1 μC (0.01 μmole) mevalonate-[^{3}H]; flasks containing clofibrate contained only acetate-[2-^{14}C] as precursor.

[b] Isolated, purified, and counted as 5,6-dibromocholestan-3β-ol.

[c] Final concentration.

[d] Percentage inhibition.

mated 50% of that present in neutral lipids. The values obtained in the present study show that, in the rat intestine, only 3% of the mevalonate radioactivity found in the neutral lipid fraction was recovered in cholesterol. These results suggest that, in rat intestinal sections, the rates of one or more of the enzymatic reactions involved in the conversion of neutral sterols (and possibly squalene) into cholesterol are slower than in the liver. This may result in the formation of small pools of cholesterol precursors (*e.g.*, 6,7) sufficient to trap and dilute the radioactivity originating from acetate or mevalonate. These results are in contrast with those of Dietschy and Siperstein (9,10), who reported that the digitonin-precipitable sterol fraction isolated from rat intestine following incubation with acetate-[^{14}C] comprised mainly cholesterol, with negligible amounts of other C_{27} sterols. This discrepancy may reflect differences in experimental technique and remains to be resolved. It should be noted that, using the experimental conditions described in the present communication, the incorporation of mevalonate into cholesterol was only 0–0.05%. This may reflect limited penetration of mevalonate to the site(s) of intestinal cholesterogenesis (cf. 21).

The results show that AY-9944, SKF 525-A, triparanol, and 22,25-diazacholestanol suppressed the incorporation of both acetate and mevalonate into cholesterol by sections of everted rat intestines. These inhibitors of cholesterol synthesis differ in their site of action: AY-9944 blocks the conversion of 7-dehydrocholesterol to cholesterol (12), while triparanol (22) and 22,25-diazacholestanol (23) inhibit the reduction of the Δ^{24}-bond of lanosterol and its metabolites. This indicates that the enzymes 7-dehydrocholesterol Δ^{7}-reductase and 24-dehydrosterol Δ^{24}-reductase are present in the rat intestine. The site of cholesterol biosynthesis inhibition of SKF 525-A has not been conclusively established (15,24). The data in Table II indicate that

SKF 525-A, while slightly depressing the incorporation of acetate and mevalonate into petroleum ether soluble neutral lipids, mainly inhibited (90%) the synthesis of cholesterol at a site after the formation of neutral lipids. This finding supports the postulate that SKF 525-A acts at least to some degree prior to the formation of squalene (15); however, its main effect is exerted after squalene formation. In any case, the rat intestine contains the enzyme systems inhibited by SKF 525-A.

These experiments demonstrate the similarity in enzymatic reactions involved in cholesterol synthesis in rat liver and intestine.

Summary. AY-9944, SKF 525-A, triparanol, and 22,25-diazacholestanol, agents known to inhibit hepatic cholesterol synthesis, suppressed the incorporation of acetate-[2-^{14}C] and mevalonate-[^{3}H] into cholesterol by sections of everted rat intestines. Clofibrate had no effect on the conversion of acetate-[2-^{14}C] into cholesterol. The results indicate the presence in rat intestine of enzyme systems involved in the saturation of the Δ^{24}-bond in lanosterol and its metabolites, the conversion of 7-dehydrocholesterol into cholesterol, and those inhibited by SKF 525-A. Hence, the pathway of intestinal cholesterol synthesis is similar to that of the liver.

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