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Sterol Metabolism

4. THE ABSORPTION OF 7-DEHYDROCHOLESTEROL IN THE RAT*

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It was shown previously that sterols undergoing absorption in the guinea-pig intestine become distributed uniformly among the cell fractions and are not confined to fatty globules (Glover & Green, 1957). The sterol composition of each cell fraction was found to be the same, indicating that an exchange and transfer of the sterols took place between them. It was suggested that this process played an important part in absorption and indicated that sterols were absorbed at the molecular level.

The present work was undertaken to see if a similar situation obtains in another species, the rat, which has been the main experimental animal used for lipid-absorption studies. A preliminary account of the work has been described (Glover & Stainer, 1957).

7-Dehydrocholesterol was selected for the test because the rat intestine, in contrast with that of the guinea pig, contained little (Glover, Glover & Morton, 1952) and its characteristic spectral absorption serves as an excellent label for tracing its movement across the cell.

EXPERIMENTAL

Materials. 7-Dehydrocholesterol was obtained from Peboc Ltd., Liverpool, and was recrystallized from methanol before use until $E^{1\%}_{1\,cm.}$ 281·8 mμ was 286–290.

Animals. Male rats in the weight range 140–265 g. were divided into groups of two having approximately the same mean weights. Four days before selection for experiment they were placed on a lipid-free diet of solvent-extracted food cubes (no. 4 diet, British Extracting Co. Ltd., Port Sunlight). Control groups (C1–C7) were fasted for 24 hr. and then killed for examination as described previously (Glover *et al.* 1952). Each animal in the test groups (D1–D8) was given a dose of 150 mg. of 7-dehydrocholesterol dispersed in 0·5 ml. of arachis oil and mixed into 10 g. of lipid-free diet. The animals were killed 5 hr. later for examination. In four experiments (D5–D8), however, each animal was given a similar dose 24 hr. before the final dose. The additional material was administered to ensure a reasonable level of absorption along the intestine at the time of examination.

Preparation of mucosal cell homogenate. The proximal half of the small intestine was removed from each animal and immediately flushed out several times with ice-cold 0·25 M-sucrose solution. The tissue was then cut into 4 in. lengths, opened to expose the mucosa and laid flat on top of large Petri dishes containing salt–ice mixture. The mucosal layer was scraped off gently with the back of a scalpel and transferred with five times its volume of 0·25 M sucrose solution into a pre-cooled Potter–Elvehjem-type homogenizer having a Teflon (Fluon) pestle. The mucosal cells were comminuted for 2 min. with the tube immersed in salt–ice mixture.

The volume of the homogenate was noted (about 25–30 ml.) and a portion, usually one tenth, taken for sterol analysis. The fractionation of the remainder was carried out in a refrigerated centrifuge at -5 to $+5°$. A modification of the procedure used by Glover & Green (1957) was introduced for the control groups C4–C7 and the dosed animals D1–D8. It was desired to obtain more concentrated groundplasm fractions for nitrogen assays and electrophoresis studies (results to be reported in another paper). Consequently, the homogenate in the centrifuge tubes was covered with a layer of light petroleum–ether (50:50, v/v) and subjected to 20 000 *g* for 90 min. The centripetally moving fatty particles collected in the organic solvent (F layer) and the supernatant groundplasm fraction (I) were then carefully separated; the latter was thus obtained without dilution with washings. The sedimented particulate material was recomminuted in the homogenizer with the same volume of 0·25 M-sucrose as before and then fractionated to yield the fractions: 'cell debris and nuclei', mitochondria, microsomes, groundplasm (II). Although the two supernatant fractions, groundplasm (I) and (II), were analysed separately, the results for sterol analysis have been combined for presentation. The above-mentioned microsome fraction is obtained

* Part 3: Glover & Green (1957).

at a relatively low g value and hence contains mainly the larger lipid-containing granules and not the smaller particles with a high nucleic acid content (Barnum & Huseby, 1948).

This modified procedure was advantageous in dealing with the mucosal homogenates from the dosed animals, which with their increased fat content tended to agglutinate, and not fractionate easily.

Extraction of lipids and sterol estimations. The lipids were extracted from each fraction after the denaturation of the proteins as previously described (Glover & Green, 1957). The total, 'slow-' and 'fast-reacting' sterols were assayed by the Moore–Baumann (1952) method. The latter group, having a double bond in the 7:8 position, are also referred to as Δ^7-stenols.

The 7-dehydrocholesterol was determined spectrographically (Glover *et al.* 1952). It should be mentioned here that for the purposes of applying the correction for irrelevant absorption, it is advisable that the standard curve on which the correction procedure is based should be obtained on the instrument used for the routine analysis, and modified correction points selected if necessary.

RESULTS

The distribution of the total sterols in the various cell fractions from the fasting and dosed animals is shown in Table 1. The variation in the mean values for the fasted groups C1–C3 compared with those for C4–C7 inclusive arises from the difference in fractionation technique. With C4–C7 the fatty layer was removed first by spinning the homogenate for 90 min. at 20 000 g. The redispersion of the sedimented particles probably breaks down the residual whole cells which had previously escaped rupture. Thus the microsomes and groundplasm fractions become larger at the expense of the 'cell debris and nuclei' fraction, which includes unbroken cells.

The mean value for the total sterol content of the mucosal homogenate from the dosed animals is approximately 3–7 mg. greater than that for the controls, but the percentage distribution of the total sterols among the various cell fractions remains much the same. This indicates that the sterol absorbed from the lumen becomes uniformly dispersed among the organelles and is not retained in the fatty globules which make up the F-layer fraction. The latter, in fact, from both the fasting and control animals, contains only 3 % of the total cell sterols.

The amount, however, by which each mucosal cell fraction increases its sterol content during absorption of 7-dehydrocholesterol is discussed in the next paper (Glover, Green & Stainer, 1959).

The contents of 'fast-reacting' Δ^7-stenols, lathosterol and 7-dehydrocholesterol, expressed as a percentage of the total sterols in each cell fraction, are given in Table 2.

Table 1. *Distribution of total sterols in the intestinal mucosal cells of fasting rats and animals dosed with 7-dehydrocholesterol*

There were two rats in each group. Dose: 150 mg. in experiments D1–D4 and 300 mg. in D5–D8. A modified fractionation procedure was used for control groups C4–C7 and for the dosed animals. Results for each fraction are expressed as a percentage of total sterols in the homogenate.

	C1–C3		C4–C7	
	Range	Mean ± S.D.	Range	Mean ± S.D.
	Control groups			
Total sterols in homogenate (mg.)	6·5–9·5	8·1	6·5–7·5	7·1
Cell fraction:				
Cell debris and nuclei	14·4–27·2	20·5 ± 6·4	6·2–15·1	9·6 ± 3·9
Mitochondria	11·1–15·4	13·2 ± 2·2	5·4–17·0	10·0 ± 5·1
Microsomes	24·4–36·9	31·3 ± 6·4	30·0–48·4	39·9 ± 8·1
Groundplasm	23·4–27·6	25·5 ± 3·0	27·1–34·4	31·9 ± 3·4
F layer	1·3–4·0	2·3 ± 1·5	0·8–5·4	2·8 ± 2·1
Recovery (%)	76–94	—	81–100	—
	D1–D4		D5–D8	
	Range	Mean ± S.D.	Range	Mean ± S.D.
	Dosed groups			
Total sterols in homogenate (mg.)	8·5–14·6	10·2	11·6–16·1	14·4
Cell fraction:				
Cell debris and nuclei	7·0–12·4	9·1 ± 2·9	6·4–10·2	8·7 ± 1·7
Mitochondria	7·2–22·5	17·2 ± 7·2	8·6–16·6	11·8 ± 3·4
Microsomes	31·7–38·9	36·2 ± 3·3	31·2–40·0	36·2 ± 3·7
Groundplasm	17·3–30·4	25·6 ± 5·9	22·4–43·5	30·7 ± 9·1
F layer	1·8–8·1	4·2 ± 2·8	1·8–2·5	2·1 ± 0·3
Recovery (%)	84–90	—	85–98	—

Table 2. *Percentage of Δ^7-stenols, 7-dehydrocholesterol and lathosterol in the total sterols of the cell fractions from the intestinal mucosa of fasting rats and animals dosed with 7-dehydrocholesterol*

Cell fraction	Total Δ^7-stenols (%)		7-Dehydrocholesterol (%)		Lathosterol (%) (by diff.)	
	Range	Mean ± S.D.	Range	Mean ± S.D.	Range	Mean ± S.D.
	Control animals (seven experiments)					
Homogenate	8·4–22·9	14·7 ± 5·4	2·9–4·3	3·6 ± 0·6	5·0–19·6	11·1 ± 5·4
Cell debris and nuclei	6·9–20·0	14·7 ± 4·9	2·1–5·3	3·5 ± 1·3	4·3–15·5	11·2 ± 4·8
Mitochondria	10·4–22·0	15·6 ± 4·3	1·9–6·9	4·4 ± 1·9	5·8–14·8	11·2 ± 3·9
Microsomes	5·5–16·7	11·7 ± 4·6	1·6–3·5	2·5 ± 0·7	3·9–14·4	9·2 ± 4·3
Groundplasm	6·5–22·4	11·1 ± 5·8	2·7–5·4	3·7 ± 0·9	1·1–19·4	7·4 ± 6·3
F layer	4·4–45·8	23·1 ± 17·1	0·8–7·5	4·6 ± 2·6	1·2–38·3	18·5 ± 15·0
	Dosed animals (eight experiments)					
Homogenate	51·7–68·4	62·7 ± 5·9	17·4–26·0	22·2 ± 2·6	34·3–46·2	40·5 ± 4·3
Cell debris and nuclei	47·1–74·4	62·8 ± 10·9	13·2–26·9	19·4 ± 4·6	20·2–58·2	43·4 ± 12·3
Mitochondria	57·8–83·5	71·0 ± 7·7	10·5–28·3	18·4 ± 5·9	15·0–56·5	52·6 ± 11·1
Microsomes	40·5–71·9	59·5 ± 10·0	20·4–34·2	25·4 ± 5·1	20·1–50·9	34·1 ± 12·0
Groundplasm	53·1–70·1	64·0 ± 5·8	12·7–26·9	19·0 ± 4·8	29·8–57·4	45·0 ± 8·7
F layer	55·1–84·7	69·8 ± 12·8	11·1–30·8	18·8 ± 7·8	36·2–69·8	51·0 ± 11·7

In the control groups, the 'fast-reacting' Δ^7-stenols make up 15% of the total, 7-dehydrocholesterol accounts for 3–4% and the remainder is regarded as being mainly lathosterol. These results are in agreement with those of Moore & Baumann (1952). In the dosed animals, the quantity of Δ^7-stenols (47%) is greater, as expected, but the increase in 7-dehydrochlolesterol (approx. 19%) accounts for only a small part of the total increment. Thus a considerable portion of the 7-dehydrocholesterol absorbed has apparently been reduced enzymically at the 5:6 double bond to form lathosterol. The content of the latter in each cell fraction is given by the difference between the values for total Δ^7-stenols and 7-dehydrocholesterol.

There was no significant increase in the cholesterol content of the intestinal mucosa of the dosed animals over that of the controls. Clearly, the different endogenous sterols must be rapidly exchanged and mixed between the cell components. The 7-dehydrocholesterol absorbed from the lumen of the intestine and its transformation products also enter into these exchange and transfer processes.

DISCUSSION

The above results for the distribution of total sterol across the intestinal mucosal cell of the rat are in general agreement with those obtained by Schotz, Rice & Alfin-Slater (1953) for rat liver, when account is taken of the higher *g* value used by these workers in obtaining the microsome fraction. They differ a little, however, from the previous results obtained in this Laboratory on the guinea pig (Glover & Green, 1957). The difference (a smaller 'cell debris and nuclei' fraction and larger groundplasm fraction) is probably due more to the modification of the technique mentioned above than to a species difference.

In keeping, however, with the results of most workers using mainly liver tissue, the microsome fraction contains the largest portion of the sterol content of the mucosal cell.

With regard to the 'fast-reacting' Δ^7-stenols, the total amount in the mucosal tissue of the fasting rat (10–20% of the total sterols) is very similar to that for the fasting guinea pig (Glover & Green, 1957). In the latter species, however, 7-dehydrocholesterol makes up half of this amount whereas in the rat it accounts for only a quarter. It is possible to change markedly the sterol composition of the tissue by prolonged feeding of a given sterol (Gould, 1955; Glover & Green, 1956), which displaces some endogenous material. The effect, if any, on cell function remains to be determined. However, soon after the administered sterol is withdrawn from the diet the characteristic sterol composition or equilibrium is restored.

The results in Table 2, showing that the distribution of the various sterols among the cell components tends to be uniform, confirm the previous findings of Glover & Green (1954, 1957) for the guinea pig. The fact that the newly formed lathosterol, which has increased from 11 to 40% of the total sterol in the dosed animals, is also evenly mixed shows that the exchange and transfer process between the lipoproteins of the various cell components is more rapid than the enzymic reduction of the 5:6 double bond of 7-dehydrocholesterol.

It is postulated that in the rat, as well as the guinea pig, the sterol-exchange and -displacement process in lipoproteins aids the transfer of the sterols across the mucosal cells and thus their absorption. The sterols finally transferred into the

lymph will therefore contain a mixture of dietary and endogenous sterols together with reduced or oxidized derivatives formed by the action of reductases (or cholesterol dehydrogenases) within the mucosa.

Recently Swell, Trout, Hopper, Field & Treadwell (1958) have also observed that dietary [4-^{14}C]cholesterol mixed with the endogenous sterols of the intestine of the rat during absorption. It should be noted, however, that this exchange and mixing process involves only the endogenous sterols of the mucosa and connective-tissue layers, and not those of the muscle layers, with which the dietary sterols scarcely come into contact. It was shown previously that the sterol compositions of these two neighbouring types of tissue are quite different and therefore do not take part in the above rapid-exchange system (Glover *et al.* 1952; Glover & Green, 1957).

SUMMARY

1. The distribution of total sterols, 7-dehydrocholesterol and lathosterol between the various cell fractions of intestinal mucosa of fasting rats and animals dosed with 7-dehydrocholesterol has been determined.

2. In both groups of animals, about 50% of the total sterols in the tissues was associated with the microsomes and mitochondria, 30% with the supernatant or groundplasm and 10% with the 'cell debris and nuclei' fraction. The mitochondrial fraction of the dosed rats contained more of the total sterol (15%) than the corresponding fraction from fasting animals (10%).

3. In agreement with previous work on the guinea pig, the various sterols were uniformly distributed among the components of the mucosal cells of both fasting and dosed groups, implying that the accumulated dietary sterol can exchange or mix with the endogenous sterols of the cell components.

4. It is also postulated that in the rat the uptake and exchange of sterols between the mucosal cell lipoprotein plays an important role in the absorption of sterols. The sterols in excess of the fasting level which are finally transferred to the lymph will, however, contain a mixture of the dietary and endogenous types displaced by the exchange process.

5. A large part of the 7-dehydrocholesterol absorbed from the intestine is also reduced within the mucosa to lathosterol.

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5. THE UPTAKE OF STEROLS BY ORGANELLES OF INTESTINAL MUCOSA AND THE SITE OF THEIR ESTERIFICATION DURING ABSORPTION

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The total sterols in the intestinal mucosa of the fasting guinea pig, previously maintained on a cholesterol-free diet, are invariably unesterified in the free alcohol form (Glover, Glover & Morton, 1952). On the other hand, it has been widely established since the work of Mueller (1916) that absorbed cholesterol appears in the lymph mainly as the ester, irrespective of the form in which it is administered. Cholesterol esters are hydrolysed mainly in the lumen of the intestine before absorption, but the position in the gut wall where resynthesis of esters commences has not yet been satisfactorily settled. Favarger & Metzger (1952) administered single doses of deuterated cholesterol oleate to rats, and found that the proportion of the labelled sterol in the free form was greater in the