THE SUBCELLULAR SITE OF CHOLESTEROL SYNTHESIS IN RAT LIVER

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Cholesterol biosynthesis from squalene and lanosterol in rat liver is found to occur mainly in the microsomal fraction (Tchenn and Bloch, 1955). However, since recent work has shown that the rat liver plasma membrane breaks up during homogenization forming vesicles which on fractional centrifugation separate in the microsomal fraction (El-Aaser and Reid, 1965), the endoplasmic reticulum could not be definitely ascribed as the site of synthesis. Plasma membranes contain substantial amounts of cholesterol (Coleman and Finean, 1965) and could participate in the synthetic process. Below, the distribution of ^{14}C-squalene, ^{14}C-lanosterol, and ^{14}C-cholesterol between the subcellular fractions of rat liver, 2, 10 and 30 minutes after the injection of rats with the terpenoid precursor [2-^{14}C]-mevalonic acid (MVA), is compared with the distribution of DNA for the nuclei and marker enzymes for the mitochondria, lysosomes, endoplasmic reticulum, plasma membranes and soluble components. The comparison shows that during the conversion of MVA to cholesterol <u>in vivo</u> the intermediates squalene and lanosterol, and the majority of the cholesterol formed, are bound to the endoplasmic reticulum indicating this to be the major site of cholesterol synthesis from squalene.

METHODS

Two approximately 150 gm. rats were used for each of the 2, 10 and 30 min. *in vivo* incubation periods, the two livers being combined in one homogenate. 10μMoles (10 μC) of [2-^{14}C]MVA in 0.2 ml. 0.85M saline was injected intravenously into each rat. After the appropriate time the rats were killed and the livers removed, homogenized, and fractionated as described by Sedgwick and Hubscher (1965) with the modifications of Michell and Hawthorne (1965). The procedures for protein estimation and assay for enzyme markers (given in figure 1) were those of Galliard *et al*. (1965) except for 5'-nucleotidase (Michell and Hawthorne, 1965) and acid phosphatase (Hubscher and West, 1965). Evidence for the validity of the enzymes as markers for subcellular structures is reviewed by de Duve *et al*. (1962). DNA was determined as by Burton (1956).

Lipids were extracted from the fractions with chloroform-methanol (Galliard *et al*., 1965) and the cholesterol, lanosterol and squalene plus cholesterol ester fractions separated by thin layer chromatography on kieselgel G (Goodman *et al*., 1963). Squalene and cholesterol ester were separated in the same system after conversion of the latter to cholesterol by saponification. Radioactivity of lipids was determined by liquid scintillation counting after elution from the kieselgel with dichloromethane. The identity of the ^{14}C-lipids, which have been previously shown to be the only significantly labelled products accumulating in rat liver under identical conditions (Goodman *et al*., 1963), was checked by cocrystallization with authentic lipid, squalene as the hexahydrochloride and lanosterol and cholesterol as the acetate derivatives.

RESULTS AND DISCUSSION

The relative incorporation of MVA into rat liver squalene, lanosterol, and cholesterol is shown in table 1.

Lipid	*In Vivo* Incubation Time (min.) 2	10	30
	% of MVA injected		
Total Lipid	00.49	1.80	3.21
	% of total lipid radioactivity		
Squalene	36.7	7.0	7.9
Lanosterol	28.5	6.3	3.4
Cholesterol	12.1	75.4	79.6

TABLE 1. Time Study of *In Vivo* Incorporation of MVA into Liver Lipids.

The rapid labelling of squalene and lanosterol together with the slower accumulation of ^{14}C-cholesterol is consistent with the role of squalene and lanosterol as precursors of cholesterol.

The marker enzyme and DNA distributions did not vary to any significant extent between the 2, 10 and 30 min. homogenates and those given in figure 1 are the mean distributions. They show that adequate fractionation of subcellular structures was obtained. The distributions of ^{14}C-squalene and ^{14}C-lanosterol are similar to that of glucose-6-phosphatase (the endoplasmic reticulum marker enzyme) especially in the 2 min. homogenate. At longer times, although the distribution of ^{14}C-lanosterol remains roughly constant, the ^{14}C-squalene content in the soluble fraction increases probably due to

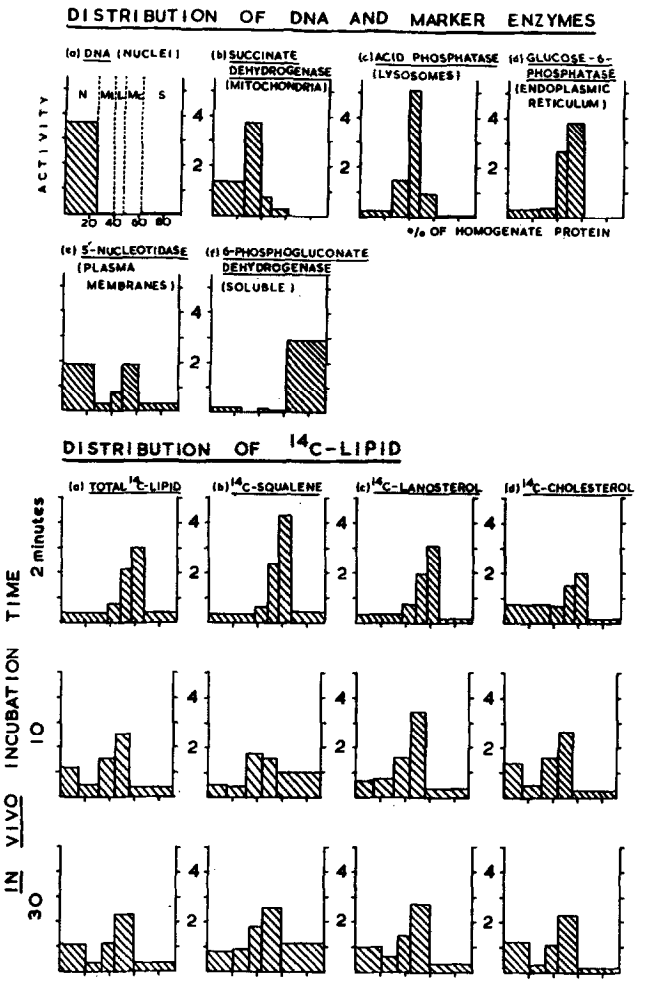

FIGURE 1. Distribution of Marker Enzymes, DNA and ^{14}C-lipids between the Subcellular Fractions of Rat Liver. The distribution of the component or enzyme assayed is depicted as a histogram each fraction being represented by a single column. The fractions are designated from left to right; N (nuclear), Mt (mitochondrial), L (lysosomal), Mc (microsomal), S (soluble). Height of the column (ordinate value) = relative specific activity of the fraction = activity of fraction per mg. protein/ activity of homogenate per mg. protein (each with respect to the component or enzyme assayed). Width of column (abscissa value) = % of homogenate protein found in fraction. Area of column thus represents the % of homogenate activity

% Recovery of homogenate activity (a) DNA 103; enzyme markers (b) 93, (c) 89, (d) 89, (e) 95, (f) 101; labelled lipids-2 min., (a) 85, (b) 97, (c) 73, (d) 77; labelled lipids - 10 min., (a) 92, (b) 88, (c) 99, (d) 91; labelled lipids-30 min., (a) 91, (b) 129, (c) 102, (d) 88.

equilibration with the large pool of "low turnover" squalene known to be present in rat liver (Loud and Bucher, 1957). ^{14}C-Cholesterol gives a mainly endoplasmic reticulum distribution though a significant amount is always found in the nuclear fraction. Since nuclei contain only low levels of cholesterol this is probably due to the presence of plasma membranes, as shown by the distribution of 5'-nucleotidase, and could indicate that limited synthesis also occurs here.

In view of these results it is attractive to suggest that, during the formation of cholesterol, lanosterol and the other intermediary sterols remain bound to one site on the endoplasmic reticulum membrane and that the reactions of the pathway are catalyzed by microsomal and perhaps soluble enzymes.

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REFERENCES

Burton, K., Biochem.J., 62, 315 (1956).
Coleman, R. and Finean, J.B., Biochem. J., 97, 31P (1965).
De Duve, C., Wattiaux, R. and Baudhuin, P., Adv. Enz., 24, 320 (1962).
El-Aaser, A.A. and Reid, E., Abstr. F.E.B.G. Meeting, Vienna, p.41 (1965).
Galliard, T., Michell, R.H., and Hawthorne, J.N., Biochim. Biophys. Acta, 106, 551 (1965).
Goodman, D.S., Avigan, J., and Steinberg, D., J. Biol. Chem., 238, 1287 (1963).
Hubscher, G. and West, G.R., Nature, 205, 799 (1965).
Loud, A.V. and Bucher, N.L.R., J. Biol. Chem., 233, 37 (1958).
Michell, R.H. and Hawthorne, J.N., Biochem. Biophys. Res. Commun., 21, 333 (1965).
Sedgwick, B. and Hubscher, G., Biochim. Biophys. Acta, 106, 63 (1965).
Tchenn, T.T. and Bloch, K., J. Am. Chem. Soc., 77, 6085 (1955).