isotopic separation of the radioactive from the non-radioactive forms of these two amino acids was observed when the effluent fractions were examined by both ^{14}C and amino acid assay techniques. The amino acid content of a portion of the lanthionine fraction was determined with the (Chinard, 1952) ninhydrin reagent. The ^{14}C content of a duplicate portion was measured to an accuracy of 2% with a Beckman liquid-scintillation spectrometer, appropriate corrections being made for loss of count by quenching. The cystine was oxidized to cysteic acid with performic acid, and pure samples of cysteic acid were isolated by high-voltage electrophoresis on paper at pH 1.85. Duplicate portions were then taken for amino acid assay by the method of Atfield & Morris (1961), and for ^{14}C content as described above. Pure specimens of LAL were isolated by high-voltage electrophoresis on paper at pH 5.2 (Atfield & Morris, 1961), and determinations of the ^{14}C and amino acid contents were made on duplicate portions. For calibration purposes pure authenticated samples of the three amino acids concerned were subjected to all the procedures described. Six analyses were carried out.

In the second Laboratory LAL was isolated from the wool hydrolysate by chromatography on a 15 cm.-wide strip of Whatman 3MM paper, eluted with butan-1-ol-acetic acid-water (4:1:5, by vol.) for 2 days. The LAL was washed from its location on the paper with water, and duplicate portions of the eluate were taken for the determination of amino acid content by the method of Atfield & Morris (1961) and ^{14}C content by the method described above. Under the paper-chromatography conditions used lanthionine and cystine run together. They were washed from the paper strip with water and separated from each other by high-voltage electrophoresis on paper at pH 1.85. The procedures used to determine their specific activities

were the same as described for LAL. Analyses were carried out in triplicate.

The specific activities obtained, expressed in the proportions cystine:lanthionine:LAL, were respectively 2.00:2.03:0.70 in the first Laboratory and 2.00:1.98:0.78 in the second. We regard the discrepancies between the two as being within the limits of experimental error.

It is therefore apparent that under the conditions of alkali treatment we have used, which are similar to those employed by Ziegler (1965) and Mellet & Swanepoel (1965), about one-quarter of the LAL produced stems from dehydroalanine residues that were not half-cystine residues, whereas all the dehydroalanine residues required for lanthionine formation come from half-cystine. There are thus at least three acid-stable cross-linkages formed in wool when it is treated with alkali: the lanthionine linkage, an LAL linkage between lysine residues and dehydroalanine residues from half-cystine, and an LAL linkage between lysine residues and dehydroalanine residues that arise by modification of other amino acid residues in wool. Some of these are probably serine residues.

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The Binding of Sterols in Cellular Membranes

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One well-established property of the unesterified cholesterol molecules of the erythrocyte membrane is the ability to exchange *in vitro* with those of soluble plasma lipoproteins (Hagerman & Gould, 1951). This has not been investigated with other cellular membranes, although many studies of

cholesterol turnover *in vivo* in the various body tissues have been made. Cellular membranes differ in morphology (Sjöstrand, 1963) and in composition (Ashworth & Green, 1966) and many types of structure have been proposed for them (e.g. Parpart & Ballentine, 1952; Vandenheuvel, 1963;

Lucy, 1964; Kavenau, 1965; Green, 1965; Whittaker, 1966). To investigate possible differences in the mode of cholesterol binding in different membranes, the exchange of unesterified cholesterol between plasma lipoproteins and a number of cellular membranes has been studied.

The following membrane preparations, obtained from the rat, were used: intestinal microvilli membranes (Eicholz & Crane, 1965); liver plasma membranes (Emmelot, Bos, Benedetti & Rümke, 1964); skeletal-muscle sarcolemma (Kono & Colowick, 1961); small myelin particles and brain mitochondria (Whittaker, 1966); kidney microsomes, kidney mitochondria, liver smooth endoplasmic reticulum and liver mitochondria (Peters, 1962); intestinal mitochondria (9000*g* fraction; Goodman & Kadis, 1965).

The method of Whittaker (1966) was adopted instead of that used for brain mitochondria by Ashworth & Green (1966) because the latter gave preparations contaminated by myelin. Sections of the brain and intestinal mitochondrial pellets were examined in the electron microscope. Contamination by other particles was found to be very small.

Exchange of cholesterol was determined at intervals over 24 hr., with either membranes or plasma lipoproteins that had been labelled with [¹⁴C]cholesterol by injecting the rats previously with [¹⁴C]mevalonic acid (Bruckdorfer & Green, 1967). In some experiments whole plasma was used, whereas in others the plasma lipoproteins were separated by raising the density of the plasma to 1.21 with solid NaCl-KBr and centrifuging for 18 hr. at 105 000*g*. The lipoproteins were removed from the top of the tube and dialysed against several changes of 0.25*M*-sucrose containing EDTA (5*mM*) and tris-HCl buffer, pH 7.4 (5*mM*). The membrane preparation was suspended directly in this medium.

The situation with certain subcellular particles is more complicated than that with erythrocytes since enzymes are present that hydrolyse the cholesterol esters of the plasma lipoproteins, and, in addition, net movement of unesterified cholesterol from plasma lipoproteins to membrane occurs. To overcome the former, di-isopropyl phosphorofluoridate was added, and to prevent the latter, the membranes concerned (liver, kidney and brain mitochondria) were first incubated with unlabelled plasma lipoproteins and allowed to take up cholesterol until 'saturated'. When these membranes were subsequently incubated with [¹⁴C]-cholesterol-labelled lipoproteins, no further net movement of cholesterol occurred. The increases in unesterified cholesterol concentration obtained during preincubation were: liver mitochondria, 42%; brain mitochondria, 95%; kidney mitochondria, 16%. The extra sterol did not appear to

affect the final level of exchange since the values for all mitochondria, including those from the intestine, were very similar. Further, in a subsequent experiment where liver mitochondria were incubated directly with radioactive lipoproteins and only a small uptake of cholesterol occurred, mitochondrial and lipoprotein unesterified cholesterol specific activities reached the same level in 6 hr.

Because of the variation in membrane yields the ratios of membrane and lipoprotein cholesterol varied quite widely. This did not affect the exchange rate, since in all experiments the specific activities of membrane and lipoprotein unesterified cholesterol achieved their final values in 6–8 hr. and did not alter significantly over the succeeding 16–18 hr.

The results (Table 1) are expressed, in the form used by Bruckdorfer & Green (1967), as the percentage of complete cholesterol exchange reached in 24 hr. This method of expressing the results is chosen because, if the final specific activities of membrane and lipoprotein unesterified cholesterol are not identical, it must be because part of the membrane unesterified cholesterol and/or part of the lipoprotein unesterified cholesterol does not participate in the exchange process: the results do not allow a decision to be made between these alternatives. However, assuming that all of the lipoprotein unesterified cholesterol exchanges, the minimum percentage of the membrane cholesterol that has exchanged can be calculated from the sterol contents and specific activities. Except for the myelin particles (59%) this value was in all cases at least 81%. Thus the sterol of all the membranes studied behaves in the same way as that of the erythrocyte, except for myelin, where presumably access of lipoproteins to the interior of the membrane spiral is restricted.

The relative proportions of cholesterol and phospholipid in different cellular membranes vary widely (Ashworth & Green, 1966; Millington & Finean, 1963), and the results of monolayer studies suggest that the way in which membrane lipids are packed will depend on these proportions (Willmer, 1961). If this is so, the differences in packing do not influence the ability of cholesterol molecules to exchange.

The high proportion of exchangeable cholesterol in mitochondria is noteworthy because the lipid composition of inner and outer mitochondrial membranes is the same (Green, 1965). As the area of the inner membrane is greater than that of the outer, most of the sterol must be present in the former. The outer membrane is normally impermeable to particles as large as plasma lipoproteins. Unless the outer membrane breaks down during the experiment, it is difficult to see, on present views of the mitochondrial structure, how the sterol of the

Table 1. *Exchange of unesterified cholesterol between cellular membranes and plasma lipoproteins*

Membrane preparations were incubated for 24 hr. at 37° with either whole plasma or a solution of plasma lipoproteins in 0.25 M-sucrose buffered to pH 7.4. The membranes contained 23–360 μg . of unesterified cholesterol/ml. and the plasma lipoproteins 33–284 μg . The intestinal microvilli and liver plasma membranes were labelled with [^{14}C]cholesterol. In all other experiments the plasma lipoproteins contained the isotope. Mitochondria from liver, kidney and brain were preincubated in unlabelled plasma as described in the text. The extent of cholesterol exchange was measured from the specific activities at 24 hr. of (a) the membrane (b) the plasma lipoprotein unesterified cholesterol (Bruckdorfer & Green, 1967).

Membrane preparation	Form of soluble lipoproteins	Cholesterol exchange (%)		
		(a)	(b)	Mean
Intestinal microvilli membranes	Plasma	89	91	90
Liver plasma membrane	Plasma	97	95	96
Muscle sarcolemma	Plasma	99	93	96
Small myelin particles	Lipoproteins	51	62	57
Kidney microsomal fraction	Lipoproteins*	84	98	91
Liver smooth endoplasmic reticulum	Lipoproteins*	90	90	90
Liver mitochondria	Lipoproteins*	85	75	80
Brain mitochondria	Lipoproteins	86	87	87
Kidney mitochondria	Lipoproteins	91	83	87
Intestinal mitochondria	Lipoproteins*	90	86	88

* Medium also contained di-isopropyl phosphorofluoridate (40 μM).

inner membrane can exchange with that of plasma lipoproteins.

These results demonstrate that cholesterol is bound in a similar way in most types of cellular membrane. Further, the fact that the lipids of all the membranes can coalesce with those of plasma lipoproteins suggests that the surface structures of the membranes must be very similar. This need not necessarily mean that all membranes have an identical structure, but the above findings must be taken into account when possible structures are being considered (see Bruckdorfer & Green, 1967).

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