Turnover of the Plasma-Membrane and Microsomal Proteins of Rat Liver containing Primary Hepatoma Nodules

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Although there is considerable literature on the isolation and characterization of rat liver plasma membranes, very little is known about their biosynthesis. On the basis of their experiments on the incorporation of radioactive leucine into rat liver membrane proteins, Ray, Lieberman & Lansing (1968) concluded that newly formed proteins are not directly added to the surface membrane. Using [^3H]leucine, Widnell & Siekevitz (1967) showed that the nuclear-membrane, plasma-membrane and endoplasmic-reticulum-membrane proteins of normal rat liver have similar turnover rates. On the other hand, Arias, Doyle & Schimke (1969), who used the non-reutilizable tracer [*guanidino*- ^{14}C]-arginine, reported that proteins of plasma membranes turn over faster than do microsomal proteins. The differences observed in the turnover rates may largely be due to the type of isotopic label used (Omura, Siekevitz & Palade, 1967; Arias *et al.* 1969). Little is known about changes in the biosynthesis of plasma membranes during carcinogenesis. In connexion with our studies on plasma membranes during carcinogenesis (Chandrasekhara & Narayan, 1970a), we have determined the degradation rates of the proteins of plasma membranes of liver tumours isolated from rats given *N*-fluoren-2-ylacetamide. In this communication we report that the proteins of the microsomal fraction and of surface membranes of rat liver containing primary hepatoma nodules are degraded more slowly than are those of normal rat liver.

Sixty male weanling rats weighing about 50 g each (Holtzman Co., Madison, Wis., U.S.A.) were divided into two equal groups. One group was fed on a 10%-corn-oil diet and the other on a 10%-corn-oil diet containing 30 mg of *N*-fluoren-2-ylacetamide/100 g for 27 weeks (Narayan, 1967). The animals were starved for 10 h and then given an intraperitoneal injection of 2.5 μCi of L-[U- ^{14}C]-leucine (210 mCi/mmol; International Chemical and Nuclear Corp., Irvine, Calif., U.S.A.)/100 g body wt. Except for those killed 2 h after injection, the

animals were re-fed 2 h after injection of the tracer and they were killed in the fed state at 12 h, 24 h, 48 h or 96 h later. Each preparation of plasma membranes, microsomal fraction and liver homogenate for each time-point (2 h, 12 h, 48 h and 96 h) was obtained from a pool of liver lobes or hepatoma nodules from four rats (5 g of tissue/rat), except in two cases where livers or tumour tissue from three rats were used (7 g of liver/rat). Although care was exercised to select the tumour portion of the liver from carcinogen-ingesting rats, non-cancerous tissue could not be entirely avoided. Therefore the term 'hepatoma' or 'tumour' used in this communication refers to tissue containing some normal and some hyperplastic liver. Portions (20 g) of pooled livers or livers containing hepatoma nodules were homogenized in 1 mM-sodium hydrogen carbonate and the 1500 g pellet was obtained by the procedure of Neville (1968). This pellet, homogenized in sucrose and with the density adjusted to 1.197, was subjected to discontinuous sucrose-density-gradient (*d* 1.197, 1.18 and 1.16) centrifugation (98 500 g for 75 min). The material at the 1.16/1.18 density interface was collected and constituted the plasma-membrane fraction. It was characterized on the basis of the activities of the membrane marker enzymes 5'-nucleotidase and Mg^{2+} + Na^{+} + K^{+} -stimulated adenosine triphosphatase, and on the basis of enzymes characteristic of mitochondria and microsomal fraction (Chandrasekhara & Narayan, 1970a). Other experiments from this laboratory have conclusively shown that isolated nuclei, mitochondria and microsomal fraction do not contribute to the plasma-membrane zone (*d* 1.16-1.18) when they are subjected to the same procedure used for the isolation of plasma membrane (Chandrasekhara & Narayan, 1970b).

For isolation of the microsomal fraction the 1500 g supernatant from the plasma-membrane preparation was centrifuged at 10 000 g for 20 min, and the supernatant from this step was then centrifuged at 100 000 g for 60 min. Samples of the plasma membranes, microsomal fraction and liver homogenate were washed to remove free labelled leucine, solubilized in NCS reagent (The Radiochemical Centre, Amersham, Bucks., U.K.) and their radioactivities counted in a Packard Tri-Carb scintil-

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Table 1. *Turnover of protein components of normal rat liver and of rat liver containing primary hepatoma nodules*

Details are given in the text.

Tissue and fraction	Regression coefficient per h	Correlation coefficient	$t_{\frac{1}{2}}$ (h)
Normal liver			
Whole homogenate	-0.00354	-0.940	85
Microsomal fraction	-0.00440	-0.869	68
Plasma membranes	-0.00450	-0.964	67
Liver containing primary hepatoma nodules			
Whole homogenate	-0.00192	-0.887	157
Microsomal fraction	-0.00260	-0.791	116
Plasma membranes	-0.00237	-0.932	127

lation spectrometer. Semi-logarithmic plots relating the decrease with time in the specific radioactivity of the total proteins of the three fractions (homogenate, microsomal fraction and plasma membranes) of normal liver and of liver containing primary hepatoma nodules were used for the estimation of half-lives. The regression lines were drawn by the method of least squares of the determined specific-radioactivity points. As indicated above, each time-point represents pooled livers or livers containing tumours from at least three animals (four rats in most cases). The regression coefficients given in Table 1 represent the slopes of the various lines. The correlation coefficient was calculated as described by Snedecor & Cochran (1965). The half-lives were calculated from the regression coefficients (slopes) by using the formula:

$$t_{\frac{1}{2}} \text{ (h)} = \frac{\log 2}{\text{Regression coefficient per h}}$$

The plasma membranes isolated from normal rat livers were observed to have a high nucleotidase activity [63.9 ± 7.5 (7) μmol of P_i liberated/h per mg of protein]. As observed by us before (Chandrasekhara & Narayan, 1970a), the plasma membranes of liver containing hepatoma nodules exhibited a lower nucleotidase activity [46.8 ± 13.5 (5) μmol of P_i liberated/h per mg of protein].

The rate of degradation of normal rat liver proteins ($t_{\frac{1}{2}}$ 85 h; Table 1) was in the range of values ($t_{\frac{1}{2}}$ 89 h and 109 h respectively) reported by Ryoo & Tarver (1968) and by Arias *et al.* (1969). The half-life ($t_{\frac{1}{2}}$ 68 h) of the proteins of normal rat liver microsomal fraction was close to that ($t_{\frac{1}{2}}$ 75 h) observed by Omura *et al.* (1967). Similarly the present value for control plasma membranes ($t_{\frac{1}{2}}$ 67 h) may be compared with that ($t_{\frac{1}{2}}$ 74 h) obtained by Arias *et al.* (1969) and that ($t_{\frac{1}{2}}$ 85 h) observed by Widnell & Siekevitz (1967). It is surprising

that the proteins of microsomal fraction, plasma membranes and total homogenate of the liver containing primary hepatoma nodules are degraded more slowly ($t_{\frac{1}{2}}$ 116 h, 127 h and 157 h respectively) than are the corresponding proteins of normal liver ($t_{\frac{1}{2}}$ 68 h, 67 h and 85 h respectively). Although the correlation is reasonably good for plasma membranes and whole homogenate of livers from both groups, there is a considerable scatter with the microsomal fractions.

The plasma membranes isolated in the present experiments consist mainly of bile canalicular portions of the surface membrane of the liver cell (Neville, 1960; Song, Rubin, Rifkind & Kappas, 1969). Further, it is well recognized that the smaller plasma-membrane fragments are present in the microsomal fraction (Chandrasekhara & Narayan, 1970b). Therefore the turnover observed by us for the proteins of plasma membranes of both the normal liver and the liver containing tumour nodules may represent the turnover of the proteins of the bile canalicular regions of the surface membranes. With the recent development of methods for the isolation of plasma membrane fragments from the microsomal fraction (Graham, Higgins & Green, 1968; Touster, Aronson, Dulaney & Hendrickson, 1970), it may be of interest to determine whether the turnover of the proteins of these membrane fragments differs substantially from that observed in this study.

With microsomal fraction and plasma membranes obtained from liver containing hepatoma nodules it was expected that the degradation rates of these proteins would be higher than those from normal liver. Warren & Glick (1968) and Warren (1969) have proposed that, for the L cell, it may make as much membrane both in the dividing and in the non-dividing states and that, in the stationary state, there is rejection of material equal to that which goes in. Warren (1969) has calculated that

the dividing cell replaces only about 7% of its surface-membrane proteins per generation. On the other hand the non-dividing cell may replace 40% of its surface-membrane proteins per equivalent generation time, which suggests a very rapid turnover. It was also indicated that this difference in the turnover in the two states was not peculiar to the surface membrane; similar overall rates of turnover were observed in the particulate fraction containing mitochondria, microsomal fraction and nuclei (Warren, 1969). As an extension of these arguments, it may be proposed that, for the economy of the dividing tumour cell, the replacement rate of membrane proteins is kept at a minimum. Thus there is apparently a lower rate of replacement of plasma membranes and microsomal proteins in the liver containing hepatoma nodules than in the normal liver. Since the liver tumours used in this study were not entirely free from non-cancerous tissue, the question arises as to the relative contribution of the hepatoma and the non-cancerous liver to the observed low rate of turnover of proteins. On the basis of the size of the hepatoma nodules used in this study and visual observations on the relative proportion of nodules to non-cancerous tissue, we believe that the half-lives reported in Table 1 represent a reasonable estimate of the rate of degradation of the proteins of a primary hepatoma. However, a further study using a transplanted tumour, such as a Morris hepatoma, may be necessary to support this tentative conclusion.

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