Sterol Metabolism

3. THE DISTRIBUTION AND TRANSPORT OF STEROLS ACROSS THE INTESTINAL MUCOSA OF THE GUINEA PIG*

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Animal tissues contain different proportions of several closely related sterols, and in the intestinal mucosa at least one of these, 7-dehydrocholesterol ($\Delta^5:7$ -cholestadien-3 β -ol), has been shown to be in equilibrium with the remainder (Glover, Glover & Morton, 1952). These sterols are composed mainly of cholesterol (Δ^5 -cholestenol) and some lathosterol (Δ^7 -cholestenol) (Moore & Baumann, 1952). It was of interest to make a more detailed study of intestinal mucosa to determine the proportion of 7-dehydrocholesterol in the various cell components; the sterols in some parts of the cell structure might not be exposed to the dehydrogenase enzymes, and hence the percentage of 7-dehydrocholesterol in the total present in those components would differ from the average value for the whole cell. A difference in the distribution of one sterol relative to another could help to establish the site of the enzyme concerned. Further information might also be obtained by observing changes in the sterol content of the mucosal-cell components while 7-dehydrocholesterol or cholesterol was being absorbed. Again, by direct examination of the fraction containing the fatty globules derived from the cell, it would be possible to test the hypothesis that the absorption of sterols was closely linked to that of fat, possibly by virtue of their solubility in it (Frazer, 1955).

Only a few studies have previously been carried out on the distribution of sterols in the cell components. Swanson & Artom (1950) demonstrated that lipid from rat-liver mitochondria contained 4.4% of cholesterol, which concentration is similar to that for the whole tissue. Chauveau, Clement, Clement-Champougny & Le Breton (1951) and recently Schotz, Rice & Alfin-Slater (1953) found cholesterol in all cell fractions obtained by differential centrifuging of liver cells. Even more recently Spiro & McKibbin (1956) have made a detailed study of the distribution of phospholipid and sterol in rat-liver cells. In our preliminary work liver cells of guinea pig were studied to check the fractionation technique, but the main investigations were concerned with the intestinal mucosa. While the present results have not clearly indicated

the localization of the dehydrogenase enzyme, a better understanding of the absorption of sterols has been obtained. The preliminary findings have already been published (Glover & Green, 1954, 1956).

EXPERIMENTAL

Animals. Female guinea pigs, 220–550 g., supplied by an accredited breeder, were placed on our standard no. 4 cube diet (British Extracting Co. Ltd., Bromborough) with a supplement of cabbage leaves until the start of the experiment; 3–4 days before selection for test, they were placed on a sterol-free diet (Glover, Leat & Morton, 1957). Then they were grouped in pairs of uniform total weight and fasted for 24 hr. The control undosed groups were killed, after anaesthetization and withdrawal of blood by cardiac puncture, and examined directly. The test animals were each given 150 mg. of cholesterol or 7-dehydrocholesterol dispersed in 1 ml. of arachis oil mixed with 5 g. of the lipid-free diet. After 24 hr. another 150 mg. of the sterol was administered in the same way and the animals were left for 5–6 hr. before killing, as described above. By this procedure it was hoped that absorption would be occurring along the whole intestine at the time of examination. The livers and small intestines were excised and the latter washed out with 0.88 M-sucrose solution. All tissues were kept in ice-cold 0.88 M-sucrose solution until required.

Preparation of mucosal cells. The two small intestines, freed from mesenteric fat, were slit longitudinally and cut into short lengths which were spread flat on a cold glass surface (0–5°), i.e. the lid of a large Petri dish containing crushed ice and a little salt. The mucosal cells were scraped off with the blunt edge of a scalpel or spatula, collected in a small beaker (10 ml.), immersed in crushed ice, and then transferred completely to the homogenizer. Histological examination of tissue sections showed clearly that this treatment removed only the uppermost cells of the villi, i.e. the cells more exposed to food in the lumen and more active in lipid absorption (Hewitt, 1954). In later experiments, to sharpen further the differences between the fasting and dosed animals, only the mucosa from the proximal third of the intestine was examined because it is better developed in this portion and in fact contains almost half the total mucosal sterol (see Table 5).

The distribution of 7-dehydrocholesterol in relation to the total sterol is fairly uniform along the intestine of the fasting animal (Glover *et al.* 1952). This was confirmed by the present work (Table 5) so that no appreciable error was introduced by sampling only the proximal third. Histological examination of the residual tissue after scraping showed that the scrapings consist mainly of mucosal cells

* Part 2: Glover, Leat & Morton (1957).

with a small amount of the underlying connective tissue, but none of the main lymph vessels of the intestine was removed.

Preparation of homogenates. Liver tissue was cut into small pieces and placed in a porcelain mortar with cold 0.25M-sucrose (1 vol.), and roughly broken up by pressure with a pestle. The resulting mince was filtered through 50-mesh nylon, diluted with 4 vol. of 0.25M-sucrose and transferred to a Potter-Elvehjem homogenizer, which was previously chilled to 0°.

Movement of the homogenizer mortar (35 ml. capacity) up and down smoothly over the rapidly rotating (4000–5000 rev./min.) Teflon (polytetrafluoroethylene) pestle about four times for a total period of 1 min. sufficed to break up the greater portion of the cells.

The homogenate of combined mucosal cells was prepared in a similar manner, the final volumes usually being 30–40 ml. Suitable portions (2–5 ml.) for analysis were im-

mediately withdrawn and the whole of the remainder was fractionated.

Separation of cell components. The fractionation procedure which follows that of Schneider & Hogeboom (1950) is shown in Fig. 1. Steps 1–3 were carried out in 15 ml. centrifuge tubes in a small centrifuge placed in a large refrigerator (–2°). The resulting ‘cell-debris’ fraction, containing unbroken cells and cell membranes in addition to the nuclei, was not fractionated further for the present work. The supernatants from the above steps were then mixed and transferred to 50 ml. plastic centrifuge tubes for the preparation of the mitochondria and microsome pellets. This section of the work was carried out in a high-speed angle-head centrifuge fitted with a refrigeration coil which maintained the temperature of the preparation between –3° and +5°. Before separation of the microsomes, however, a layer of light petroleum (b.p. 40–60°)–diethyl ether (1:1, v/v) was carefully placed on top of the suspension

Fig. 1. Method of fractionating cell components by centrifuging the tissue homogenate in 0.25M-sucrose at –3° to +5°.

(stage 6) so that, during the sedimentation process, fatty droplets migrating centripetally were collected in the organic solvent and not left sticking to the side of the centrifuge tube. A capillary pipette enabled the latter fraction (called F-layer) to be removed cleanly. The mitochondrial pellet, which had a pale-grey appearance, consisted of a fairly compact portion with a lighter fluffy layer on top. Microscopic examination of a dilute suspension showed that it was free from nuclei and broken cells but probably contained some large microsomes. The translucent microsome pellet was dull pink and very compact. The transparent pale yellow-brown supernatant (groundplasm) was easily separated by decantation. In view of the relatively low g value employed here compared with that in other Laboratories, it is probable that some of the very small microsomes (less than 50 $m\mu$ diam.) would be left in this fraction. These, however, have a much smaller amount of lipid than the larger ones (Barnum & Huseby, 1948).

Extraction of lipids. The various pellets were separately ground with anhydrous sodium sulphate and a little washed sand in a mortar; each mixture was transferred to a beaker and extracted four times with 50 ml. of boiling, dry peroxide-free ether. A further extraction with boiling ethanol did not remove any more sterol. The combined ether extracts were evaporated to dryness under nitrogen on a hot-water bath and the residues dissolved in spectroscopically pure ethanol for assay of the 7-dehydrocholesterol.

The groundplasm fraction was poured into sufficient ethanol to make the final alcohol concentration 70%, to denature the proteins. The lipids were then extracted twice with light petroleum (b.p. 40–60°), once with diethyl ether-light petroleum (1:1) and once with ether. These extracts were combined, washed twice with water and dried over anhydrous sodium sulphate. The solvent was evaporated off and the lipid residue dissolved in a suitable volume of spectroscopically pure ethanol (10–25 ml.).

Estimation of 7-dehydrocholesterol. The ultraviolet-absorption spectra of the above solutions suitably diluted were recorded over the range 230–350 $m\mu$. The amount of 7-dehydrocholesterol present was then calculated from the extinction of the solution at 281.5 $m\mu$ after correction for irrelevant absorption (Glover *et al.* 1952).

Estimation of total sterol. A portion of the lipid solution in ethanol was taken to dryness, redissolved in chloroform and the sterol content determined by the method described by Kenny (1952), an EEL colorimeter being used. The range of sterol concentration for minimum error with this instrument was between 0.3 and 1.3 mg./10 ml. of final solution. For

values below this range, the modification of the Kenny method as used by Green, Lowe & Morton (1955) was employed. In this procedure the extinction of the coloured solution at $\lambda_{\max}$, 410 $m\mu$ was read in a Unicam SP.600 photoelectric spectrophotometer.

Estimation of Δ^7 -cholesterol (lathosterol). The colorimetric method, described by Moore & Baumann (1952) for total fast-reacting sterols (Δ^7 -cholesterol and $\Delta^{5,7}$ -cholesta-dienol), was followed except that the volumes of the reacting solutions were increased 1.5 times to suit the EEL colorimeter cells. Filter 607 was used.

Pure crystalline 7-dehydrocholesterol and cholesterol, purified via the dibromide process (Schwenk & Werthessen, 1952), and chromatography on alumina to remove traces of Δ^7 -cholesterol contaminants, were employed for the standardization of the procedure.

The total content of fast-reacting sterols in the fraction minus that of 7-dehydrocholesterol gives the amount of lathosterol present.

RESULTS

Liver. The experiments with liver tissue were of a preliminary nature in order to evolve a suitable technique of cell fractionation which permitted good recovery of the sterols. In four experiments, the sterol distribution was found to be in general agreement with the results of Schotz *et al.* (1953) for rat liver. The recovery of sterol, however, was only 75–88% of the total in the homogenate. The losses were found to be in large part due to inadequate refrigeration during the fractionation, particularly in the high-speed centrifuge where the temperature of the material in the cups at the end of a 90 min. run rose to about 25–30°. For the work on the intestine, the refrigeration was improved so that the temperature of the tissues was kept between –3° and +5° and recoveries were much better (90–107%).

Intestine. The distribution of total sterol in the various fractions of intestinal-mucosal cells from fasting guinea pigs is indicated in Table 1. In those cases where total recovery was greater than the value obtained by analysis of the original homogenate, the higher figure was taken for the calculation of the percentage distribution.

Table 1. *Distribution of total sterols, mainly cholesterol, in the various cell fractions of intestinal mucosa of the fasting guinea pig*

Absolute amounts of sterol found in each fraction are given together with the mean values of the amounts expressed as percentages of the total sterols in the homogenate.

Fraction	Total sterol (mg.)							Mean $\pm$ s.d. (% of total)
	I	II	III	IV	V	VI	VII	
Homogenate	7.65	7.32	24.1	17.64	29.2	9.13	10.23	—
Debris and nuclei	0.72	1.60	3.58	3.41	5.80	2.96	2.00	18.9 $\pm$ 2.8
Mitochondria	0.68	1.36	5.90	3.27	5.43	1.76	2.72	18.4 $\pm$ 5.7
Microsomes	4.04	4.40	13.75	5.73	9.77	3.76	4.08	43.2 $\pm$ 10.5
Groundplasm	0.76	0.54	4.44	2.59	8.40	0.68	0.80	13.3 $\pm$ 8.4
F-layer	0.09	0.20	0.04	0.02	0.10	—	—	0.9 $\pm$ 1.0
Recovery (%)	82.4	107.0	98.0	101.0	85.0	100.3	94.0	—

On the whole the distribution pattern is quite similar to that found for liver tissue. The greatest portion of the sterol in the cell is associated with the microsome fraction. It is of interest that an appreciable amount of the sterol is dispersed in the transparent groundplasm fraction, presumably conjugated with protein. Only a very small amount of cholesterol is present in the F-layer of the intestine compared with that from the liver. This is to be expected, since the latter fraction contains mainly cholesterol in the esterified form (Schotz *et al.* 1953) known to be absent from the intestine of the fasting guinea pig (Glover *et al.* 1952).

The results for animals absorbing cholesterol or

7-dehydrocholesterol are given in Table 2. They show little variation from the controls. In experiment VI the groundplasm and F-layer fractions were much higher than the others. In this instance the whole of the intestinal mucosa was taken, the fat content was higher and consequently may have affected the separation.

Relative distribution of the various sterols in mucosal-cell fractions. The ultraviolet-absorption spectra of the lipids from the various fractions except the F-layer of the fasting animal show clearly the characteristic absorption bands of 7-dehydrocholesterol (Fig. 2) in each. Whereas it is absent from the F-layer in the fasting animal it was

Fig. 2. Ultraviolet-absorption spectra of lipids from the cell components of intestinal mucosa fractionated at 0°. Cell debris and nuclei, ---; mitochondria,; microsomes —; groundplasm, ---.

Fig. 3. Ultraviolet-absorption spectra of chromatographic fractions of lipids from mucosal-cell components fractionated at room temperature. Original lipids, —; fraction A, ---; fraction B, -.-.-; 7-dehydrocholesterol, fraction C, For preparation of fractions see Results section.

Table 2. *Distribution of total sterols in the various cell fractions of intestinal mucosa of female guinea pigs dosed with 150 mg. each of cholesterol and 7-dehydrocholesterol*

Amount of total sterol in each cell component is expressed as a percentage of the total recovered. The total sterol (in mg.) in the original homogenate and the percentage recovered are also included.

Fraction	Cholesterol			7-Dehydrocholesterol		
	I	II	III	IV	V	VI
Homogenate (mg. of sterol)	30.0	11.0*	32.9	10.3*	8.3*	28.3
		(% of total)			(% of total)	
Debris and cell nuclei	31.3	18.9	24.6	21.3	29.7	12.6
Mitochondria	16.2	24.7	14.3	29.4	17.1	12.6
Microsomes	41.2	42.2	37.7	38.6	38.9	29.7
Groundplasm	10.0	12.2	23.4	10.7	14.3	33.7
F-layer	1.3	2.9	—	—	—	11.3
Recovery (%)	97	101	107	96	105	95

* Mucosa was taken only from proximal third of the intestine.

detected in that fraction from the animal dosed with 7-dehydrocholesterol.

In some of the preliminary experiments, marked destruction of 7-dehydrocholesterol occurred during the fractionation and subsequent isolation processes. The absorption spectra of fractions where the recovery of 7-dehydrocholesterol was poor are characterized by a greater extinction at $271.5\text{ m}\mu$ than at the λ_{max} , $281.5\text{ m}\mu$ (Fig. 3). Since the absorption spectra of the tissue lipids dissolved in ethanol did not change appreciably when left in the dark at room temperature for 7 days, the change must have occurred during the cell-fractionation procedure. This was confirmed by leaving a mitochondrial preparation at room temperature for 2 hr. before extraction, when it lost about 30% of its 7-dehydrocholesterol content. The separated lipoproteins containing the sterols are stable, on the other hand, for a few hours at room temperature. The change appears therefore to be enzymic since, by maintaining the preparations at less than 5° during the cell fractionation, 90–100% recovery of this relatively labile sterol was always obtained.

When the contribution due to 7-dehydrocholesterol absorption is subtracted from the spectrum of the tissue lipids of mitochondria which have been kept at room temperature for a few hours, the residual absorption shows maxima at 260 and $272\text{ m}\mu$.

Chromatography of the lipids on alumina [weakened by adding 2% (w/w) water] yielded, apart from known sterols, two impure fractions *A* and *B* (eluted with 4% ethyl ether in light petroleum and 6% ether in light petroleum respectively), each of which had a single maximum at 272 and $260\text{ m}\mu$ respectively. The former chromophore was previously reported (Glover *et al.* 1952) in the same chromatographic fraction of guinea-pig intestinal lipids. The substance responsible was first obtained in purified form by Festenstein (1951), and has been designated SA by Festenstein, Heaton, Lowe & Morton (1955). It is not yet fully characterized. The substance giving rise to the chromophore in the second fraction is also unknown. A third fraction *C*

(eluted with 8% ether in light petroleum) contained most of the 7-dehydrocholesterol free from selectively absorbing constituents.

It is an odd feature, however, of the spectra of the mitochondria and microsome fractions that although the recovery of 7-dehydrocholesterol was excellent, often the extinction at $271.5\text{ m}\mu$ is just higher than that at $281.5\text{ m}\mu$, although for the original homogenate it is invariably less. No satisfactory reason has yet been found for the increase in the irrelevant steeply rising end-absorption of the component lipids over that of the homogenate. It could possibly be due to a more efficient extraction of the lipids after the mechanical breakdown of the cells, or perhaps to some oxidative process during the cell fractionation.

Fasting animals. In seven experiments with animals maintained on the sterol-free diet for a few days before examination, the recoveries of 7-dehydrocholesterol from the cell fractions were satisfactory and the percentage in relation to total sterol in each was calculated. The results (Table 3) show that the distribution of the 7-dehydrocholesterol (which makes up about 9% of the total sterol in each fraction) is very uniform across the cell.

Dosed animals. The results for the percentage of 7-dehydrocholesterol in the various mucosal-cell fractions from animals dosed with cholesterol and 7-dehydrocholesterol are set out in Table 4. The values for the former group are all lower and those for the latter all higher than the controls. Apparently, each fraction has taken up some of the administered sterol, causing a change in the relative amounts of the sterols present, but at the same time the distribution of the 7-dehydrocholesterol across the cell remains quite uniform. In Expts. II, IV and V only the proximal thirds of the intestines were examined. It was thought that by restricting the examination to the absorbing proximal third of the intestine, any difference between fasting and dosed animals would be accentuated. It is seen from Table 5, where the percentage of 7-dehydrocholesterol in the proximal

Table 3. *Distribution of 7-dehydrocholesterol in the cell fractions of intestinal mucosa of the fasting guinea pig*

Fraction	7-Dehydrocholesterol (% of total sterol)							Mean $\pm$ s.d.
	I	II	III	IV	V	VI	VII	
Homogenate	8.7	9.4	9.2	9.9	8.5	9.3	—	9.2 ± 0.5
Debris and nuclei	11.3	11.5	8.4	9.4	8.8	8.6	10.1	9.7 ± 1.3
Mitochondria	6.2	8.9	8.5	6.5	10.4	10.4	10.4	8.8 ± 1.8
Microsomes	11.4	8.1	11.4	7.5	9.8	9.3	10.8	9.9 ± 1.3
Groundplasm	6.7	9.6	Very low	8.9	—	3.2	7.7	7.4 ± 2.8 (8.2 ± 1.3)*

* Mean value of the four highest results.

Table 4. *Distribution of 7-dehydrocholesterol in the cell fractions of intestinal mucosa of animals dosed with cholesterol (group A) and 7-dehydrocholesterol (group B)*

Dose was 150 mg. to each of the two animals per experiment.

7-Dehydrocholesterol (% of total sterol)

Fraction	Group A				Group B			
	I	II	III	Mean $\pm$ s.d.	IV	V	VI	Mean $\pm$ s.d.
Homogenate	6.6	6.8	7.3	6.9 $\pm$ 0.4	14.5	14.3	—	14.4 $\pm$ 0.1
Debris and nuclei	5.9	6.1	8.1	6.7 $\pm$ 1.2	12.7	14.9	18.9	15.5 $\pm$ 2.9
Mitochondria	5.3	7.0	7.2	6.5 $\pm$ 1.1	10.9	15.4	20.0	15.4 $\pm$ 4.5
Microsomes	7.6	6.7	7.6	7.3 $\pm$ 0.5	14.9	15.4	20.4	16.9 $\pm$ 3.1
Groundplasm	10.5	5.5	4.2	6.7 $\pm$ 3.3	13.2	11.6	13.3	12.7 $\pm$ 1.0
F-layer	—	—	—	—	—	—	9.4	—

Table 5. *Distribution of tissue sterols between the proximal third and distal two-thirds of the length of the small intestine of the guinea pig*

Tissue section	Total sterol (mg.)	7-Dehydrocholesterol	
		(mg.)	(% of total sterol)
Mucosa			
Proximal third	11.0	1.02	9.3
Distal two-thirds	13.0	1.03	7.9
Total	24.0	2.05	8.5
Muscle			
Proximal third	12.8	0.32	2.5
Distal two-thirds	16.0	0.38	2.4
Total	28.8	0.70	2.4
Whole intestine	52.8	2.75	5.2

Table 6. *Distribution of slow- and fast-reacting sterols in the cell fractions from intestinal mucosa*

Values were obtained by the method of Moore & Baumann (1952).

Fraction	Controls			Cholesterol fed			7-Dehydrocholesterol fed		
	Slow (mg.)	Fast (mg.)	Fast (%)	Slow (mg.)	Fast (mg.)	Fast (%)	Slow	Fast	Fast (%)
Homogenate	—	—	—	22.2	2.85	11.4	4.60	6.40	58.2
Debris and nuclei	5.98	1.81	23.2	7.1	0.98	12.1	0.68	1.00	59.5
Mitochondria	0.77	0.17	18.1	3.2	0.36	10.1	0.70	0.89	56.0
Microsomes	3.60	1.10	23.4	7.4	1.2	13.9	1.40	2.30	62.0
Groundplasm	0.42	0.10	19.2	2.0	0.31	13.4	1.50	1.50	50.0
F-layer	—	—	—	—	—	—	0.46	0.18	28.1

Table 7. *Distribution of lathosterol in cell fractions of the intestinal mucosa of the guinea pig*

Results, obtained by deducting the amount of 7-dehydrocholesterol from the total amount of fast-reacting sterols, are expressed as a percentage of total slow and fast.

Fraction	Controls	Cholesterol fed	7-Dehydrocholesterol fed
Homogenate	—	1.4	15.4
Debris and nuclei	10.1	2.8	21.4
Mitochondria	0.0	0.0	13.2
Microsomes	8.7	2.2	16.2
Groundplasm	5.8	0.0	9.7

third and the distal two-thirds mucosal fractions are compared, that the selection of the proximal third does not cause an appreciable change in the control value for the percentage concentration of 7-dehydrocholesterol.

The distribution of the total fast-reacting sterols, lathosterol and 7-dehydrocholesterol, was also studied in two experiments. In Table 6 are listed the percentages of fast- and slow-reacting sterols obtained for each fraction in mucosal cells from controls and animals given (a) cholesterol and (b) 7-dehydrocholesterol. Again, the distribution of the total fast-reacting sterols (relative to the slow

reacting) appears to be remarkably uniform, which supports the previous results. The percentages of lathosterol in the various mucosal fractions of the above animals are given in Table 7. The increase above the control level observed in the latter group indicates some conversion of the dietary 7-dehydrocholesterol into lathosterol in the mucosa.

DISCUSSION

The values for the overall distribution of cholesterol across the guinea-pig intestinal-mucosal cells (Table 1) show that the microsomes contain the greatest proportion and the mitochondria the next. These are in general agreement with the results for the liver cells and also with those obtained from rat liver (Schotz *et al.* 1953; Spiro & McKibbin, 1956). The cholesterol content of the F-layer from mucosal cells, however, is very small compared with that from liver cells, which contains mainly esterified cholesterol. The latter is only occasionally detectable in the mucosal fraction from fasting animals (unpublished observations). Since the sterols in the intestinal mucosa are unesterified (Glover *et al.* 1952) and not in solution in the fatty globules, they are presumably in association with other lipids in the formation of the different characteristic membrane-like structures similar to those observed histologically by Sjöstrand & Rhodin (1953) in mitochondria and groundplasm fractions of mouse-kidney-tubular cells and in other tissue cells.

With regard to the uniform distribution of 7-dehydrocholesterol relative to total sterols between the cell fractions, the results in Table 3 show firstly that there is no preferential localization of one sterol in a particular fraction. Hence the location of the dehydrogenase enzyme, which brings about the formation of the related sterols, cannot yet be determined by this procedure. Secondly, they indicate a rapid turnover or exchange of the greater part of the various sterols between their lipoprotein components. Support for this view comes from the fact that in the dosed animal also, despite the change in the percentage content of 7-dehydrocholesterol in the total sterols of each fraction, the values for the different cell fractions still remain at a uniform level. In fact, this exchange or transfer process must take place in the cell faster than the oxidative-reductive steps responsible for maintaining the normal equilibrium between the sterols; otherwise it would not have been possible to demonstrate a change from the fasting level to the new values obtained in the dosed animals. Again, during the absorption of 7-dehydrocholesterol, Δ^7 -cholestenol was formed and was found in uniform relation to the other sterols present. This finding has recently been confirmed in the rat (Glover & Stainer, 1956).

Gould (1951) has previously observed a rapid equilibration between plasma and blood cells of unesterified sterol attached to protein. Again, Gould (1955) found that the free cholesterol of plasma equilibrated much faster with the free cholesterol of liver than did the corresponding esters. Thus the exchange of the sterol between lipoproteins is faster than that promoted by cholesterol esterase between the esterified and free cholesterol. Phospholipids attached to protein have also been shown to exchange quite rapidly (Hahn & Hevesy, 1939). The above findings show that dietary sterol on absorption is taken out of the oily medium, in which it is administered, by the lipoproteins of the organelles and groundplasm and rapidly exchanged between them; only a relatively small amount of the sterol appears in the fatty globules dispersed within the cytoplasm. This leads to the conclusion that the lipid must be dispersed to the molecular level during the absorption process. It is also significant that esterified cholesterol must be hydrolysed to the free alcohol form before entry into the cell (Kim & Ivy, 1952; Favarger & Metzger, 1952). Further experiments on the form of cholesterol within the cell during absorption will be discussed more fully in a later paper.

It would appear therefore that the sterols are transported across the mucosal cell by means of this exchange between lipoproteins rather than by their migration in fatty globules. The latter are apparently temporary reservoirs of fat rather than carriers of dissolved sterol, the main absorption stream occurring at the molecular level on lipoproteins.

Whereas it has been established since the work of Mueller (1916) that dietary cholesterol appears in the lymph in large part esterified, and that fatty acids, particularly oleic acid, aid sterol absorption (Kim & Ivy, 1952), the main role of fatty acids seems to be concerned with the acceleration of sterol transfer from the mucosal cell into the lacteals. Esterification, however, does not appear to be the main controlling factor since *epi*cholesterol is absorbed unesterified (Hernandez, Chaikoff, Dauben & Abraham, 1954). Again, dihydrocholesterol, which is less well absorbed than cholesterol (Gould, Lotz & Lilly, 1956), is more readily esterified than the latter (Swell, Field & Treadwell, 1955). Moreover, in work in this Laboratory to be described in later papers, the above exchange and transfer process has been confirmed with [14 C]cholesterol *in vivo* and *in vitro*. Furthermore, the various lipoproteins from the particulate fractions concerned have been resolved by paper electrophoresis and shown to carry the dietary sterols (Green, 1955).

With regard to the mechanism of the absorption process the transfer of the sterol across the cell must

involve the lipoproteins of the various cell fractions and is probably mediated by its adsorption and desorption on them in the same manner in which the endogenously synthesized sterols become associated with the various cell proteins. It would seem reasonable that sterol presented to the external side of the mucosal-cell membrane would exchange or readily displace a similar molecule within the membrane and hence penetrate the cell. Then further transfer of the sterol to the other lipoproteins could occur whereby the molecules would be passed across the mucosal cells to the connective tissue containing the lymph capillaries. Esterification of the sterol may then occur to hasten the transfer into the lacteals.

The finding of Biggs, Friedman & Byers (1951), that the specific activity of [^{14}C]cholesterol administered orally to rats is diluted approximately tenfold with endogenous sterol by the time it reaches the intestinal lymph, lends strong support to the mechanism proposed above.

Furthermore, the above concept of sterol absorption enables a satisfactory explanation of the poor absorption of phytosterols to be suggested. The tendency of the intestinal mucosa to reject the latter, which are only slightly different from cholesterol structurally, implies that stereochemistry is the main issue and not solubility in other lipids or chemical reactivity. It would seem that the phytosterols do not fit on to the acceptor lipoproteins so readily as the natural animal sterols and that they enter into exchanges more slowly than the latter, hence their transfer across the mucosal cells becomes inhibited.

The inhibition of cholesterol absorption by phytosterols, initially observed by Peterson (1951), could also be explained by assuming that the acceptor lipoprotein becomes partially blocked with the foreign sterol, thereby reducing its capacity for the transfer of the natural animal sterol.

SUMMARY

1. The distribution of total sterols and 7-dehydrocholesterol among the components of intestinal-mucosal cells of young fasting female guinea pigs and animals actively absorbing cholesterol and 7-dehydrocholesterol has been studied.

2. In the fasting animal, the sterols are associated with each of the fractions examined: cell membranes and nuclei, mitochondria, microsomes, groundplasm and fatty globules (F-layer). The microsomes contained about 40 % of the total in the cell, the mitochondria about 20 % with the remainder distributed equally between the cell membranes and nuclei fraction and the groundplasm. The amount in the F-layer was less than 0.5 %.

3. The sterols undergoing absorption were observed to enter each fraction but the number of results obtained was insufficient to determine unequivocally if the uptake represented an absolute increase in each fraction or merely an exchange between the dietary and endogenous molecules.

4. The distribution of 7-dehydrocholesterol in relation to total sterols and of fast-reacting to slow-reacting sterols was uniform across the mucosal cells of the fasting animals and tended towards uniformity in the dosed animals. These findings imply a rapid exchange of the dietary with the endogenous sterols during the passage of the former across the intestinal mucosa.

5. The above evidence leads to the conclusion that the absorption of sterols takes place at the molecular level via a rapid exchange and transfer process between the various lipoproteins of the cell membrane, organelles and groundplasm.

6. An explanation for the poor absorption of phytosterols and their inhibition of cholesterol absorption in relation to the above hypothesis is discussed.

7. Evidence was obtained for the reduction of 7-dehydrocholesterol to lathosterol during the absorption of the former across the intestine.

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The Mechanism of the Irreversible Inhibition of Rat-Liver Monoamine Oxidase by Iproniazid (Marsilid)

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The physiological role of monoamine oxidase in the oxidation of adrenaline, 5-hydroxytryptamine and related amines is still the subject of controversy (Furchgott, 1955). Many investigators have used monoamine oxidase inhibitors such as choline *p*-tolyl ether, and particularly iproniazid (1-*iso*-nicotinoyl-2-*isopropylhydrazine* phosphate, Marsilid), as tools in studies of this important problem (e.g. Zeller, Barsky & Berman, 1955*a*; Schayer, Smiley & Kaplan, 1952; Brodie, Pletscher & Shore, 1956; Griesmer & Wells, 1956). Despite the widespread use of iproniazid as an irreversible inhibitor, both *in vivo* and *in vitro*, little is known about the mechanism of the inhibition reaction.

In this paper the original observations of Zeller *et al.* (1955*a*), showing that pre-incubation of liver monoamine oxidase with iproniazid is necessary to obtain maximum inhibition, have been extended. Inhibition is easily prevented by the addition of substrate. In the absence of substrate, the irreversible inhibition reaction was found to be progressive and first-order, to require oxygen, and to have a high apparent energy of activation. These findings suggest that iproniazid reacts chemically at or near the enzyme active centre, and a hypothesis is advanced which accounts not only for this possibility but also for the structural requirements for inhibitors as shown by Zeller, Barsky, Fouts & Lazanas (1955*b*).

EXPERIMENTAL

Monoamine oxidase determinations. Livers from adult albino rats were chilled with ice, washed and suspended in Sørensen's 0.067 M-phosphate buffer, pH 7.2 (20%, w/v), by means of a Potter-Elvehjem glass homogenizer. The suspension was centrifuged at room temperature for 2 min. at the maximum speed (approx. 4000 g) of a bench centrifuge (Baird and Tatlock Ltd.) and the resultant supernatant was then recentrifuged at 8500 g for 10 min. at 10° in a refrigerated centrifuge. The mitochondria so obtained were resuspended in the 0.067 M-phosphate buffer [equivalent to 300 mg. (wet wt.) of liver/ml.]. The enzyme activity was determined on 1 ml. of mitochondria suspension by using tyramine (0.01 M final concentration) as a substrate and adjusting the volume in the flasks to 4 ml. with buffer. Substrate and inhibitors were dissolved in the phosphate buffer. The flasks were flushed with oxygen before equilibration (total time 5 min.) and addition of the substrate. The bath temperature was 37°. Potassium hydroxide was not present in the centre well.

Kinetic measurements. The methods were similar to those of Davison (1956). The standard technique was to place 0.5 ml. of inhibitor solution and 0.25 ml. of tyramine solution in side arms of double-armed flasks and, after flushing with oxygen and equilibrating (5 min.), to add inhibitor from the side arm at different times. In these experiments the flasks containing enzyme were shaken at 37° for the same time and only the time of addition of inhibitor was varied. At the end of the incubation the substrate was added to all the flasks and manometer readings were taken every 5 min. for 35 min. The mean oxygen uptake was calculated by the method of least squares (Aldridge, Berry & Davies, 1949). Residual enzyme activity was expressed as a percentage of a control suspension

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