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Factors modifying cholesterol uptake by intestinal rings

The role of the intestinal plasma membrane in determining intestinal absorption of cholesterol was investigated in jejunal slices from hamsters. The linearity of uptake with concentration of micellar cholesterol, and low Q_{10} suggested uptake by simple diffusion[1]. Requirement of metabolic energy, membrane integrity and cholesterol exchange were then investigated for evidence of carrier-mediated processes.

Sodium taurodeoxycholate (Maybridge Chemical Co., Tintagel, England), cholesterol (Nutritional Biochemicals Corp., Cleveland, Ohio), 1-monoolein (The Hormel Institute, Austin, Minn.) and [4-^{14}C]- and [^{3}H]cholesterol (New England Nuclear Corp., Boston, Mass.) were 98 % pure or better, verified by thin-layer chromatography. Enzymes and metabolic inhibitors were obtained commercially. Micellar solutions (5.4 mM monoolein, 0.25 mM cholesterol, 6.0 mM sodium taurodeoxycholate, Krebs–Ringer phosphate buffer, pH 6.3) were prepared with a Branson Sonifier Model S125, 7.5 A, 5 min. Jejunal rings were mixed from hamster pairs of one sex fasted 24 h. Rings (100–200 mg) were incubated in 1 ml micellar solution, with 10 μmoles glucose in a Dubnoff metabolic shaker, at 37°, for 30 min, in 100 % O_2. The fluid was decanted at termination. The rings were rinsed with chilled bile salt buffer.

Experimental modifications were: omission of glucose; comparison of N_2 for O_2 gas phases using a double hood; substitution of buffer ions; addition of test substances, 200 and 500 μg. Rings in modified media were incubated simultaneously with rings under control conditions. Results of experiments performed in duplicate 3 times yielded means and standard errors.

Lipids were extracted from homogenized rings and fluids before and after incubation. Lipid classes were separated by thin-layer silicic acid chromatography. The radioactivity of cholesterol was assayed by liquid scintillation counting[2]. Mean recovery of radioactivity from tissues and media after incubation was 94 % of the activity recovered from initial incubation solutions, entirely as free sterol. Uptake was calculated as mμmoles cholesterol taken up per 100 mg intestinal rings.

The mean uptake $\pm$ S.E. for 12 pairs of incubations of intestine with 0.25 mM cholesterol (250 mμmoles) was 41.6 $\pm$ 1.106 mμmoles (range 35.2–49.2)/100 mg per 30 min, averaging 16.6 % of the sterol incubated (Table I).

Metabolic activity. When glucose was omitted, cholesterol uptake diminished by 19 %. In an atmosphere of N_2, intestinal slices took up 30 % less sterol than in O_2. When sodium azide was added, uptake of cholesterol decreased by 38 %. Iodoacetamide increased cholesterol uptake by 57 %.

Membrane effects. Phospholipase A (phosphatide acyl-hydrolase, EC 3.1.1.4) addition to the incubation medium decreased sterol uptake 50 %. With 2 mM lysolecithin added to 1 mM cholesterol micellar solutions, sterol uptake after 8 min was 101 mμmoles/100 mg (control, 202 mμmoles) and at 30 min it was 136 mμmoles (control, 224 mμmoles). Inclusion of amphotericin B in incubations doubled cholesterol uptake.

Hypocholesteremic drugs. With nicotinic acid, sterol uptake increased by 50 %.

Modifications where sterol uptake approximated simultaneous control values (range 30.6–49.9 mμmoles). Replacing buffer Na^+ by K^+; addition of 2.7 mM Ca^{2+} and 1.3 mM Mg^{2+}; NaF, KCN, *N*-ethylmaleimide; dimethylsulfoxide 1.0 % or 2.5 %;

TABLE I

CHOLESTEROL UPTAKE

Cholesterol uptake is expressed in mμmoles ± S.E. per 100 mg (incubation, 30 min with 250 mμmoles cholesterol). Values in parentheses are the number of paired incubations. $P < 0.001$ for all experimental values compared to control using Student's t test. Control value: 41.6 ± 1.01 (12).

Modification of incubation mixture	*Cholesterol uptake (mμmoles ± S.E. per 100 mg)*
No glucose	33.3 ± 1.01 (3)
100% N_2	29.1 ± 1.20 (3)
Sodium azide (200 μg)	25.8 ± 3.50 (3)
Iodoacetamide (200 μg)	65.3 ± 4.21 (3)
Phospholipase A (200 μg)	21.8 ± 1.71 (3)
Amphotericin B (200 μg)	89.0 ± 5.00 (3)
Amphotericin B (500 μg)	73.2 ± 4.60 (3)
Nicotinic acid (200 μg)	62.4 ± 0.88 (3)

vitamin A; neomycin, cetrimide, ethanol; phospholipase C (phosphatidylcholine cholinephosphohydrolase, EC 3.1.4.3), phospholipase D (phosphatidylcholine phosphatidohydrolase, EC 3.1.4.4).

Exchange between cholesterol previously taken up and sterol in the medium. Intestinal rings were incubated 8 min in 4 beakers with 1 ml 0.25 mM micellar cholesterol containing 124, 850 counts/min [^{3}H]cholesterol and rinsed. Tissues from 2 beakers were transferred immediately to beakers containing 17500 counts/min [4-^{14}C]cholesterol and incubated an additional 8 min. Radioactivity was measured in tissues incubated in [^{3}H]cholesterol without and with subsequent transfer to [^{14}C]cholesterol solutions (Table II). Rings took up labeled cholesterol from both media but released about half the [^{3}H]cholesterol taken up in the first 8 min into the second incubation medium in the succeeding period, demonstrating exchange.

TABLE II

EXCHANGE OF CHOLESTEROL BETWEEN TISSUE AND MEDIUM

Incubation	*Uptake of label*			
	Counts/min per 100 mg		%	
	^{14}C	^3H	^{14}C	^3H
First with [^{3}H]cholesterol	0	8134	0	6.5
Second with [^{14}C]cholesterol	1942	3881	10.5	3.1

Inhibition of energy-yielding reactions had small influence on cholesterol uptake by intestinal slices. Kinetic data did not support cholesterol accumulation "uphill" against a gradient, or active transport[1]. Segments perfused *in vitro* took up cholesterol equally from mucosa or serosa, with no asymmetric transport[6]. These data indicate that micellar cholesterol is taken up by the intestine by diffusion. Similar conclusions were reached for fatty acid uptake[3,4]. In contrast, under anaerobic conditions, and using metabolic inhibitors intestinal absorption of most sugars and amino acids was depressed profoundly implying "active" transport[5].

Phospholipase A releases hydrophobic segments of the liquid crystalline bimolecular lipid leaflet of the cell membrane, and decreases cell permeability. Lysolecithin, the product of phospholipase A action on lecithin, decreased sterol absorption. Neither phospholipases C nor D, affecting hydrophilic groups of phospholipids influenced sterol uptake. Lysolecithin may affect the membrane, the micellar dispersion of cholesterol, or its adsorption[7]. Where intestine was incubated with lysolecithin in similar concentration, it was taken up and acylated indicating viability of the preparation[8]. Amphotericin B disrupts lipid structures containing sterols with a free hydroxyl group[9] when in contact with the sterol-containing surface. Interaction with membrane sterols affecting sterol orientation increases cell permeability[10]. Conformational changes induced may secondarily affect binding affinity and transport velocity of sterols.

The cholesterol in the intestinal plasma membrane is large relative to phospholipid, with a ratio of 1.11 (ref. 11) resembling that for the erythrocyte. Exchange of cholesterol between intestinal membrane and medium in the present study, and between erythrocyte membranes and serum lipoproteins[12], indicates processes differing from those in culture lymphocytes which showed no exchange of cholesterol between cells and medium[13]. In that system, cholesterol was taken up by adsorption.

Cholesterol is absorbed from a micellar phase of intestinal content containing conjugated bile salts, fatty acids, monoglycerides and lecithin (or lysolecithin). Fatty acids, monoglycerides and cholesterol may be absorbed by diffusion from a molecular phase, rather than a micellar phase[14]. The mechanism of entry of individual micellar components such as cholesterol into intestinal epithelial cells is as yet unknown and warrants further investigation at the cell, organ and whole animal level.

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