Unaltered Catabolism of Desialylated Low-Density Lipoprotein in the Pig and in Cultured Rat Hepatocytes

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Removal of the terminal sialic acid residues from many serum glycoproteins results in exposure of their penultimate galactose residues and rapid clearance from circulation by the liver. Low-density lipoprotein is a glycoprotein containing 21 galactose and 9 sialic acid residues per particle. Studies in this laboratory and others have shown that both the liver and extrahepatic tissues contribute to the degradation of low-density lipoprotein. This study was undertaken to determine whether desialylation of pig low-density lipoprotein alters its removal from circulation. Low-density lipoprotein was incubated at 37°C with an agarose-bound neuraminidase, proteinase-free, from *Clostridium perfringens*. After 18 h at pH 5.0, 70% of the sialic acid residues were removed. The desialylated ¹³¹I-labelled and native ¹²⁵I-labelled low-density lipoproteins were simultaneously injected into a pig, and their disappearance from plasma was followed for 96 h. The turnovers of the two were identical. In contrast, neuraminidase-treated fetuin was cleared about 200-fold faster than native fetuin. Studies were also performed in cultured rat hepatocytes. Rates of degradation of native and neuraminidase-treated low-density lipoprotein were similar, whereas asialo-fetuin was degraded at six to ten times the rate of native fetuin. Thus desialylation does not appear to alter low-density-lipoprotein catabolism by hepatic or extrahepatic cells.

Early studies of the organ distribution of injected ¹²⁵I-labelled LD lipoprotein led to the conclusion that the liver is the major site of LD-lipoprotein catabolism (Hay *et al.*, 1971; Calvert *et al.*, 1975). Subsequently, evidence for a potentially important role for extrahepatic tissues in the catabolism of LD lipoprotein emerged from studies both *in vivo* and *in vitro*. The work of Sniderman *et al.* (1974), in which it was shown that the rate of LD-lipoprotein catabolism in pig was not decreased by hepatectomy, demonstrated that extrahepatic tissues have a large capacity for LD-lipoprotein degradation *in vivo*. Cultured cells derived from several extrahepatic tissues, including fibroblasts (Goldstein & Brown, 1974), lymphocytes (Ho *et al.*, 1976), endothelial cells (Reckless *et al.*, 1978; Stein & Stein, 1976) and smooth-muscle cells (Weinstein *et al.*, 1976; Albers & Bierman, 1976; Goldstein & Brown, 1975), have been shown to bind, internalize and degrade LD lipoprotein via the receptor-mediated LD-lipoprotein pathway described by Goldstein & Brown (1977). However, liver indeed is capable of degrading

LD lipoprotein, as shown in perfused rat liver (Sigurdsson *et al.*, 1978) and in isolated rat hepatocytes (Pangburn & Weinstein, 1978). Using a new approach to the measurement of lysosomal degradation (Pittman & Steinberg, 1978) *in vivo*, we have recently found that in the pig the liver degrades LD lipoprotein to a significant extent (R. C. Pittman, A. D. Attie, T. E. Carew & D. Steinberg, unpublished work).

The pioneering work of Ashwell and his co-workers revealed the importance of the carbohydrate moieties of glycoproteins in determining their removal from circulation (Ashwell & Morell, 1974). They discovered that exposure of penultimate galactose residues of several plasma glycoproteins by removal of terminal sialic acid residues results in their rapid clearance by the liver.

Apoprotein B, which comprises more than 98% of LD-lipoprotein protein (Schaefer *et al.*, 1978), is a glycoprotein containing 5% carbohydrate, of which 10% is sialic acid (Swaminathan & Aladjem, 1976; Azuma *et al.*, 1977). The sialic acid-containing carbohydrate units in LD lipoprotein differ from those of other glycoproteins in that they have a molar ratio of galactose to sialic acid of more than 2:1

Abbreviation used: LD lipoprotein, low-density lipoprotein.

(Swaminathan & Aladjem, 1976), whereas the ratio in most serum glycoproteins is 1:1 (Kornfeld & Kornfeld, 1976). This suggests that LD lipoprotein may contain galactose residues that are exposed as terminal sugar residues, whereas other serum glycoproteins have few if any exposed galactose residues. This study was undertaken to determine whether the catabolism of LD lipoprotein, like that of many other glycoproteins, is altered by the removal of terminal sialic acid residues and consequent exposure of galactose. The pig was chosen as an animal model for studies *in vivo*. This species resembles man in that LD lipoprotein is the major circulating carrier of cholesterol, LD-lipoprotein concentrations are high relative to those of the other serum lipoproteins and pigs generally respond to dietary and drug interventions as do humans. Liver parenchymal cells have been identified in rats and rabbits as the site of uptake of asialo-glycoproteins (Tolleshaug *et al.*, 1977). Therefore the uptakes of native and desialylated LD lipoprotein and fetuin were also studied in monolayer cultures of isolated rat hepatocytes.

Experimental

Lipoproteins

Human and pig LD lipoproteins of density 1.019–1.063 were isolated from fresh plasma (containing 0.1 mg of EDTA/ml) as previously described (Carew *et al.*, 1976). The lipoproteins were washed twice by refloatation at d 1.063 in an SW41 rotor. No contaminating lipoproteins were detected by agarose-gel electrophoresis of the intact purified lipoproteins.

Desialylation

Fetuin (foetal bovine; Sigma type IV; Sigma Chemical Co., St. Louis, MO, U.S.A.) or LD lipoprotein was incubated with agarose-bound neuraminidase (*N*-acetylneuraminidase glycohydrolase, EC 3.2.1.18; from *Clostridium perfringens*; Sigma) and 20 mg of substrate protein per unit of enzyme activity (one unit liberates 1.0 μ mol of *N*-acetylneuraminic acid/min at pH 5.0 and 37°C) for 18 h at pH 5.0 and 37°C in 0.2 M-sodium acetate buffer, containing 0.9% NaCl, 0.02% NaN_3 and 0.01% EDTA. Neuraminidase was checked for proteinase activity with ^{14}C -labelled haemoglobin as substrate. The [^{14}C]haemoglobin (Sigma) was prepared by reductive methylation, by using [^{14}C]formaldehyde (New England Nuclear, Boston, MA, U.S.A.) and NaBH_4 (Murthy *et al.*, 1975). Final specific radioactivity of the haemoglobin after exhaustive dialysis against distilled water was 1100 c.p.m./ μg . Haemoglobin (200 μg) was incubated with neuraminidase (0.5 unit) under the conditions described above. Proteinase activity was assayed in terms of ^{14}C -labelled pro-

ducts not precipitable by trichloroacetic acid (final concentration, 10%, w/v) in the presence of 50 μg of bovine albumin (Sigma) as carrier protein.

Characterization of asialo-proteins

Carbohydrate content was analysed by g.l.c. as previously described (Freeze *et al.*, 1978). Proteins were assayed by the method of Lowry *et al.* (1951), with bovine serum albumin (Sigma) as the reference standard for LD lipoprotein and desialylated LD lipoprotein, and fetuin as the reference standard for fetuin and asialo-fetuin.

Iodination of proteins

Proteins were iodinated by a modification (Carew *et al.*, 1976) of the iodine monochloride method of MacFarlane (1958). The proteins were then exhaustively dialysed against 0.02 M-sodium phosphate. (pH 7.4)/0.9% NaCl/0.01% EDTA. Specific radioactivities ranged from 50 to 550 c.p.m./ng of protein, representing no more than 0.32 atom/ 10^5 daltons of LD-lipoprotein protein and 0.09 atom/44000 daltons of fetuin. More than 99% of the radioactivity was precipitable by 10% (w/v) trichloroacetic acid in the case of the lipoproteins and desialylated lipoproteins, and by a combination of 10% (w/v) trichloroacetic acid and 2% (w/v) phosphotungstic acid in the case of fetuin and asialo-fetuin.

Turnover studies

Castrated male Duroc or Hampshire pigs (30–60 kg), maintained on a standard chow diet (Purina Complete Sow Chow; Ralston Purina, St. Louis, MO, U.S.A.), were used throughout.

Under general anaesthesia (sodium pentobarbital), Tygon catheters were inserted into both external jugular veins, and the distal ends were brought out through stab incisions at the back of the neck. The animals were allowed to recover for 1–2 days with free access to food and to water containing Lugol's iodine solution (100 g of KI/litre and 50 g of iodine/litre).

Homologous LD lipoprotein (^{125}I -labelled) and desialylated LD lipoprotein (^{131}I -labelled) were simultaneously injected (25–50 μCi of each label) through one catheter. At indicated times, blood samples were withdrawn through the other catheter into tubes containing EDTA (1 mg/ml final concentration). The turnover of ^{125}I -labelled fetuin and of ^{125}I -labelled asialo-fetuin were studied in separate animals. Plasma radioactivity was determined in an automatic gamma-counting system (Searle, model 1197). Counting efficiencies were 82% for ^{125}I and 40% for ^{131}I . The plasma radioactivity-decay results were analysed by the method of Matthews (1957) as applied by Carew *et al.* (1976).

Rat hepatocytes

Female Sprague-Dawley rats (150–200g; Hilltop Lab Animals, Chatsworth, CA, U.S.A.) kept on a 12h light cycle (07:00–19:00h) and fed on ordinary rat chow and water *ad libitum* were used at about 09:00h. Hepatocytes were isolated by a modification of the method of Berry & Friend (1969). With the use of aseptic technique, the portal vein was cannulated, the abdominal vena cava cut, and the liver immediately perfused (10ml/min by gravity flow) *in situ* with 80ml of Hanks calcium-free buffer (Hanks & Wallace, 1949). Perfusion buffer was warmed and oxygenated by passage through silicone tubing (Cole-Parmer, Chicago, IL, U.S.A.) immersed in a 41°C water bath saturated with O₂/CO₂ (19:1). After the initial perfusion, which blanched the liver, the abdominal vena cava was clamped, the thoracic inferior vena cava was cannulated through the atrium, and collagenase (0.67mg/ml; Sigma, type 1) in Hanks buffer was recirculated for 20min at 10ml/min. The liver was then removed, cut into small pieces and incubated for 5min in 20ml of the collagenase solution in a 37°C shaking water bath (60cycles/min). The separated cells were drawn off with a 10ml pipette and transferred to cold (4°C) arginine-free Dulbecco's modified Eagle's medium (DME; Grand Island Biochemical Co., Santa Clara, CA, U.S.A.) containing 20% heat-inactivated foetal calf serum. The suspension was centrifuged at 40g for 1min in round-bottomed polycarbonate tubes to obtain a cell pellet that was washed twice by suspension and centrifugation in the same manner. The yield of hepatocytes was 2×10^8 – 4×10^8 cells per liver with 92–96% Trypan Blue exclusion (dye concentration, 0.04%). Cells were then plated in 60mm Lux contour plastic dishes at 3×10^6 cells per dish in 3ml of arginine-free DME medium containing foetal bovine serum (20%), insulin (10µg/ml; Sigma), penicillin G (100units/ml; greater than 1435 units/mg) and streptomycin sulphate (100µg/ml). Cells were maintained at 39°C in an atmosphere containing air/CO₂ (19:1). A 24–48h recovery period was allowed before use. Characterization of these cells as metabolically functional hepatocytes has already been reported (Davis *et al.*, 1979).

Uptake studies

The maintenance medium was aspirated and replaced with DME medium containing 5% human lipoprotein-deficient serum, the labelled protein under study and insulin and antibiotics as described above. Parallel incubations were carried out in plates containing no cells, providing a measure of spontaneously generated acid-soluble radioactive iodide, which was subtracted from the values obtained in the presence of cells. After incubation at 39°C for the indicated times, dishes were placed on ice, the media

removed, and proteins immediately precipitated by addition of cold acid [final concentrations: 10% (w/v) trichloroacetic acid for LD lipoprotein and desialylated LD lipoprotein; 10% (w/v) trichloroacetic acid containing 2% (w/v) phosphotungstic acid for fetuin and asialo-fetuin]. Precipitates were centrifuged for 30min at 3000g at 4°C. The supernatant fractions were cleared of residual particulate matter by passage through a 0.2µm filter (Millipore Corp., Bedford, MA, U.S.A.) and analysed for radioactivity. Cells were scraped from plates with a 'rubber policeman', collected by centrifugation at 3000g for 15min and finally dissolved in 1M-KOH for protein determinations (Lowry *et al.*, 1951). A deiodinase present in hepatocytes can liberate ¹²⁵I from [¹²⁵I]monoiodotyrosine (Dumas *et al.*, 1973), thus inorganic iodide was not removed before acid-soluble radioactivity was assayed in the medium. In the present studies it was found that iodide represents a quite constant proportion of trichloroacetic acid-soluble radioactivity with both the native proteins and the desialylated proteins.

Binding studies

Cells were placed on ice in a 4°C cold-room and radiolabelled protein was added in fresh cold culture medium. After a 2h incubation at 4°C, the medium was aspirated and the cells were washed six times with 2ml of cold Dulbecco's phosphate-buffered saline (Grand Island Biochemical Co., New York, NY, U.S.A.). Cells were then scraped off and collected as above, and cell-associated radioactivity was determined.

Results

Neuraminidase treatment of proteins

Neuraminidase treatment of both human and pig LD lipoprotein removed 70% of the sialic acid residues (Table 1). There was no detectable change in the concentration of the other carbohydrate residues in neuraminidase-treated pig LD lipoprotein and little or no change in neuraminidase-treated human LD lipoprotein. Analysis of the carbohydrate composition of fetuin after neuraminidase treatment also showed a 70% decrease in sialic acid content (results not shown).

Neuraminidase preparations often contain proteinase activities, which could modify the protein moiety of glycoproteins. Therefore preparations used were screened for proteinase activity by using ¹⁴C-labelled haemoglobin as substrate. Even with a relatively high enzyme to substrate ratio (see the Experimental section) no proteinase activity could be detected. Neuraminidase treatment of radioiodinated proteins used in this study revealed no

Table 1. Carbohydrate composition of pig and human LD lipoprotein

Samples of delipidated LD lipoprotein (250–500 µg of protein) were dissolved in 0.1 M-sodium decyl sulphate, and dialysed against 100 vol. of 2 mM-sodium decyl sulphate. Arabinol (5 µg) was added as an internal standard, and the samples were methanolysed at 85°C for 24 h in 1.5 M-HCl/methanol, re-*N*-acetylated, dried over KOH and extracted with diethyl ether twice. The methyl glycosides were converted into their trimethylsilyl esters and analysed by g.l.c. on a column packed with 3% SE-30 on Supelcoport by using a temperature programme of 125–230°C at 2°C/min. Further details are given in Freeze *et al.* (1978). Abbreviation: N.D., none detected; less than 0.13 µg/100 µg of native LD-lipoprotein protein and less than 0.5 µg/100 µg of desialylated LD-lipoprotein protein.

	Carbohydrate composition (µg/100 µg of LD-lipoprotein protein)			
	Pig LD lipoprotein		Human LD lipoprotein	
	Native	Neuraminidase-treated	Native	Neuraminidase-treated
Mannose	2.18	2.17	2.38	2.12
Galactose	1.30	1.37	1.23	1.06
Glucose	0.55	0.51	0.23	0.18
<i>N</i> -Acetylglucosamine	1.39	1.45	1.77	1.51
Fucose	0.14	0.15	N.D.	N.D.
Sialic acid	0.55	0.17	0.51	0.14

Fig. 1. Representative plasma disappearance curves for simultaneously injected ¹²⁵I-labelled LD lipoprotein (a) and ¹³¹I-labelled desialylated LD lipoprotein (b)

The decay of plasma radioactivity was biexponential and could be resolved into two monoexponential components by curve-peeling. The lower curve (○) was obtained by subtracting the extrapolated second exponential (●) phase from the experimental values. All data were normalized to the initial plasma radioactivity at 2 min, which equalled 25 600 c.p.m. of ¹²⁵I/ml and 26 900 c.p.m. of ¹³¹I/ml.

Table 2. Catabolism in vivo of native and neuraminidase-treated pig LD lipoprotein: fractional catabolic rates, slopes (b) and intercepts (C) of biexponential plasma disappearance curves

Three different preparations (25–50 µCi) of ¹²⁵I-labelled LD lipoprotein and ¹³¹I-labelled desialylated LD lipoprotein were simultaneously injected into three different pigs. Disappearance of radioactivity from plasma was followed for 96 h. Kinetic analysis was performed as described by Matthews (1957). Plasma radioactivity (normalized so that 2 min time value equals 1.0) = $C_1e^{-b_1t} + C_2e^{-b_2t}$ and the fractional catabolic rate = $(C_1/b_1 + C_2/b_2)^{-1}$.

Pig	Native LD lipoprotein					Desialylated LD lipoprotein				
	Fractional catabolic rate (h ⁻¹)	b ₁ (h ⁻¹)	b ₂ (h ⁻¹)	C ₁	C ₂	Fractional catabolic rate (h ⁻¹)	b ₁ (h ⁻¹)	b ₂ (h ⁻¹)	C ₁	C ₂
1	0.042	0.033	0.205	0.739	0.261	0.044	0.030	0.215	0.632	0.368
2	0.028	0.022	0.161	0.751	0.249	0.029	0.022	0.139	0.723	0.277
3	0.054	0.033	0.154	0.503	0.497	0.048	0.033	0.148	0.608	0.392
Mean	0.041	0.029	0.173	0.664	0.336	0.040	0.028	0.167	0.654	0.346
± s.d.	0.013	0.006	0.028	0.140	0.140	0.010	0.006	0.042	0.061	0.061

Fig. 2. Representative plasma disappearance curves for ^{125}I -labelled fetuin (○) and ^{125}I -labelled asialo-fetuin (●).

Experiments were carried out in separate pigs. The data were normalized to the initial plasma radioactivity determined at 1.5 min, which equalled 27200 c.p.m./ml in the case of ^{125}I -labelled fetuin and 40000 c.p.m./ml in the case of ^{125}I -labelled asialo-fetuin.

Fig. 3. Degradation of ^{125}I -labelled LD lipoprotein (○) and ^{125}I -labelled desialylated LD lipoprotein (●) by cultured rat hepatocytes.

The accumulation of acid-soluble radioactivity in the culture medium is shown as a function of time at a constant lipoprotein concentration of 20 $\mu\text{g}/\text{ml}$ (above) and as a function of the concentration of added lipoprotein after 24 h (below). The same native lipoprotein preparation was used to prepare both labelled LD-lipoprotein species. The data represent means $\pm$ s.d. for three culture dishes.

proteinase activity, as determined by the production of acid-soluble ^{125}I or ^{131}I .

Plasma turnover studies

The plasma decay or tracer doses of radioiodinated LD lipoprotein and desialylated LD lipoprotein in pig were biexponential, as shown in Fig. 1. It has been previously demonstrated in the case of native LD lipoprotein that the initial rapid phase represents equilibration with extravascular pools, the liver accounting for the major part of the extravascular LD-lipoprotein pool, the slower phase representing irreversible disappearance from the plasma pool (Sniderman *et al.*, 1975). The kinetics of decay of simultaneously injected ^{125}I -labelled LD lipoprotein and ^{131}I -labelled desialylated LD lipoprotein were not significantly different. Neither the fractional rate of decay of the slow component (b_1 in Table 2) nor the formally calculated fractional catabolic rate were altered by the neuraminidase treatment. On the other hand, asialo-fetuin was cleared very rapidly ($t_{1/2}$ 90 s), whereas intact fetuin was cleared much more slowly ($t_{1/2}$ 42 h), as shown in Fig. 2.

Studies in rat hepatocytes

The site of uptake of asialo-glycoproteins has been identified in rats and rabbits as liver parenchymal cells (Tolleshaug *et al.*, 1977). Therefore uptake of native and desialylated human LD lipoprotein and fetuin was studied in monolayer cultures of isolated rat hepatocytes. Degradation of labelled glycoproteins was measured in terms of the appearance of acid-soluble labelled metabolites in the medium, as described in the Experimental section. The degradation rates of native and desialylated LD lipoprotein were indistinguishable (Fig. 3). In contrast, asialo-fetuin was degraded at six to ten times the rate of native fetuin (Fig. 4). The higher rate of catabolism of asialo-fetuin was associated with greater cell-surface binding, determined at 4°C to minimize endocytosis and subsequent metabolism (Fig. 5).

Fig. 4. Degradation of ^{125}I -labelled fetuin (○) and ^{125}I -labelled asialo-fetuin (●) by cultured rat hepatocytes. Appearance of acid-soluble radioactivity in the culture medium is shown as a function of concentration of added protein in 24 h (a) and as a function of time at a constant protein concentration of 20 μg/ml (b). The same native protein preparations were used to prepare both labelled species. The data represent means $\pm$ s.d. for three culture dishes.

Fig. 5. Binding of ^{125}I -labelled fetuin (○) and ^{125}I -labelled asialo-fetuin (●) to cultured rat hepatocytes.

The accumulation of cell-associated radioactivity after a 2 h incubation at 4°C is shown as a function of the concentration of added radiolabelled protein. The data represent means $\pm$ s.d. for three culture dishes.

Discussion

The carbohydrate analyses of human and pig LD lipoproteins showed that neuraminidase treatment

selectively removed 70% of the sialic acid residues from both lipoproteins. Since there are an estimated 5×10^5 daltons of protein per LD-lipoprotein particle (Jackson *et al.*, 1976), each particle contains about nine sialic acid and 21 galactose residues (Table 1). Thus, on the average, six sialic acid residues per LD-lipoprotein particle were removed by the neuraminidase treatment. Van den Hamer *et al.* (1970) showed that, in the case of caeruloplasmin, removal of any two out of ten sialic acid residues resulted in rapid clearance of the protein from the circulation. This clearly was not the case with LD lipoprotein. A Poisson distribution analysis, as used by Van den Hamer *et al.* (1970), indicates that more than 97% of the LD-lipoprotein particles lost at least two residues as a result of neuraminidase treatment, 6.9% had no remaining sialic acid residues and 10.3% had only one, assuming that all residues are equally subject to attack by neuraminidase. If any one exposed galactose residue were critical for signalling rapid clearance, the predicted presence of a significant fraction of LD-lipoprotein particles with exposed markers would have resulted in a readily apparent early phase in the plasma radioactivity-decay curve. Such was not observed. Moreover, the ratio of radiolabelled desialylated LD lipoprotein to native LD lipoprotein found in the plasma at later time points should have differed from

the ratio shortly after injection. No such difference was evident. The ratio was constant with a standard deviation of 1.2% ($n = 7$) for times up to 90 min. It may be inferred from the Poisson distribution data that even exposure of all galactose residues does not result in rapid clearance of pig LD lipoprotein. However, we cannot rule out the possibility that some of the three remaining sialic acid residues cover critical galactose residues and that these sialic acid residues are resistant to the action of neuraminidase.

An hepatic galactose-specific receptor with high affinity for asialoglycoproteins has been isolated from liver membrane fractions (Pricer & Ashwell, 1976). In the present study the degradation rate of asialo-fetuin by hepatocytes was considerably higher than that of native fetuin in a process showing saturation kinetics (Fig. 4), consistent with receptor-mediated uptake. The striking difference between fetuin and asialo-fetuin in terms of degradation rates is paralleled by differences in their binding and degradation both in freshly isolated hepatocytes (Tolleshaug *et al.*, 1977) and in the long-term hepatocyte cultures used here. However, a cultured hepatocyte has lost its orientation *in situ*, no longer has defined sinusoidal and canalicular surfaces and has been stripped of surrounding diffusion barriers. Thus caution should be exercised in inferring catabolic rates *in vivo* from degradation rates in cultured hepatocytes. Nevertheless these results illustrate the usefulness of the culture system in characterizing recognition markers involved in receptor-mediated membrane transport processes.

When first examined, transferrin was believed to be an exception to the model predicting rapid clearance of asialo-glycoproteins (Morell *et al.*, 1971). However, in longer turnover studies, Regoeczi & Hatton (1974) found that removal of two of the four sialic acid residues from human transferrin increased its rate of catabolism in rabbits. In contrast, catabolism of bovine transferrin, which has only a single carbohydrate chain (Hatton *et al.*, 1978), was not affected by desialylation. One explanation for these results is that there is a threshold density of exposed galactose residues necessary for rapid uptake. The two exposed galactose residues per caeruloplasmin molecule necessary for rapid clearance represent a calculated density of one galactose per 30 nm² of surface area. Similarly, for human transferrin, which is 50% desialylated, the value is one galactose residue per 21 nm². Native LD lipoprotein contains 12 exposed galactose residues (data from Table 1), which represents a density of one galactose residue per 93 nm². Calculations assume a prolate ellipsoid shape with an axial ratio of 4.8 for transferrin (Phelps & Cann, 1956), 11 for caeruloplasmin (Sutton & Jamieson, 1972) and 1.243 for LD lipoprotein (Smith, 1970). Neuraminidase treatment

yielded an LD-lipoprotein particle with 18 out of 21 galactose residues exposed (86% of total galactose) or one galactose residue per 62 nm². If complete desialylation were achieved, the calculated density would be one residue per 53 nm². It is unlikely that this small increment would be critical in signalling rapid clearance. A similar lack of rapid clearance in the rat of LD lipoprotein that was 95% desialylated has been described in a preliminary report (Swaminathan & Aladjem, 1974). It therefore appears that LD lipoprotein is not capable of achieving the galactose density apparently necessary for rapid hepatic uptake. Of course, this does not rule out the possibility of other specific carbohydrate-recognition sites on the LD-lipoprotein molecule. Further evidence indicates that some receptors recognize carbohydrates such as mannose (Kawasaki *et al.*, 1978) and fucose (Prieels *et al.*, 1978). However, Shireman & Fisher (1978) found that enzymic removal of virtually all carbohydrate residues from human LD lipoprotein did not alter its binding to cultured human fibroblasts.

If carbohydrate moieties are not involved in regulating catabolism of LD lipoprotein, what other roles might they play? Kornfeld and others (Hickman *et al.*, 1977) have considered that glycosylation might be an essential prelude to secretion of glycoproteins, as originally hypothesized by Eylar (1965). They found that tunicamycin, an antibiotic that inhibits asparagine-linked glycosylation of nascent proteins (Takatsuki & Tamura, 1971), also inhibits the secretion of certain serum glycoproteins. However, Struck *et al.* (1978), studying the secretion of very-low-density lipoprotein, a precursor of circulating LD lipoprotein (Berman *et al.*, 1978), found that tunicamycin did not inhibit its secretion by cultured chick hepatocytes, suggesting that glycosylation is not required for secretion of this lipoprotein. The issue is not completely resolved, however; it has been postulated that orotic acid inhibition of lipoprotein secretion may be due to incomplete glycosylation of newly synthesized apoproteins (Pottenger *et al.*, 1973).

Exposure of specific carbohydrate moieties appears to be a biological strategy for targeting circulating glycoproteins for hepatic uptake. LD-lipoprotein cholesterol plays a prominent role in regulating cholesterol biosynthesis in a variety of extrahepatic tissues (Anderson & Dietschy, 1977) as well as in liver cells (Davis *et al.*, 1978). The exclusive targeting of LD lipoprotein for the liver might not be expected, yet LD lipoprotein does bind to high-affinity receptors in many types of cultured cells and must therefore have recognition markers, albeit not carbohydrate.

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