SYNTHESIS OF THE PLASMA MEMBRANE OF THE LIVER CELL*

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The steps involved in the synthesis of a plasma (surface) membrane are completely unknown. For the newly-synthesized protein constituents of the membrane, four possibilities have been considered. First, newly-formed proteins are added directly to the membrane; second, they mix with a soluble pool and are then incorporated into the membrane; third, they are assembled into complex units that are then built into the membrane; and fourth, they are incorporated into a precursor membrane that is later converted into a plasma membrane.

The work presented here shows that proteins may be formed several hours before they are built into the liver plasma membrane. Thus, a direct incorporation of all new protein can be ruled out. It is not yet possible, however, to distinguish among the remaining three possibilities.

EXPERIMENTAL PROCEDURE

Male albino rats, obtained locally, received food and water ad libitum and were used when they weighed 180 to 200 g. Plasma membranes were isolated from liver by a modification of the procedures of Neville (1960) and Emmelot (1964). The purity of

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each preparation was checked by electron microscopic scanning of thin sections[1] and by the estimation of nucleoside triphosphate pyrophosphohydrolase (Lieberman et al., 1967) (minimum acceptable activity, 60 units/mg of protein), glucose-6-phosphatase (maximum acceptable activity, 1.0 unit/mg of protein), and RNA (maximum acceptable content, 0.02 mg/mg of protein). Smooth and rough endoplasmic reticulum (glucose-6-phosphatase, 15-17 and 8-10 units/mg of protein, respectively) were prepared from liver by the method of Dallner et al. (1966). The ranges of RNA (mg/mg of protein) in smooth and rough endoplasmic reticulum and in total microsomes were 0.09-0.11, 0.58-0.65, and 0.25-0.30, respectively. To measure the incorporation of ^{3}H-leucine (L-leucine-4,5-^{3}H, 5 mC/μmole, New England Nuclear) into protein, the samples were washed extensively with trichloracetic acid, ethanol, and ether and counted in a Hyamine-phosphor solution. Protein was estimated according to Lowry et al. (1951), RNA according to Mejbaum (1939).

RESULTS AND DISCUSSION

The kinetics of incorporation of ^{3}H-leucine into the protein of the plasma membranes of rat liver are shown in Fig. 1. As can be seen from the figure, the labeling of the plasma membranes increased for about 4 h. For comparison, the figure also shows the more rapid labeling obtained with total liver protein (see Peters, 1962) and smooth endoplasmic reticulum. Not shown are the data obtained with rough endoplasmic reticulum and the total microsome fraction. The results were identical to those with the smooth membranes.

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1) The authors gratefully acknowledge the technical assistance of Mr. James V. Apicella.

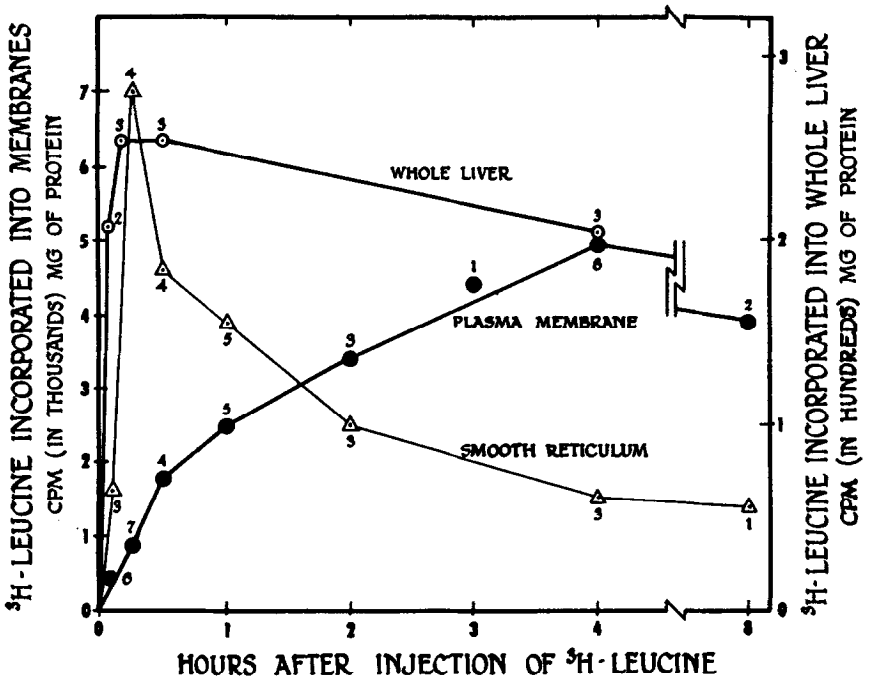

Fig. 1. The kinetics of the incorporation of ^{3}H-leucine into plasma membranes, smooth endoplasmic reticulum, and total liver protein of rat liver. For the measurements with plasma membranes and smooth endoplasmic reticulum, each rat received 100 µC of ^{3}H-leucine in the tail vein, and for total liver protein, 15 µC was injected. Liver samples were removed at the indicated times and the membranes were isolated and counted as described in "Experimental Procedure". Each point represents the average of the individual results obtained with one to eight preparations as shown.

The kinetic results suggested that a long period of time may intervene between the synthesis of a protein and its incorporation into the plasma membrane. To study this point further, animals were given ^{3}H-leucine and, after 5 min, protein synthesis was stopped with cycloheximide. The appearance of radioactivity in the plasma membranes was then followed for 4 h (Fig. 2). The dose of cycloheximide used (5 mg/100 g of rat) reduced the rate of liver protein synthesis by 97% within 10 seconds of the injection and a similar level of inhibition was found 4 h later.

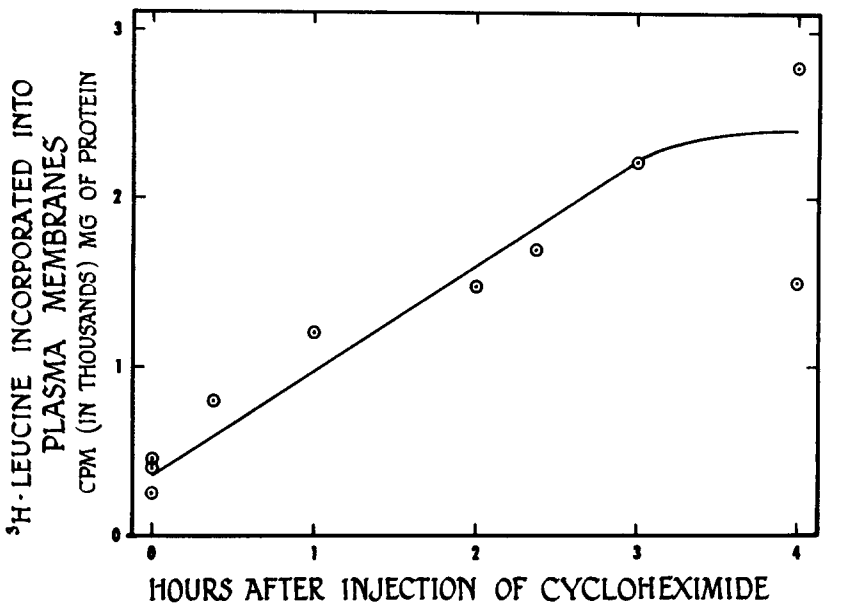

Fig. 2. The kinetics of the incorporation of ^{3}H-leucine into the plasma membranes of rat liver after treatment of the animal with cycloheximide. Each rat received 100 μC of ^{3}H-leucine in the tail vein and cycloheximide (5 mg/100 g of rat, tail vein) was given 5 min later. Liver samples were removed at the times shown and the plasma membranes were isolated and counted as described in "Experimental Procedure". Each point describes the result obtained with one membrane preparation.

As the figure shows, in spite of the inhibition of protein synthesis, the incorporation of labeled protein into the plasma membranes continued for at least 3 h.

The possibility was considered that the increase in radioactivity of the plasma membranes shown in Fig. 2 resulted from adsorption of non-membrane proteins. This possibility was considerably weakened by extracting the isolated plasma membranes with a strong salt solution (Table I). As the table shows, the extracted protein (about one-half of the total) had a lower specific activity than the residual membrane protein.

These observations do not define any of the intermediate

TABLE I

EXTRACTABILITY OF RADIOACTIVE PROTEIN FROM PLASMA MEMBRANES

Each rat received ^{3}H-leucine (100 μC) and, after 5 min, cycloheximide (5 mg/100 g of rat). Injections were made in the tail vein. At the times indicated, liver samples were removed and plasma membranes were isolated. The membranes were extracted with 2 M NaCl - 0.05 M Tris buffer (pH 7.4) at 37° for 1 h with occasional shaking. At the end of this time, the residual membranes were collected by centrifugation at 12,500 x g for 20 min. The unbracketed values describe the specific activities of the fractions, the bracketed values, the protein contents.

Time after cycloheximide	Plasma membranes	Extract	Residue
h		cpm/mg of protein	
0	370 (0.41)	305 (0.18)	400 (0.24)
1	1200 (0.61)	760 (0.28)	1790 (0.30)
2	1450 (1.19)	550 (0.52)	1780 (0.57)
3	2220 (0.44)	980 (0.20)	2450 (0.21)
4	2800 (0.40)	1200 (0.20)	3870 (0.18)

steps in plasma membrane synthesis. They do, however, provide a means for studying the steps involved in building proteins into the membrane.

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