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Some observations on the mechanism of absorption of cholesterol in rats

Several aspects of the absorption of cholesterol have been actively studied for quite some time now, and yet very little was known about the actual mechanism of its absorption till GLOVER AND GREEN[1] and GANGULY *et al.*[2] put forward the hypothesis that cholesterol is probably absorbed by a process in which the sterol presented to the external side of the mucosal cell readily displaces a similar molecule already present in the lipoproteins of the cell membrane and thereby penetrates the cell. The above hypothesis was based upon the observation that no significant changes can be noticed in the sterol contents of the intestinal-mucosal cells of rats following either starvation, or feeding of cholesterol. A direct proof for the hypothesis was rendered difficult by the fact that the endogenous cholesterol is always present in large quantities in the mucosal cells, almost entirely in the free form[2], and that it is in this form that the sterol enters the cell[3]. However, the endogeneous cholesterol can readily be differentiated from the newly absorbed sterol by feeding ^{14}C-labelled cholesterol. Using such ^{14}C-labelled cholesterol it is now shown here that 74–77% of the newly absorbed sterol is recovered from the microsomal particles of the mucosal cell homogenate, the supernatant fraction being almost free of it.

[4-^{14}C]Cholesterol (0.4 μC), obtained from Radiochemical Centre, Amersham, after dilution with 3 mg of cholesterol, was dissolved in minimal amounts of diethyl ether. The other solution was poured on 500 mg of a normal stock diet and the solvent was allowed to evaporate off, after which the diet was thoroughly mixed. Normal male rats of this Institute strain weighing 120–130 g were starved for 18 h and individually offered 500 mg of the cholesterol-mixed diet. Usually the rats consumed the entire diet within 5 min of offering. Water was given *ad libitum*. At the given time intervals the rats were killed by direct heart puncture, while under ether anaesthesia. The small intestine was immediately removed and transferred into a beaker previously placed in crushed ice. It was then processed according to GANGULY *et al.*[2] with the difference that the microsomal fraction was collected by centrifuging the mitochondrial supernatant at 104000 $\times$ *g* for 60 min in a Spinco model L ultracentrifuge. The lipids of the sub-cellular particles of the mucosa and of the muscles of the small intestine were extracted by boiling for 10 min with 3 volumes of an alcohol–ether mixture (3:1, v/v), followed by two extractions with light petroleum

(40–60°) in a Waring blendor. The solvent was removed by evaporating under suction and the free and ester forms of cholesterol were separated by chromatography on silicic acid columns according to FILLERUP AND MEAD[4]. After proper dilutions, the sterol was plated on aluminium planchets and the radioactivity was measured with a Geiger–Müller tube (made by General Electric Co. Ltd., England, Type E.H.M.I/S, window weight 2.3 mg/cm^2, operating voltage 1520 V), attached to a PANAX Scaler (Type 100 C, Redhill, Surrey). Chemical estimation of cholesterol was carried out by means of the Liebermann–Burchard reaction.

It is shown in Table I, where the results of two typical experiments are given, that in the mucosa, the net amount of free [^{14}C]cholesterol was higher at the 2-h interval, as compared to the 4-h values. However, its relative distribution in the various subcellular fractions was similar at both the time intervals and the microsomal fraction always contained almost all of it (74–77 %). The counts due to the ester fraction in the mucosa were comparatively very low, being 2.5–8 % of the total, and of these, the maximum was always associated with the microsomal particles. In separate experiments, where no fat or 20 % fat (the labelled sterol was previously dissolved in the fat) was present in the diet, the pattern of intracellular distribution of the absorbed [^{14}C]cholesterol was essentially similar. It is interesting that BORGSTROM *et al.*[5] have mentioned in one of their papers that 5 h after a dose of labelled cholesterol its relative distribution in the mitochondrial, microsomal and supernatant fractions of the mucosal cell homogenate was 30, 60 and 10 %, respectively. These results thus broadly agree with the present values.

These experiments, when continued upto 48 h, showed a similar relative intracellular distribution of the free and esterified forms of the newly absorbed sterol at all time intervals studied. However, the net amount of the [^{14}C]cholesterol of the mucosa began to decline after 2 h and almost disappeared at 48 h. In the intestinal muscles, there was some [^{14}C]cholesterol in the 2-h and 4-h samples, but it was mostly in the free form. At 48 h, when the mucosa was almost free of the labelled sterol, there was considerable amount of it in the intestinal muscles, again in the free form.

TABLE I

DISTRIBUTION OF [4-^{14}C]CHOLESTEROL IN THE CONTENTS AND MUSCLES, AND WITHIN THE MUCOSAL CELLS OF THE SMALL INTESTINE OF RATS

Values are expressed per small intestine and each point represents an average of two rats. Counts are given as obtained in the instrument, which has an overall efficiency of 2 %.

	Cholesterol							
	Free		*Esterified*		*Free*		*Esterified*	
	2 h (counts/min)	*4 h (counts/min)*	*2 h (counts/min)*	*4 h (counts/min)*	*2 h (mg)*	*4 h (mg)*	*2 h (mg)*	*4 h (mg)*
Contents	2745	3037	194	70	1.888	2.012	0.328	0.288
Mucosa:								
nuclei	133	102	11	6	0.188	0.164	0.060	0.052
mitochondria	708	507	36	22	0.880	0.668	0.060	0.060
microsomes	3220	2430	94	175	2.712	2.448	0.200	0.144
supernatant	300	130	76	66	0.368	0.288	0.160	0.144
Muscles	152	190	20	31	4.480	4.400	0.192	0.224

Some of the striking features of these preliminary results are, that the labelled sterol is almost quantitatively localised in the microsomal particles of the mucosal cell homogenate, the supernatant fraction is practically free of it, and that the esterified [^{14}C]cholesterol is present only in very small amounts in the microsomal and supernatant fractions and in the intestinal muscles throughout the entire period of 15 min to 48 h. This is in sharp contrast with our observations on the absorption of vitamin A, where most of it was recovered from the supernatant fraction, and in the esterified form.

Clearly, during absorption, cholesterol is thrown out of fat solution and is subsequently absorbed at a molecular level, otherwise one would expect it to be present in the supernatant fraction, if it were to be absorbed in a state of solution in fats. The almost quantitative localization of the newly absorbed sterol in the microsomal particles and in the free form, lends strong support to the hypothesis that it is absorbed by a process of displacement of the existing cholesterol of the lipoproteins of the cell, because during its absorption no net increase in the cholesterol contents of the mucosal cell could be noticed by GANGULY *et al.*[2]. The microsomal particles are currently being considered to consist of, among other constituents, the endoplasmic reticulum and the membrane of the cell[6,7]. In that case, the endoplasmic reticulum, in addition to the membrane of the mucosal cell, would appear to have added significance in the absorption of cholesterol. Since the absorbed cholesterol appears mostly as the ester in the lymph[3], the virtual absence of the [^{14}C]sterol ester from the mucosal cells and the intestinal muscles would appear to support the theory of GLOVER AND GREEN[1] that it is esterified at the time of its transfer into the lymph.

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