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Hepatic receptor that specifically binds oligosaccharides containing fucosyl α1→3 *N*-acetylglucosamine linkages

(fucose/lactoferrin/glycoprotein/lectin)

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ABSTRACT **Evidence is presented suggesting that hepatocytes contain a receptor that binds glycoproteins specifically through fucose in α1→3 linkage to *N*-acetylglucosamine. Human lactoferrin, which contains this type of linkage, is rapidly cleared from the circulation of mice after intravenous injection, and greater than 90% of the injected material is found in hepatocytes. Binding of lactoferrin is mediated through its carbohydrate groups, since its clearance is prolonged after periodate oxidation or after its oligosaccharide groups are extensively degraded with glycosidases. In addition, glycopeptides from lactoferrin inhibit lactoferrin clearance. That lactoferrin clearance is mediated through binding to its fucosyl groups is suggested for several reasons. First, transferrin and asialotransferrin, whose oligosaccharide groups are essentially structurally identical to those of lactoferrin but devoid of fucose, are not cleared on intravenous injection. Second, when fucose is incorporated into asialotransferrin by α1→3 *N*-acetylglucosamine fucosyl transferase, the resulting fucosylated derivative is cleared rapidly. Neither mannan nor derivatives of orosomucoid that are cleared by binding to receptors for galactose, *N*-acetylglucosamine, or mannose, inhibit clearance of lactoferrin although clearance is inhibited by fucoidin. Finally, glycoproteins containing fucose in α1 → 2 linkage to galactose or α1 → 6 linkage to *N*-acetylglucosamine do not inhibit lactoferrin clearance by the liver. Since clearance of other glycoproteins, such as human lactoperoxidase, also appears to be mediated through binding to the same hepatocyte receptor as lactoferrin, it is concluded that the fucose-specific receptor studied here may fulfill other functions than binding lactoferrin. Preliminary studies with liver homogenates and detergent extracts of liver show binding *in vitro*.**

After the initial observations of Ashwell and coworkers (1, 2) that mammalian hepatocytes contain a protein that specifically binds asialoglycoproteins through exposed galactose residues, several other animal binding proteins were reported. A β-galactoside binding protein has been described from calf heart and lung (3), chick embryo thigh muscle (4), and the electric organ of *Electrophorus electricus* (5), but they have properties that differ somewhat from the hepatocyte protein and perhaps from one another. Avian liver also contains a protein that binds glycoproteins with exposed *N*-acetylglucosamine (6), and considerable evidence has been reported for mannose-specific binding proteins (7).

This report provides evidence suggesting the presence of a receptor in hepatocytes that specifically binds oligosaccharides in glycoproteins through a fucosyl α1→3 *N*-acetylglucosamine group. These studies were prompted by the observations that human lactoferrin (L_f) was rapidly cleared from the circulation when injected intravenously in rats and mice, and that over 90% of the injected protein was recovered in hepatocytes.

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Much is known about L_f structure although its biological role is obscure (8). L_f, like serotransferrin (T_f), has two iron-binding sites, and iron binding is dependent on bicarbonate (9). Its amino acid sequence is homologous with that of T_f (10, 11) and ovotransferrin (11) and it contains two oligosaccharide chains, which are structurally similar to those of serotransferrin (Fig. 1), except that additional fucose residues are in α1→3 linkage with the *N*-acetylglucosamine residues adjacent to galactose and those chains containing fucose are devoid of sialic acid (11, 12, and unpublished observations). Although the studies reported here may reveal unknown aspects of L_f function, it is likely that the existence of a fucose-specific receptor in liver is not limited in its function to binding only L_f.

MATERIALS AND METHODS

Human (13) and bovine L_f (14) were purified to homogeneity by published procedures. Human T_f (homogeneous on sodium dodecyl sulfate gel electrophoresis; molecular weight 77,000) was from Sigma. Human lactoperoxidase was purified as described for the bovine enzyme (15). Human milk was obtained from the lactarium of the Oeuvre Nationale de l'enfance, Brussels, Belgium. Orosomucoid (α_1-acid glycoprotein) was a homogeneous preparation kindly supplied by Karl Schmid, Boston University. Human IgG and IgM were generous gifts from C. D. Buckley, III (Duke University) and Frank Putnam (University of Indiana, Bloomington), respectively. Porcine submaxillary mucin and asialomucin were prepared as described earlier (16). Commercial preparations of fucoidin (K & K Chem. Co.) and mannan (Sigma) were used without further characterization. GDPfucose α1→3 *N*-acetylglucosamine fucosyltransferase was prepared from human milk (17, 18) and had a specific activity of 0.4 μmol of fucose transferred to lactose/min per mg of enzyme. GDP[^{14}C]fucose (New England Nuclear) was used in conjunction with that synthesized by published methods (19). Neuraminidase, β1→4 galactosidase, β-*N*-acetylglucosaminidase, and endo-β-*N*-acetylglucosaminidase D were purified from *Streptococcus pneumoniae* as described earlier (16). A partially purified mixture of glycosidases containing neuraminidase, fucosidase, β-galactosidase, endo-β-*N*-acetylglucosaminidase, and β-*N*-acetylhexosaminidase were prepared from culture filtrates of *Clostridium perfringens*.† *C. perfringens* neuraminidase was prepared as reported earlier (16).

Abbreviations: L_f, lactoferrin; ^{125}I-L_f, radioactive iodinated lactoferrin; T_f, transferrin.

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† This mixture of glycosidases was obtained from fractions that were isolated during preparation of α-*N*-acetylgalactosaminidase (20) and fucosidase (16).

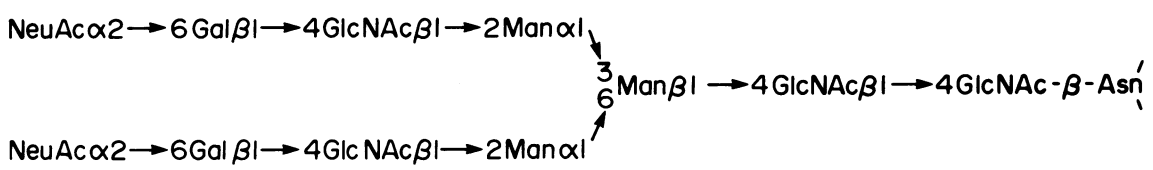

FIG. 1. Structure of the oligosaccharide groups in human transferrin. Human lactoferrin is reported to contain fucose linked α1→3 to the *N*-acetylglucosaminyl residues adjacent to the galactose (11, 12), but each oligosaccharide group contains only about 1 fucose residue. The fucose-containing chains of L_f are devoid of sialic acid (unpublished observations).

Orosomucoid, partially deglycosylated orosomucoids, lactoferrin, lactoperoxidase, and transferrin were labeled with ^{125}I (New England Nuclear) as described earlier for growth hormone (21) and had specific radioactivities between 2 and 10 μCi/μg. Iodination did not degrade the L_f, as judged by gel filtration and gel electrophoresis. Periodate oxidation of glycoproteins was performed by published methods (7). Lactoferrin glycopeptides were isolated from Pronase digests and purified by chromatography in water, first on columns (4 × 150 cm) of Sephadex G-25 (superfine; Pharmacia), second on columns (2 × 120 cm) of Sephadex G-50 (fine; Pharmacia), and then on columns (0.5 × 4 cm) of carboxymethyl-Sephadex C-25 (Pharmacia) equilibrated with 0.05 M sodium cacodylate (pH 6) and eluted with the same buffer. The glycopeptides contained the following amino acids in a mole ratio relative to aspartic acid (1): glutamic acid (0.5), threonine (0.38), glycine (0.15), and serine (0.11). Deglycosylation of orosomucoid (40 mg/ml) was performed for 72 hr at 37° in 0.02 M cacodylate buffer (pH 6) with 0.08 unit of neuraminidase (*C. perfringens*) per ml to give asialo-orosomucoid, with 0.08 unit of neuraminidase per ml and 0.025 unit of β1→4 galactosidase (*S. pneumoniae*) per ml to give asialoagalacto-orosomucoid, and with 0.08 unit of neuraminidase per ml, 0.025 unit of β1 → 4 galactosidase per ml, and 0.33 unit of β-*N*-acetylglucosaminidase (*S. pneumoniae*) per ml to give asialoagalactoahexosamino-orosomucoid. The glycosidases were removed from deglycosylated glycoproteins by affinity adsorbents (16) or by heating at 80° for 15 min for the *C. perfringens* enzymes. L_f was also deglycosylated with *S. pneumoniae* glycosidases in addition to 0.08 unit of endo-β-*N*-acetylglucosaminidase D per ml to remove additional mannose, *N*-acetylglucosamine, and fucose. The glycosidases were removed by adsorption of L_f on carboxymethyl-agarose under conditions (0.01 M sodium cacodylate, pH 6/0.1 M NaCl) that do not adsorb the enzymes. Asialotransferrin (0.86 mg/100 μl) was fucosylated by incubation with 80 nmol of GDP[^{14}C]fucose (6290 cpm/nmol), 2.5 μmol of morpholinopropane sulfonate (pH 7.5), 2 μmol of $MnCl_2$, 0.02 unit of fucosyl transferase, and 1 mg of bovine serum albumin. The reaction was allowed to proceed at 37° for 19 hr; 0.02 unit of fucosyl transferase, 80 nmol of GDP[^{14}C]-fucose, and 1 mg of bovine serum albumin in a final volume of 60 μl were added at 4 and 9 hr. The product was isolated by gel filtration on a column (0.7 × 13 cm) of Sephadex G-50 (Pharmacia, fine) equilibrated in 2.5 mM morpholinopropane sulfonate, pH 7.5/25 mM sodium chloride, followed by passage through two columns (0.4 × 5 cm) of DEAE-Sepharose (Pharmacia) equilibrated in the same buffer in order to absorb and remove bovine serum albumin. The fucosylated derivative of asialotransferrin contained 4.0 mol of fucose per mol of T_f.

The fucose content (22) of the proteins and peptides were as follows (residues per molecule): human L_f (2.6), L_f degraded extensively with *S. pneumoniae* glycosidases (1.3) or *C. perfringens* glycosidases (1.1), human T_f (<0.2), bovine L_f (0.6), human lactoperoxidase (1.2), human IgG (0.75), and human IgM (0.35). Porcine asialomucin contained 53 mmol of fucose per 100 g. Human L_f also had the following carbohydrates (residues per molecule based on molecular weight of 79,000): sialic acid (1.8), galactose (4.0), *N*-acetylglucosamine (8.2), and mannose (not quantitated).

Clearance studies in albino mice (CD-1, female, 9–12 weeks old, Charles River Labs, North Wilmington, MA) were performed by injection of radiolabeled derivatives in the tail vein (10–500 μl); blood samples (25 μl) were removed at intervals in heparinized capillary tubes from the orbital venous plexus (23), and ^{125}I radioactivity was measured in a γ counter. Hepatocytes and Kupffer cells were separated by published methods (24), but livers were perfused prior to cell fractionation by the method of Seglen (25). Carbonyl iron was first injected, followed by ^{125}I-labeled L_f (^{125}I-L_f).

RESULTS

Lactoferrin injected intravenously into mice and rats is rapidly cleared from the circulation, as shown in Fig. 2 for the ^{125}I-labeled derivative (^{125}I-L_f). Less than 10% of the ^{125}I-L_f is in the circulation 10 min after injection, and a large majority of the radioactivity injected was found in the liver. In rats killed 9 min after injection, 85% of the radioactivity was in the liver and the remainder in blood (10%), the kidneys (3.6%), and the spleen (0.9%). Ninety-eight percent of the radioactivity that is cleared by the liver is in hepatocytes, and only 1.3% is in Kupffer cells (Table 1).

Considerable evidence has been obtained to suggest that the clearance of L_f by hepatocytes is mediated by specific interaction with the asparaginyl-linked oligosaccharide groups of L_f. First, clearance of ^{125}I-L_f is inhibited by L_f, reduced and aminoethylated L_f, and the glycopeptides from L_f (Fig. 2). Periodate-oxidized glycopeptides are much less effective in inhibiting the clearance of ^{125}I-L_f. Second, ^{125}I-L_f oxidized with periodic acid and L_f enzymically deglycosylated with a mixture of glycosidases from either *S. pneumoniae* or *C. perfringens* were not cleared as rapidly as ^{125}I-L_f.

L_f clearance does not appear to involve those proteins in liver that are specific for binding glycoproteins containing galactose, *N*-acetylglucosamine, or mannose at the nonreducing termini of their oligosaccharide groups. As shown in Fig. 3, L_f clearance

Table 1. Cellular localization of ^{125}I-L_f in rat liver

Cell type	Yield cells/g liver	Contamination by other cell type, %	Cellular content of ^{125}I-L_f, % of injected sample
Hepatocytes	55×10^6	4	98
Kupffer cells	11×10^6	0.02	1.3

^{125}I-L_f (20 μg; 10 Ci/g) was injected intravenously and hepatocytes and Kupffer cells were isolated (24) after perfusion of the livers with collagenase. The cells were separated 25 min after injection of ^{125}I-L_f. Endothelial cells were not separated from Kupffer cells.

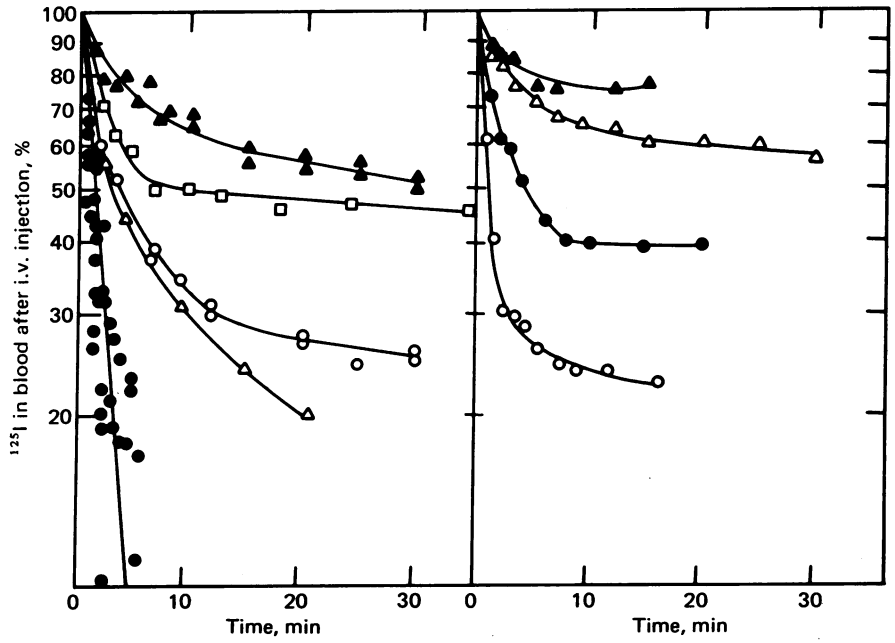

FIG. 2. Clearance from blood of intravenously injected L_f under various conditions. ^{125}I-L_f (1 μg, 2–10 Ci/g) or one of its chemically or enzymically modified derivatives, was injected into mice, in some cases along with unlabeled L_f or a L_f derivative. Blood samples were collected at intervals and radioactivity was measured. (*Left*) ^{125}I-L_f (●); ^{125}I-L_f + L_f (4–10 mg) (▲); ^{125}I-L_f oxidized with periodic acid (□); ^{125}I-L_f extensively degraded with glycosidases from *S. pneumoniae* (○) or *C. perfringens* (△). (*Right*) ^{125}I-L_f (○); ^{125}I-L_f + reduced and aminoethylated L_f (▲); ^{125}I-L_f + L_f glycopeptides (△); ^{125}I-L_f + periodate-treated glycopeptides (●). Nephrectomized mice were used in △ and ○, but control studies with non-nephrectomized mice gave substantially the same results with the glycopeptides.

is not inhibited by asialo-orosomucoid, asialoagalacto-orosomucoid, or asialoagalactoahexosamino-orosomucoid, which terminate predominantly in galactose, *N*-acetylglucosamine, and mannose, respectively. In addition, the clearance of each of the ^{125}I-labeled orosomucoids is not inhibited by L_f, although their clearance is inhibited specifically by the homologous orosomucoid derivative (data not shown).

Since a unique structural feature of the oligosaccharide groups of lactoferrin is fucose in α1→3 linkage with *N*-acetylglucosamine (Fig. 1), studies were performed to determine whether this linkage was required for L_f clearance. In support of this view is the observation that fucoidin, which contains some α1→3 linked fucose (26), prolonged the clearance of ^{125}I-L_f, although mannans, which inhibit clearance of glycoproteins terminating in mannose (7) had little effect (Fig. 3). More conclusive evidence implicating fucose in the clear-

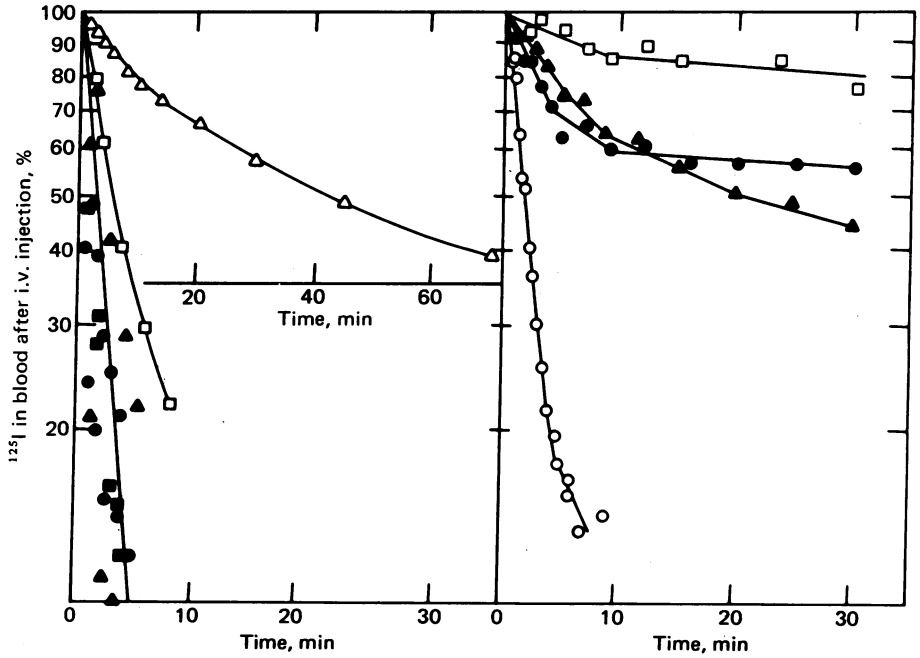

FIG. 3. Clearance from blood of intravenously injected L_f and T_f under various conditions. All ^{125}I-labeled proteins injected (1 μg) were 2–10 Ci/g. Clearance was measured as described in Fig. 2. (*Left*) ^{125}I-L_f: + 0.7 mg of fucoidin (△), + 2 mg of mannan (□), + 3 mg of asialo-orosomucoid (▲), + 2 mg of asialoagalacto-orosomucoid (■), + 1.8 mg of asialoagalactoahexosamino-orosomucoid (●). (*Right*) Human ^{125}I-T_f (□), human ^{125}I-asialo-T_f (●), human fucosylated ^{125}I-asialo-T_f (○), human fucosylated ^{125}I-asialo-T_f + 1.5 mg of human lactoferrin (▲).

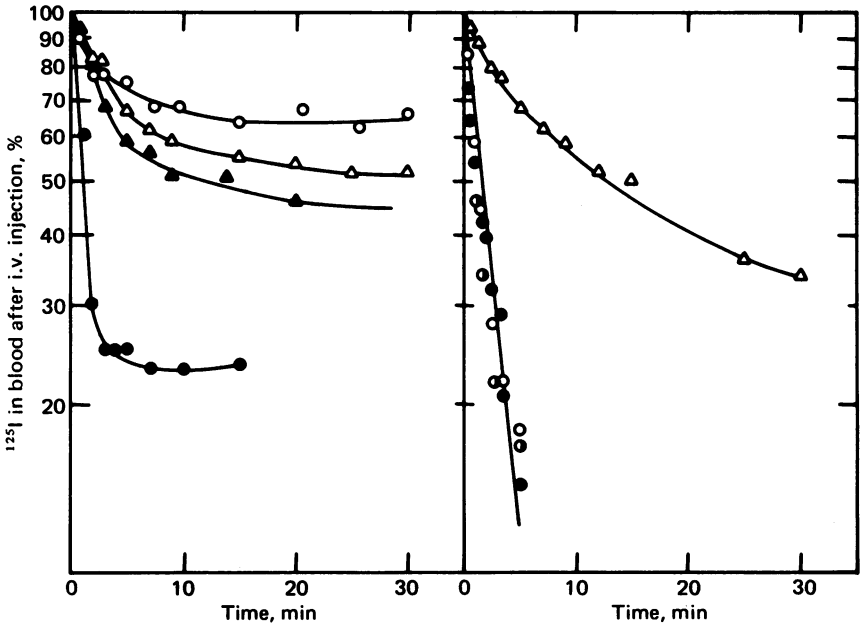

FIG. 4. Clearance from blood of intravenously injected human L_f, bovine L_f, and human lactoperoxidase under various conditions. Clearance was measured as described in Fig. 2. (*Left*) ^{125}I-Lactoperoxidase (●), periodate-treated ^{125}I-lactoperoxidase (○), ^{125}I-L_f + 6 mg of lactoperoxidase (△), ^{125}I-lactoperoxidase + L_f glycopeptides (▲). (*Right*) ^{125}I-L_f + 3.3 mg of bovine L_f (△), + 0.7 mg of asialomucin (●), + 2.8 mg of human IgM (○), + 6 mg of human IgG (◑).

ance of L_f was obtained with studies on T_f and asialo-T_f, which are not cleared rapidly on intravenous injection (Fig. 3). T_f oligosaccharides are devoid of fucose and contain two residues of sialic acid per chain (Fig. 1); otherwise they are identical to those of L_f. Thus, if fucose is involved in clearance of L_f, T_f with fucose incorporated into α1→3 linkage into its *N*-acetylglucosaminyl groups should be cleared. This was the case, as shown in Fig. 3, since asialo-T_f that was fucosylated with GDP[^{14}C]-fucose and GDPfucose α1→3 *N*-acetylglucosaminyl fucosyltransferase was cleared more rapidly than asialo-T_f itself. Moreover, clearance of the fucosylated transferrin was inhibited by L_f (Fig. 3). The fucosylated asialo-T_f is not cleared by the hepatic galactoside receptors (1), since it did not bind with the pure galactoside binding protein (data not shown).

Few glycoproteins containing the fucosyl α1→3 *N*-acetylglucosamine linkage are known; however, human milk lactoperoxidase, which has a fucose-containing oligosaccharide group, was also cleared rapidly (Fig. 4). In addition, its clearance was prolonged after periodate oxidation and was inhibited by glycopeptides from L_f. Moreover, the clearance of ^{125}I-L_f was markedly inhibited by lactoperoxidase (Fig. 4). Fig. 4 also shows that ^{125}I-L_f clearance is not inhibited by either human IgG or IgM, which contain fucose in α1→6 linkage to *N*-acetylglucosamine (27), or by porcine submaxillary asialomucin, which contains fucose in α1→2 linkage to galactose (27). Also, since bovine L_f inhibited the clearance of human ^{125}I-L_f, which was used throughout these studies, it is likely that bovine L_f also contains fucosyl α1→3 *N*-acetylglucosamine groups. These observations suggest that other glycoproteins with oligosaccharide groups similar to those of L_f and lactoperoxidase will be cleared by the same means as these two glycoproteins and that clearance is specific for one type of fucose linkage in oligosaccharides.

Preliminary studies have revealed ^{125}I-L_f binding to insoluble microsomal preparations of mouse liver homogenates, and binding is inhibited by L_f. Moreover, gel filtration (Ultragel RC34) of Triton X-100 extracts of homogenates prepared from livers obtained 10 min after intravenous injection of 1 μg of ^{125}I-L_f into mice revealed one radioactive peak at the void volume, whereas L_f itself was retained under the same conditions. This suggests that binding of L_f is mediated by a macromolecular species, most likely a protein, but further knowledge of the properties of the binding protein awaits its purification and characterization.

DISCUSSION

The data reported here support the view that L_f clearance from the circulation is dependent on its oligosaccharide groups rather than constituents in its polypeptide chain. Periodate oxidation or extensive enzymic deglycosylation prolongs its clearance. Clearance is also inhibited by glycopeptides from L_f, but periodate-treated glycopeptides do not inhibit as extensively. Other data support the view that clearance is mediated specifically by binding to hepatocytes through fucose in α1→3 linkage to *N*-acetylglucosamine in the oligosaccharide groups. Binding proteins that are specific for galactose, *N*-acetylglucosamine, or mannose do not appear to be involved since L_f clearance is not inhibited by partially deglycosylated orosomucoid derivatives that are cleared by the three different binding proteins. Also, fucoidin, but not mannan, prolongs L_f clearance. Most significant, however, is that human T_f, which has two oligosaccharide groups per molecule that are structurally similar to those of L_f but is devoid of fucose, is not cleared until fucose is enzymically incorporated into its oligosaccharides. In addition, the clearance of fucosylated asialo-T_f is inhibited by L_f. Pure fucosidases that will specifically remove fucose from L_f are presently unavailable, and it is impossible to test whether L_f clearance is prolonged when only its fucose is removed, but L_f treated with mixtures of glycosidases that extensively degrade its oligosaccharides and remove fucose is not cleared as rapidly as L_f.

Although the clearance specificity has not been extensively examined, noteworthy is the observation that L_f clearance is not inhibited by either porcine asialomucin, which contains fucose in α1→2 linkage to galactose (27), or human IgG and

IgM, which contain fucose in $\alpha 1 \rightarrow 6$ linkage to *N*-acetylglucosamine (27). Thus, although clearance is dependent on fucose in the oligosaccharide, its location and its glycosidic linkage appear to be very critical.

The nature of the enzymically fucosylated T_f used in these studies requires special comment. Unpublished studies reveal that native T_f with its full complement of sialic acid (Fig. 1) is not an acceptor for the $\alpha 1 \rightarrow 3$ *N*-acetylglucosamine fucosyltransferase, but that asialo-T_f is an excellent acceptor. Four residues of fucose per molecule are readily incorporated by the transferase into asialo-T_f, which is equal to one fucose residue per *N*-acetylglucosamine residue in the sequence $Gal\beta 1 \rightarrow 4GlcNAc\beta 1 \rightarrow 2Man$. The resulting product contains <0.04% of the fucose incorporated in $\alpha 1 \rightarrow 2$ linkage with galactose, as judged by its inability to serve as an acceptor for the A blood-group-specific *N*-acetylgalactosaminyltransferase (28). In addition, the fucosylated asialo-T_f is not an acceptor for the β-D-galactoside sialyltransferase (29), nor will it bind the purified rabbit liver lectin of Ashwell and Morell (1). Thus, sialic acid linked $\alpha 2 \rightarrow 6$ to galactose prevents fucosylation, and fucose linked $\alpha 1 \rightarrow 3$ to *N*-acetylglucosamine prevents sialylation. These studies will be reported in detail elsewhere, but they not only illustrate the reciprocal action of fucosyl and sialyl transferases in oligosaccharide biosynthesis, but also the critical nature of the location and linkage of fucose for clearance of fucose containing glycoproteins by liver receptors.

The biological significance of hepatocyte-binding proteins, which are analogous to the plant lectins, remains obscure although some may serve in the regulation of mammalian plasma glycoprotein homeostasis, as suggested earlier (1). This may not be the case for the fucose-specific lectin, since fucose is always terminal in mammalian glycoproteins and the $\alpha 1 \rightarrow 3$ fucosidic linkage to *N*-acetylglucosamine has not been reported to be present in many plasma proteins (27). Nevertheless, the present studies indicate a biological role for fucose not evident heretofore, and that this function is widely distributed in mammals, since rabbits, as well as mice and rats, rapidly clear intravenously injected L_f via the liver. It could be speculated that the fucose-lectin in hepatocytes is involved in some as yet unknown way in iron metabolism, but whether or not this is the case, the fucose-lectin may serve other functions in view of its ability to clear lactoperoxidase and other proteins which appear to contain fucose in $\alpha 1 \rightarrow 3$ linkage to *N*-acetylglucosamine. Whatever functions the animal lectins fulfill, their presence in animal cells along with the great diversity of oligosaccharide structures with the potential for specifying considerable molecular information suggests that other animal lectins, presently unknown, will be discovered and that they could play vital roles in a variety of intra- and intercellular processes.

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1. Ashwell, G. & Morell, A. G. (1974) *Adv. Enzymol.* **41**, 99–128.
2. Kawasaki, T. & Ashwell, G. (1976) *J. Biol. Chem.* **251**, 5292–5299.
3. DeWaard, A., Hickman, S. & Kornfeld, S. (1976) *J. Biol. Chem.* **251**, 7581–7587.
4. Den, H. & Malinzak, D. A. (1977) *J. Biol. Chem.* **252**, 5444–5448.
5. Teichberg, V., Silman, I., Beitsch, D. D. & Resheff, G. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1383–1387.
6. Kawasaki, T. & Ashwell, G. (1977) *J. Biol. Chem.* **252**, 6536–6543.
7. Kaplan, A., Achord, D. T. & Sly, W. S. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2026–2030.
8. Arnold, R. R., Cole, M. F. & McGhee, J. R. (1977) *Science* **197**, 263–265.
9. Aisen, P. & Liebman, A. (1972) *Biochim. Biophys. Acta* **257**, 314–323.
10. MacGillivray, R. T. A., Mendez, E. & Brew, K. (1977) in *Proteins in Iron Metabolism*, eds. Brown, E. B., Aisen, P., Fielding, J. & Crichton, R. C. (Grune and Stratton, New York), pp. 133–141.
11. Spik, G. & Mazurier, J. (1977) in *Proteins of Iron Metabolism*, eds. Brown, E. B., Aisen, P., Fielding, J. & Crichton, R. C. (Grune and Stratton, New York), pp. 143–151.
12. Dorland, L., Haverkamp, J., Schut, B. L., Vliegenthart, J. F. G., Spik, G., Strecker, G., Fournet, B. & Montreuil, J. (1977) *FEBS Lett.* **77**, 15–20.
13. Querinjean, P., Masson, P. L. & Heremans, J. F. (1971) *Eur. J. Biochem.* **20**, 420–425.
14. Castellino, F. J., Fish, W. W. & Mann, K. G. (1970) *J. Biol. Chem.* **245**, 4269–4275.
15. Groves, M. L. & Kiddy, C. A. (1964) cited by Groves, M. L. (1971) in *The Milk Proteins*, ed. McKenzie, H. A. (Academic, New York), Vol. II, p. 388.
16. Glasgow, L. R., Paulson, J. C. & Hill, R. L. (1977) *J. Biol. Chem.* **252**, 8615–8623.
17. Beyer, T. A., Prieels, J. P. & Hill, R. L. (1978) *Fourth International Symposium on Glycoconjugates*, in press.
18. Prieels, J. P., Beyer, T. A. & Hill, R. L. (1977) *Biochem. Soc. Trans.* **5**, 838–839.
19. Schachter, H., Hanako, I. & Heath, E. C. (1972) in *Methods in Enzymology*, ed. Ginsberg, V. (Academic, New York), Vol. 28, pp. 285–287.
20. Hill, H. D., Jr., Reynolds, J. A. & Hill, R. L. (1977) *J. Biol. Chem.* **252**, 3791–3798.
21. Greenwood, F. C., Hunter, W. M. & Glover, J. S. (1963) *Biochem. J.* **89**, 114–123.
22. Spiro, R. G. (1966) in *Methods in Enzymology*, eds. Neufeld, E. F. & Ginsberg, V. (Academic, New York), Vol. 8, pp. 3–26.
23. Chase, M. W. (1967) in *Methods in Immunology and Immunochemistry*, eds. Williams, C. A. & Chase, M. W. (Academic, New York), Chap. 2, Sect. D, Vol. I, pp. 252–306.
24. Wincek, T. J., Hupka, A. L. & Sweat, F. W. (1975) *J. Biol. Chem.* **250**, 8863–8873.
25. Seglen, P. O. (1973) *Exp. Cell Res.* **82**, 391–398.
26. Percivol, E. (1970) in *The Carbohydrates*, eds. Pigman, W. & Horton, D. (Academic, New York), Vol. IIB, 2nd Ed., pp. 537–568.
27. Kornfeld, R. & Kornfeld, S. (1976) *Annu. Rev. Biochem.* **45**, 217–237.
28. Schwyzer, M. & Hill, R. L. (1977) *J. Biol. Chem.* **252**, 2346–2355.
29. Paulson, J. C., Rearick, J. I. & Hill, R. L. (1977) *J. Biol. Chem.* **252**, 2363–2371.