The Distribution and Localization of the Fucose-binding Lectin in Rat Tissues and the Identification of a High Affinity Form of the Mannose/N-Acetylglucosamine-binding Lectin in Rat Liver*

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A small-scale affinity chromatographic procedure was developed to screen for the presence of fucose and mannose/N-acetylglucosamine-binding lectins in small amounts of rat tissues. Of all tissues examined, only the liver contained the fucose-binding lectin, whereas both liver and blood serum contained the mannose/N-acetylglucosamine lectin. By means of immunocytochemical methods using antibodies to hepatic lectins, the fucose lectin was shown to be uniquely present in Kupffer cells and absent in all other types of rat macrophages examined. The binding and uptake of different neoglycoproteins by nonparenchymal cell fractions of liver indicated that the fucose-binding lectin was either not responsible for the uptake or that more than one lectin was acting. With the identification of another lectin (M₀ = 180,000) by the above screening procedure for hepatic lectins and the results of studies in the following paper (Haltiwanger, R. S., and Hill, R. L. (1986) J. Biol. Chem. 261, 7440-7444) two lectins appear to be involved.

A small amount of the hepatic mannose/N-acetylglucosamine lectin was found by the above screening procedure to have a higher affinity for L-fucosyl-bovine serum albumin-Sepharose than the majority of the lectin in hepatocytes. This lectin, called the high affinity form, was purified and its properties examined. On a weight basis the high affinity form bound 7-12 times more ligand than the normal form. Its Kₛ for L-fucosyl-bovine serum albumin was 2.3 x 10⁷ M⁻¹ compared to 3.8 x 10⁸ M⁻¹ for the normal form. Moreover, the concentrations of monosaccharides required to inhibit the high affinity form were about 3 times less than those required to inhibit binding of the normal form. The two forms, however, have identical molecular weights (32,000) under reducing and nonreducing conditions, bind anti-lectin antibodies in the same way, and give identical peptide maps after V-8 protease digestion. The structural basis for the different binding affinities of the two forms remains unknown.

The preceding papers describe the purification, some structural properties (1), and the binding specificity (2) of a lectin from rat liver with a high affinity for ligands containing nonreducing terminal L-fucose or D-galactose. It differs in its structural properties from the other known lectins of liver, including those with a binding specificity for galactose (3, 4) and mannose/N-acetylglucosamine (5, 6). Although it binds L-fucose and D-galactose derivatives strongly, its affinity for N-acetylglucosamine is very low. The mannose/N-acetylglucosamine lectin, however, binds L-fucose in addition to mannose and N-acetylglucosamine but has a low affinity for galactose. In contrast, the galactose lectin has a high affinity for galactose and has a low affinity for L-fucose, mannose, and N-acetylglucosamine.

In order to understand the possible functions of these hepatic lectins, including their participation in the uptake of circulating glycoproteins from blood (7, 8), the tissue distribution of the fucose lectin and its cellular localization in the liver were studied. The results of these studies, reported here, show that the fucose lectin is present in hepatic Kupffer cells, in contrast to the galactose (7) and N-acetylglucosamine lectins (9), which are in hepatocytes. Moreover, since no other rat tissue or type of macrophage contains the fucose lectin, it appears to be a protein unique to Kupffer cells.

Also reported here is the isolation and characterization of a form of the mannose/N-acetylglucosamine lectin that has a higher affinity for its ligands than the normal form isolated from liver extracts. This lectin, called the high affinity form, was discovered during the course of purification of the fucose lectin (1) and was easily identified in a screening procedure developed for detecting lectins in small amounts of rat tissue. The screening procedure also disclosed a hepatic protein (M₀ = 180,000) with a high affinity for Fuc-BSA. Since the pattern of binding of neoglycoproteins to nonparenchymal liver cells could not be explained by the binding specificity of the fucose lectin, another yet unidentified lectin, perhaps corresponding to the protein with a M₀ = 180,000, was suggested to be involved. As shown in the following paper (10), which describes the purification and partial characterization of an alveolar macrophage lectin (M₀ = 180,000) with a high affinity for N-acetylglucosamine and L-fucose, this proved to be the case.

EXPERIMENTAL PROCEDURES

Materials—Rabbit antibodies to the rat liver fucose lectin and the mannose/N-acetylglucosamine lectin were prepared as described previously.

Neoglycoproteins are abbreviated with the standard symbols for monosaccharides and bovine serum albumin. Thus, Fuc-BSA is L-fucosyl-bovine serum albumin. Unless otherwise designated all monosaccharides used here were of the D-configuration except fucose which was of the L-configuration. The other abbreviation used is: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
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vioulsly (1). Antiserum to the rat liver galactose lectin was prepared in the same manner except that 76 μg of the purified lectin was injected following by two boosts of 75 μg each. The IgG in antiserum was isolated on Protein A-Sepharose 4B columns (11). F(ab′)2 fragments were prepared as described (12) by pepsin digestion. Goat anti-rabbit IgG was labeled with fluorescein isothiocyanate by published procedures (13).

Sprague-Dawley male rats (200–400 g) were obtained from Charles River Breeding Laboratories, Boston, MA. The following materials were obtained as indicated. Protease type XIV (Pronase B), types I and IV collagenase, deoxyribonuclease I, Pronase, HEPES, and fluorescein isothiocyanate (Sigma); type II and type III collagensases (Worthington Diagnostic Systems, Inc.); Na[131]I (Amersham Corp.); goat anti-rabbit IgG and Tissue-Tek II OCT embedding compound (Miles Laboratories, Inc.); Hanks' balanced salt solution (Worthington Diagnostic Systems, Inc.); Na[131]I (Amersham Corp.); goat anti-rabbit IgG and Tissue-Tek II OCT embedding compound (Miles Laboratories, Inc.); Hanks' balanced salt solution, Eagle's modified minimal media, and fetal calf serum (Gibco Laboratories); nylon mesh (100 μm) (Nyctex) and colloidal copper (S-P special, 3–4 μm, GAF Corp.).

The normal form of the mannose/N-acetylgalcosamine lectin was prepared as described earlier (1). The high affinity form was prepared from the material obtained in step 5 of the normal purification procedure (1) in which the lectin was adsorbed on anti-lectin IgG-Sepharose. The adsorbent was suspended in 1–2 volumes of 0.2 M glycine-HCl, pH 2.2, for 60 min at 23°C. The eluate was adjusted to pH 7.5, made 0.01 M in calcium chloride and purified on a column (1.2 x 2.5 cm) of Fuc-BSA-Sepharose as described in step 4 of the purification procedure (1). The anti-lectin-IgG-Sepharose was regenerated by exhaustive washing with 0.01 M Tris-Cl, pH 7.8, containing 0.01 M sodium chloride and 0.02 M sodium azide, and stored at 4°C.

All other materials were obtained as described earlier (1, 2).

Distribution of Lectins in Rat Tissues—The alternate procedures (9) for preparation of the fucose lectin was scaled down and used to prepare the lectins in different tissues. Tissue (0.5 g) was homogenized in 3–4 ml of extraction buffer exactly as in the step 1 of the purification procedure. The homogenate was then centrifuged through step 2 (precipitation with polyethylene glycol) exactly as described. The material from step 2 was applied to 1 ml of Fuc-BSA-Sepharose 100 mg on Fuc-BSA/ml in a column (0.4 x 2 cm) equilibrated with buffer A (1). The flow rate of the column was reduced to <1 ml/min to prevent nonspecific filtering. After washing with 10 ml of Buffer A the column was eluted with 5 ml of Buffer B. The column was then regenerated by washing with 5 ml of 0.05 M sodium acetate, pH 4.0, and 5 ml of Buffer B containing 0.05 M N-acetylglucosamine. The Buffer B eluate (5 ml) were made 0.05 M in N-acetylglucosamine and 0.01 M in calcium chloride and applied to the regenerant column. The eluate (5 ml) contained the normal form of the mannose/N-acetylgalcosamine lectin and was combined with the first 3 ml of the eluate obtained by the column with 10 ml of Buffer A containing 0.05 M N-acetylglucosamine. After elution with 10 ml of Buffer A, the columns were eluted with 4 ml of Buffer A containing 0.04 M galactose followed by 4 ml of Buffer B.

TheBuffer B eluate contained the high affinity form of the mannose/N-acetylgalcosamine lectin. The columns were then resealed above for reuse. The lectins in each fraction were mixed with 2 ml of Eagle's medium, 2.5 mM CaCl2, and 20 mM HEPES, pH 7.4 to 4°C. The cells were incubated at 37°C for 1-2 h at 37°C with 10 ml of Buffer A and 0.05 M NaCl, 0.02% NaN3 (PBS). Each section was washed three times for 10 min each in 10 mM sodium phosphate, pH 7.4, 0.15 M NaCl, 0.02% NaN3 (PBS). Each section was then covered with a drop of PBS plus 1% BSA containing 50 μg/ml of the appropriate IgG and incubated for 2 h at 37°C. After washing as before, the sections were incubated with a drop of PBS plus 1% BSA and 50 μg/ml fluorescein isothiocyanate-labeled goat anti-rabbit IgG for 2 h at 3°C. Sections were then rinsed for a final time, mounted, and viewed with a Zeiss photomicroscope III with fluorescence microscopy. Pictures were taken with Ektachrome 400 film which were punch printed on to ASA 100 film.

Western Blots—Western blots were performed essentially as described earlier (16, 17) except that 9% electrophoretic gels were used and 125I-goat anti-rabbit IgG was used rather than 125I-protein A. Tissues or cells were homogenized in 10 volumes of 0.65 mM Tris-HCl, pH 6.8, containing 2% sodium dodecyl sulfate and 10% glycerol.

Any undissolved material was removed by centrifugation. Bromphenol blue and 2-mercaptoethanol were added to the samples to give final concentrations of 0.00125 and 5%, respectively, before heating each sample to 100°C for 5 min.

Pronase Digestion of Liver—Liver slices were hydrolyzed with Pronase as described (18). After filtering the digested liver through 100-μm nylon mesh, the Kupffer cells and parenchymal cells were removed by centrifugation at 259 X g for 10 min and washed twice with Eagle's modified essential medium containing 10% fetal calf serum, 2.5 mM CaCl2 and 20 mM HEPES, pH 7.4. Typically 1–5 X 10⁶ nonparenchymal cells were recovered per rat.

Collagenase Digestion of Liver—Collagenase digestion of rat liver was performed by recirculating perfusion (19, 20). Type III collagenase from Worthington was found to have the least adverse effects on the lectin and was used for routine perfusions. After digestion, the disrupted liver was filtered through 100-μm nylon mesh and the parenchymal cells removed by centrifugation at 10 X g for 5 min. The nonparenchymal cells collected by centrifuging at 250 X g for 10 min and washed three times with Hanks' balanced salt solution containing 0.0005% deoxyribonuclease I. The nonparenchymal cells were enriched in Kupffer cells with Percoll gradient centrifugation as described (21). Approximately 5–10 X 10⁶ cells were layered onto each Kupffer cell density gradient and centrifuged at 37°C for 15 min. The gradient corresponding to densities of 1.045 and 1.062 g/ml were collected, diluted 5-fold with Hanks' solution and centrifuged at 250 X g. These cells were then resuspended in Hanks' solution and used immediately. Typically 3–14 X 10⁶ cells were isolated per rat and more than 90% were judged to be Kupffer cells by the presence of cytoplasmic trypsin blue. In a routine preparation, 38% of the cells were peroxidase positive as determined by the method of Page and Garvey (22).
proteins, but another fraction of this same lectin is tightly bound to the Fuc-BSA adsorbent and is obtained in step 6. This procedure for separating the lectins was scaled down so that a Triton X-100 extract of 0.5 g of rat liver was fractionated or a 0.4 x 2-cm column of Fuc-BSA adsorbent. Each of the fractions was then analyzed by gel electrophoresis in sodium dodecyl sulfate, and the protein species corresponding to each lectin detected with silver nitrate, as shown in Fig. 2. Silver nitrate detects as little as 10-20 ng of lectin; thus, this method is more sensitive than detection of the lectin by a binding assay.

In view of the sensitivity of the above procedure for detecting the fucose lectin, it was used to determine the distribution of the lectin in rat tissues. Of all the tissues examined, including kidney, heart, lung, brain, testes, serum, spleen, pancreas, thymus, and skeletal muscle, only liver contained the fucose lectin. The yield of lectin was about 800 ng/g of liver.

In other studies, samples (0.5 g) of mouse and human liver were analyzed (Fig. 1), and each contained silver nitrate reactive species in the fractions that contain fucose lectin. The molecular weights of these species, however, did not correspond exactly to those of rat fucose lectin (rat, 88,000 and 77,000; mouse, 95,000 and 90,000; human, 25,000, 45,000, and 60,000).

**Localization of the Fucose Lectin in Hepatic Cells**—Antibodies to the fucose lectin were used to determine which type of hepatic cells contained the lectin. Antibodies to the galactose and the mannose/N-acetylglucosamine lectins of hepatocytes were also used as controls. Fig. 3 shows Western blots of the three lectins with their homologous antibodies and indicates the high specificity of the antibodies employed. Fig. 4 shows frozen sections of rat liver treated initially with F(ab')2 fragments of either the anti-fucose lectin IgG or the anti-galactose lectin IgG and then with fluorescein-labeled F(ab')2 fragments of goat anti-rabbit IgG. When the same experiments were performed with F(ab')2 fragments from anti-mannose/N-acetylglucosamine IgG, no fluorescence was observed, in accord with the observation that this lectin is not bound to membranes (9). The pattern of staining by the galactose and fucose lectins is quite different. Since the galactose lectin is in hepatocytes, it would appear that the fucose lectin is in another cell type. This was confirmed in other experiments in which colloidal iron was injected intravenously into rats and frozen sections of the liver examined as shown in Fig. 5. Iron is specifically taken up by Kupffer cells and not by hepatocytes nor endothelial cells (23). Since the fluorescent cells are coincident with those containing iron, it is concluded that the fucose lectin is in Kupffer cells.

Further studies were performed to determine if macro-

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**Fig. 1.** Affinity chromatographic procedure for analysis of the lectin in small samples of tissue. The details are given under "Experimental Procedures" and "Results." PEG, polyethylene glycol.

**Fig. 2.** Polyacrylamide gel electrophoretic analysis of the protein species in different fractions obtained as shown in Fig. 1. A, protein standards (0.2 μg) of human transferrin, bovine serum albumin, ovalbumin, and trypsinogen. B, pure fucose lectin. C, the unadsorbed proteins at step 4 (Fig. 1). D, the proteins (fucose lectin) eluted from Fuc-BSA-Sepharose at step 5 (Fig. 1) with 0.04 M galactose. E, the proteins in the eluate at step 6 (Fig. 1) including the high affinity form of the mannose/N-acetylglucosamine lectin (Mr = 32,000) and a high molecular weight (Mr = 180,000) protein, presumably another lectin. F, the high molecular weight (Mr = 180,000) protein (Lane E) after removal of the mannose/N-acetylglucosamine lectin on anti-lectin IgG-Sepharose. Silver nitrate was used to detect the proteins.

**Fig. 3.** Polyacrylamide gel electrophoresis in sodium dodecyl sulfate and Western blots of the hepatic lectins. Duplicate samples of the three lectins (0.2 μg) were electrophoresed in sodium dodecyl sulfate under reducing conditions and one of each of the lectins stained with silver nitrate (left). The other lectin was blotted on nitrocellulose, reacted with the appropriate antibodies, and radiodistographed (right). S, standards: human transferrin (Mr = 77,000), bovine serum albumin (Mr = 66,000), ovalbumin (Mr = 45,000), and trypsinogen (Mr = 24,000). 1, fucose lectin; 2, galactose lectin; 3, mannose/N-acetylglucosamine lectin.
FIG. 4. Frozen sections of rat liver reacted with F(ab')₂ fragments of anti-galactose lectin (left) or antifucose lectin (right) and then with fluorescein-labeled F(ab')₂ fragment of goat anti-rabbit IgG (× 200 magnification).

FIG. 5. Frozen sections of rat liver from rats injected with colloidal iron particles. After infusion of a rat with colloidal iron, the liver was sectioned and the frozen sections reacted with fucose lectin antibodies as in Fig. 4. The sections were examined by fluorescence (left) or phase contrast (right) microscopy of the same field to reveal the labeled antibody and colloidal iron particles, respectively.

Phages other than Kupffer cells contained the fucose lectin. Accordingly, lung, spleen, peritoneal macrophages, and blood monocytes were examined with anti-lectin F(ab')₂ and fluorescein-labeled anti-rabbit F(ab')₂ as described above, but no fluorescence was observed. These tissues and cells were also extracted with sodium dodecyl sulfate and the extracts submitted to Western blotting. Fig. 6 shows that only Kupffer cells contained the fucose lectin. A protein with a lower molecular weight than the lectin cross-reacted with the antibody and was found in all macrophage preparations.

Further studies were performed to determine whether the fucose lectin was on the external surface of Kupffer cells, as would be expected if it is involved in receptor-mediated endocytosis. Nonparenchymal cells freshly isolated from collagenase-perfused rat livers had little or no lectin on their surfaces as judged by binding with F(ab')₂ fragments of anti-lectin IgG. It was found, however, that the lectin was particularly susceptible to hydrolysis by different collagenase preparations as judged by reactions of thin sections of liver with the enzyme and staining for lectin as in Figs. 4 and 5. Nonparenchymal cells from Pronase-digested liver slices also contained little lectin as judged by binding of labeled F(ab')₂ fragments. But if the cells were incubated 1–2 h in tissue culture medium at 37 °C, then lectin appeared as judged by binding of labeled antibody, as shown in Fig. 7.

**Binding and Uptake Neoglycoprotein by Nonparenchymal Cells from Liver**—Fig. 8 shows that freshly isolated nonparenchymal cells regained their ability in time to bind to Fuc-BSA, Gal-BSA and GlcNAc-BSA when incubated in tissue culture medium at 37°C. When cells were incubated in me-
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FIG. 6. Western blots of sodium dodecyl sulfate extracts of macrophages and tissues with anti-fucose lectin antibodies. 1, hepatocytes; 2, nonparenchymal cells of liver; 3, alveolar macrophages; 4, peritoneal macrophages; 5, plasma; 6, spleen; 7, pure fucose lectin (0.2 μg).

FIG. 7. Binding of 125I-anti-fucose lectin F(ab')2 fragments to nonparenchymal cells. Cells were incubated at 37 °C for 1-2 h, brought to 4 °C, and mixed with F(ab')2 fragments from preimmune (O) or immune IgG and incubated a further 2 h at 4 °C.

FIG. 8. Binding of neoglycoproteins to nonparenchymal cells. Freshly isolated cells were reacted at 37 °C with 125I-labeled neoglycoproteins in the presence or absence of a 1000-fold excess of unlabeled neoglycoprotein and the binding of labeled neoglycoprotein measured after 1 h as described under "Experimental Procedures."

Table I lists the amounts of 125I-labeled Fuc-BSA, GlcNac- BSA, and Gal-BSA bound to the cells and the percentage of inhibition of binding by a 1000-fold excess of the unlabeled neoglycoprotein. In accord with the above results (Fig. 8) these data show that the pattern of inhibition is also not that predicted by the binding specificity of the fucose lectin. This suggests that the fucose lectin does not mediate binding and uptake or that another lectin is also acting.

Characterization of a High Binding Affinity Mannose/N-Acetylglucosamine Lectin—A small fraction of the mannose/N-acetylglucosamine lectin had a high binding affinity for Fuc-BSA in both procedures (1) for purification of the fucose lectin. Indeed this was the major protein to contaminate the fucose lectin. Its high affinity is illustrated in Fig. 2, which shows that it is the last species to elute from the affinity adsorbent, and rather drastic conditions, removal of Ca2+, are required for elution. It was ordinarily removed by adsorption on anti-lectin-IgG-agarose adsorbents (1) and was obtained during preparation of the fucose lectin by elution of the anti- lectin IgG-adsorbent at pH 2.2 (see "Experimental Procedures"). Thus, its properties could be examined and compared with those of the mannose/N-acetylglucosamine lectin that did not have a high affinity for fucosyl-BSA adsorbents.

Fig. 9 shows the binding of 125I-Fuc-BSA by the two forms of the lectin. On a weight basis the high affinity lectin bound 7-12 times more ligand than the normal species. Moreover, binding by a mixture of the two forms was the sum of the two separately. Fig. 10 shows Scatchard plots (25) of ligand binding for the two species. The high affinity form had an apparent $K_d = 2.3 \times 10^9$ M$^{-1}$ with $2.7 \times 10^{-1}$ sites/monomer ($M_c = 32,000$), whereas the normal form had an apparent $K_d = 3.5$

Table I

| Neoglycoproteins      | Amount of neoglycoprotein bound to cells | Inhibition of binding by 1000-fold excess of unlabeled neoglycoprotein |
|------------------------|-----------------------------------------|---------------------------------------------------------------------|
|                        | ng/10^6 cells                           | %                      | Fuc-BSA | GlcNac-BSA | Gal-BSA |
| 125I-Fuc-BSA           | 5.97                                    | 50                     | 89      | 15        |
| 125I-GlcNac-BSA        | 3.36                                    | 100                    | 100     | 7.5       |
| 125I-Gal-BSA           | 1.68                                    | 17.1                   | 4.2     | 100       |

Fig. 9. Comparison of the binding of 125I-Fuc-BSA to the normal and high affinity forms of the mannose/N-acetylglucosamine lectins. Binding of ligand was determined by assay 1 as a function of lectin concentration. The two lectins were obtained from the same preparation of rat liver. O, high affinity form of the lectin; ●, normal form of the lectin. In O, 15.4 ng of the high affinity form was mixed with 35 ng of the normal form and assayed.
×10⁸ M⁻¹ and 1.4 sites/monomer. These results show that the high affinity form has about a 6-fold increase in the association constant and about twice the number of binding sites.

Table II lists the concentrations for four monosaccharides required to inhibit by 50% the binding of ¹²⁵I-Fuc-BSA to both forms of the lectin. Clearly, 2.8–3.6 times lower concentrations of monosaccharide were necessary for 50% inhibition of the higher affinity form. The binding specificity, however, was the same for both forms.

The two forms of lectin appear to have indistinguishable structures. Thus, the high affinity and normal forms are readily adsorbed to anti-lectin IgG-agarose columns (1). In addition, both show the same protein species on gel electrophoresis in sodium dodecyl sulfate either in the presence or absence of reducing agent. Only a single species (M₀ = 180,000) is observed in reducing agent although species corresponding to dimers and trimers of 32,000 appear in the absence of reducing agent. Finally, peptide maps of V-protease digests of each species were indistinguishable.

Another Hepatic Lectin—Fig. 2 shows that another protein species with a M₀ = 180,000 was observed on gel electrophoresis in sodium dodecyl sulfate of the proteins obtained in the eluate at step 6 (Fig. 1) of the small scale analysis of liver extracts. The high affinity form of the mannose/N-acetylglucosamine lectin could be removed from this fraction by adsorption on anti-lectin-IgG-Sepharose leaving the M₀ = 180,000 species in pure form. Insufficient material, however, was obtained to determine its properties. On the basis of the results presented in the following paper (10), it is now apparent that this species is likely another lectin derived from alveolar macrophages or nonparenchymal liver cells.

**TABLE II**

| Monosaccharide         | Concentration required for 50% inhibition | Normal affinity | High affinity |
|------------------------|------------------------------------------|-----------------|---------------|
| L-Fucose               | 17                                       | 6.0             | 2.8           |
| D-Mannose              | 22                                       | 6.4             | 3.4           |
| N-Acetyl-D-glucosamine | 23                                       | 7.4             | 3.1           |
| D-Galactose            | 120                                      | 33              | 3.6           |

**DISCUSSION**

The studies reported here show clearly that the fucose lectin is unique to Kupffer cells, the stationary macrophages in the sinusoidal spaces of the liver. The immunocytological methods used for cellular localization of the lectin showed that the galactose lectin is only in hepatocytes, in accord with earlier reports (7). The mannose/N-acetylglucosamine lectin, however, could not be detected by the immunocytological methods, in agreement with the report that this hepatocyte lectin is not bound to membranes (9).

The fucose lectin appears to be at least partly on the external surface of Kupffer cells, since it cannot be detected in nonparenchymal cells freshly isolated from collagenase-perfused livers but is detectable after the cells have been incubated in tissue culture medium for 1–2 h. Its surface location suggests that it is bound to plasma membranes, in accord with the fact that it cannot be solubilized from liver homogenates except on extraction with detergents such as Triton X-100 (1).

The fucose lectin is also found exclusively in Kupffer cells, since it could not be detected in other rat macrophages or tissues by either a small-scale screening procedure that detects nanogram amounts of the lectin or by immunological methods. Thus, it may well prove to be useful in the future as a protein marker of Kupffer cells.

It seems reasonable to assume that the fucose lectin serves in the receptor-mediated endocytosis of glycoconjugates by Kupffer cells. The studies reported here show that nonparenchymal hepatic cells do indeed bind and take up neoglycoproteins, in accord with this suggestion. But the specificity of binding and uptake is not in accord with the binding specificity of the fucose lectin. Thus, either the lectin is not involved or another lectin is also acting in endocytosis of glycoconjugates by nonparenchymal cells. The results presented in the following paper indicate that the latter is likely the case, since a lectin has been identified in alveolar macrophages that is very similar in molecular weight to a protein (lectin) also identified in liver by the small scale screening procedure for hepatic lectins used here (2). More importantly, antibodies to the alveolar lectin react with nonparenchymal cells in immunocytological studies (10). These results suggest that unlike alveolar macrophages, which contain only the M₀ = 180,000 lectin, Kupffer cells contain the fucose lectin and perhaps the same lectin found in alveolar macrophages. Others have recently reported the occurrence of the M₀ = 180,000 lectin in alveolar macrophages (26).

The studies reported here indicate that at least three hepatic lectins participate in the uptake of glycoconjugates from blood of mammals, viz. the galactose lectin of hepatocytes and two lectins in nonparenchymal cells. One of the two nonparenchymal cell lectins is the fucose lectin found exclusively in Kupffer cells and the lectin first identified in alveolar macrophages that is also present in either Kupffer cells or endothelial cells, or both. Based on the different binding specificities of the three lectins, a wide variety of glycoconjugates with different nonreducing terminal monosaccharides would be readily removed from the circulation by the liver, in accord with earlier observations (7, 8). It should be emphasized, however, that the mannose/N-acetylglucosamine lectin of mammalian hepatocytes, which is not on the cell surface (9), is likely not involved in clearance of glycoconjugates from blood. In view of its similar binding specificity to the alveolar lectin, it has been assumed that it was present in macrophages...
and involved in uptake (5, 6, 27).

Finally, a high affinity form of the mannose/N-acetylglucosamine lectin was detected by the affinity chromatographic procedure used here to screen small amounts of tissue for the fucose lectin. This form is structurally indistinguishable from the normal form and represents as little as 1–2% of the total lectin isolated. But it clearly has a higher affinity for its ligands, with a $K$ about 5 times the normal form. The structural basis for the high affinity form and its function remain unknown and will require further study.

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