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LOCALIZATION OF AN ENDOGENOUS LECTIN IN CHICKEN LIVER, INTESTINE, AND PANCREAS

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ABSTRACT

Extracts of adult chicken liver, pancreas, and intestine contain high levels of a lectin which appears to be identical to one previously purified from embryonic chick muscle. This lectin is virtually absent from adult muscle, but is highly concentrated in cells lining liver sinusoids, intestinal goblet cells, and the extracellular spaces surrounding pancreatic acini. These findings suggest that the lectin may play different roles in different tissues and at different times in the life of a chicken.

KEY WORDS lectin · liver sinusoids · goblet cells · secretory vesicles · extracellular space

Developing muscle (4, 11), brain, and liver (9) contain a divalent carbohydrate-binding protein. Because it agglutinates erythrocytes and its agglutination activity is inhibited by several saccharides, including lactose, it is referred to as a lectin. In both muscle and brain, lectin activity is very high at ~12 d of embryonic life and falls markedly in the ensuing weeks (9). This suggested a role for the lectin in differentiation of these tissues. However, this conclusion appeared inconsistent with the fact that lectin activity in liver increased markedly after hatching (9) and persisted at very high levels in the adult. Yet it seemed possible that some cellular component of liver was in a continuous process of differentiation and accounted for the high lectin levels in this organ. To evaluate this possibility, we sought to localize the lectin in adult liver by immunohistological techniques. In the present report we show localization of this lectin in chicken liver. We also show that it is abundant in extracts of two other visceral organs, pancreas and intestine, and is strikingly localized in these organs as well. The results challenge the generalization that the lectin always functions in differentiation, and suggest that the lectin plays different roles in different tissues.

MATERIALS AND METHODS

Assays of Lectin Activity

Agglutinins from various adult chicken tissues were extracted with 9 vol of phosphate-buffered saline (PBS), pH 7.2, containing 4 mM β-mercaptoethanol, 2 mM EDTA, and 0.3 M lactose as previously described (12). The extracts were centrifuged at 100,000 *g* for 1 h, and the supernate was dialyzed against the same buffer without lactose. Hemagglutination activity was determined using serial twofold dilutions of extract in microtiter V-plates (Cooke Engineering Co., Alexandria, Va.) with trypsinized, glutaraldehyde-fixed rabbit erythrocytes, as described previously (12). Each well contained 25 μl of erythrocyte suspension, 25 μl of extract, 25 μl of 1% bovine serum albumin in 0.15 M NaCl, and 25 μl of 0.15 M NaCl. To study the effects of lactose or antibodies on hemagglutination, 0.3 M lactose or 1:100 diluted gamma globulin solution in 0.15 M NaCl was added to each well in 25-μl aliquots, replacing the equivalent volume of saline. Hemagglutination titer is the highest dilution of extract causing agglutination. Lectin specific activity is defined as the reciprocal of the titer of a sample divided by milligrams of protein per milliliter of the sample. Protein was determined by the method of Bradford (2).

Lectin Purification and Antibody Preparation

Lectin from adult liver was purified by affinity chromatography either on lactoside-derivatized Sepharose, as described previously for embryonic muscle (12), or on asialofetuin-derivatized Sepharose by a modification of

the methods of DeWaard et al. (5) and Den and Malinzak (3). In the latter procedure, which proved more economical and simpler, desialylated fetuin was coupled to Sepharose-4B using cyanogen bromide, the crude lectin extract was applied to a column of this material, and the column was washed thoroughly with extraction buffer and then eluted with 0.3 M lactose. Both procedures yielded highly purified material of comparable purity which was used as antigen. Rabbits were immunized by intradermal injection of 30 μg of antigen in Freund's complete adjuvant followed 2 wk later by an intravenous injection of 10 μg of purified lectin. Serum was harvested 10 d later. Rabbits were later boosted by an intravenous injection of 100 μg of lectin and bled after 10 d. A gamma globulin fraction was prepared by precipitation in 33%-saturated ammonium sulfate and reconstituted to the same volume as the serum from which it was derived. Double immunodiffusion was conducted as described previously in agarose containing 0.3 M lactose (10). Antibody absorption was performed by reacting 200 μl of gamma globulin solution with 1.0 ml of polyacrylamide beads (Biogel P300; Bio-Rad Laboratories, Richmond, Calif.) to which 0.5 mg of pure lectin had been coupled by the method of Ternynck and Avrameas (14). After incubation for 1 h at room temperature, the beads were removed by centrifugation and the protein concentration was adjusted to match the unabsorbed sample.

Immunofluorescence Studies

Fixation and ultracryotomy were performed by procedures described previously (15). Briefly, liver, pancreas, or intestine was removed from adult white Leghorn chickens and placed in 0.1 M phosphate buffer, pH 7.4, containing 3% formaldehyde and 0.1% glutaraldehyde (0.2% for electron microscopy). Tissues were immediately minced into cubes of ~1 mm^3. After 1 h in fixative, tissues were stored in 0.3% formaldehyde until ready for use.

Tissue blocks were trimmed and reacted with 0.5 mg/ml $NaBH_4$ in 0.1 M phosphate buffer for 10 min to reduce glutaraldehyde autofluorescence (18). They were then infused with 1.0 or 1.2 M sucrose for 30 min and mounted on copper rods for freezing in liquid nitrogen. The rods were transferred to the precooled cryokit bowl of a Sorvall Porter-Blum MT-2B ultramicrotome (DuPont Instruments-Sorvall, DuPont Co., Newtown, Conn.) at −60°C. 0.5-μm sections were cut with a glass knife at temperatures between −50° and −60°C. The sections were picked up with a small wire loop containing a semifrozen drop of 2.3 M sucrose. The droplet was brought to room temperature and transferred to a glass slide. Slides containing sections were washed for 10 min in an excess volume of PBS and 10 mM glycine. The sections were incubated with a small droplet of immune gamma globulin (50 μg/ml), absorbed immune gamma globulin (50 μg/ml), or nonimmune gamma globulin (200 μg/ml) for 15 min at room temperature. The slides were washed again for 15 min in PBS containing 10 mM glycine. The sections were reacted for 15 min at room temperature with a 1:40 dilution of rhodamine-conjugated goat anti-rabbit IgG (N. L. Cappel Laboratories Inc., Cochranville, Pa.). After several washes in PBS and glycine, coverslips were mounted in 90% glycerol. The sections were examined with a Leitz Dialux epifluorescence microscope with a ×40 (NA 1.3) oil immersion lens.

Immunoelectron Microscopy

Ultracryotomy and immunoferritin labeling were performed as described previously (15–17). Briefly, ultrathin sections were cut at −70° to −80°C, picked up with a droplet of a sucrose-gelatin mixture, and placed on grids. Sections were incubated for 10 min with immune gamma globulin, washed thoroughly with PBS and 10 mM glycine, incubated for 10 min with ferritin-conjugated goat anti-rabbit IgG, and washed again with PBS and glycine. The sections were post-fixed with glutaraldehyde, positively stained with uranyl acetate, and then embedded in methylcellulose. Electron microscopy was performed with a Philips EM-300 electron microscope.

RESULTS

Assay of Lectin Activity in Adult Chicken Tissues

Extracts of chicken liver, intestine, and pancreas contained large amounts of lectin activity (Table I) which was markedly inhibited by lactose. The other tissues tested, including pectoral muscle, contained little or no lectin activity. The purified lectin from liver gave one band with an apparent mol wt of ~15,000 on polyacrylamide gel electrophoresis, as previously described for muscle (12) and chicken liver (10). In some preparations from liver purified with asialofetuin-Sepharose, a minor lower molecular weight band was also observed, as described before (10, 12). This band was not present in the preparation used for raising antibody for these studies.

The specificity of the antiserum raised against the purified lectin was examined by immunodiffusion. A single precipitin arc, which merged without spurring, was formed when purified liver lectin or crude extracts of liver, pancreas, or intestine were allowed to diffuse against gamma globulin prepared from this antiserum (Fig. 1). No precipitin band was formed with gamma globulin from nonimmune serum. The immune serum also produced a single precipitin arc, without spurring, when concurrently reacted against lectin from embryonic and adult liver and embryonic muscle, thus confirming previous evidence (10) that these

TABLE I

Lectin Activity in Adult Tissue Extracts and Inhibition by Lactose and Immune Gamma Globulin

Tissue	Specific agglutination activity		
	Control	Lactose	Immune gamma globulin
	titer^{-1}/mg protein/ml		
Liver	67	0.5	4
Pancreas	51	0	3
Intestine	20	0.6	5
Spleen	2	0.5	0.2
Heart	5	1.8	*
Brain	3	0	*
Pectoral muscle	0.2	0.2	*
Gizzard	0	0	*

Tissues were homogenized and lectin activity was assayed as described in Materials and Methods. Inhibition of lectin activity was tested by adding lactose (final concentration 75 mM) or an immune gamma globulin solution first reconstituted to the same volume as the initial serum and then added at a final concentration in the assay of 1:400. An equal amount of nonimmune gamma globulin had no effect on the hemagglutination activities.

* Not tested.

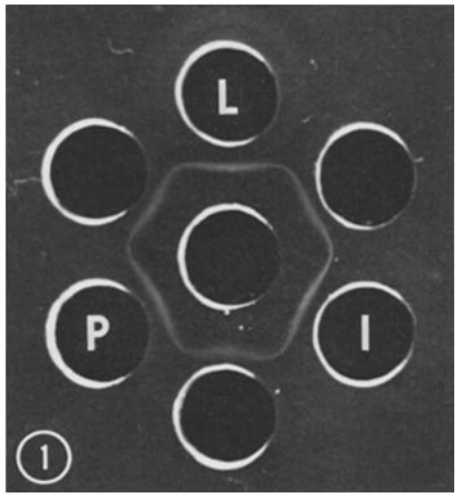


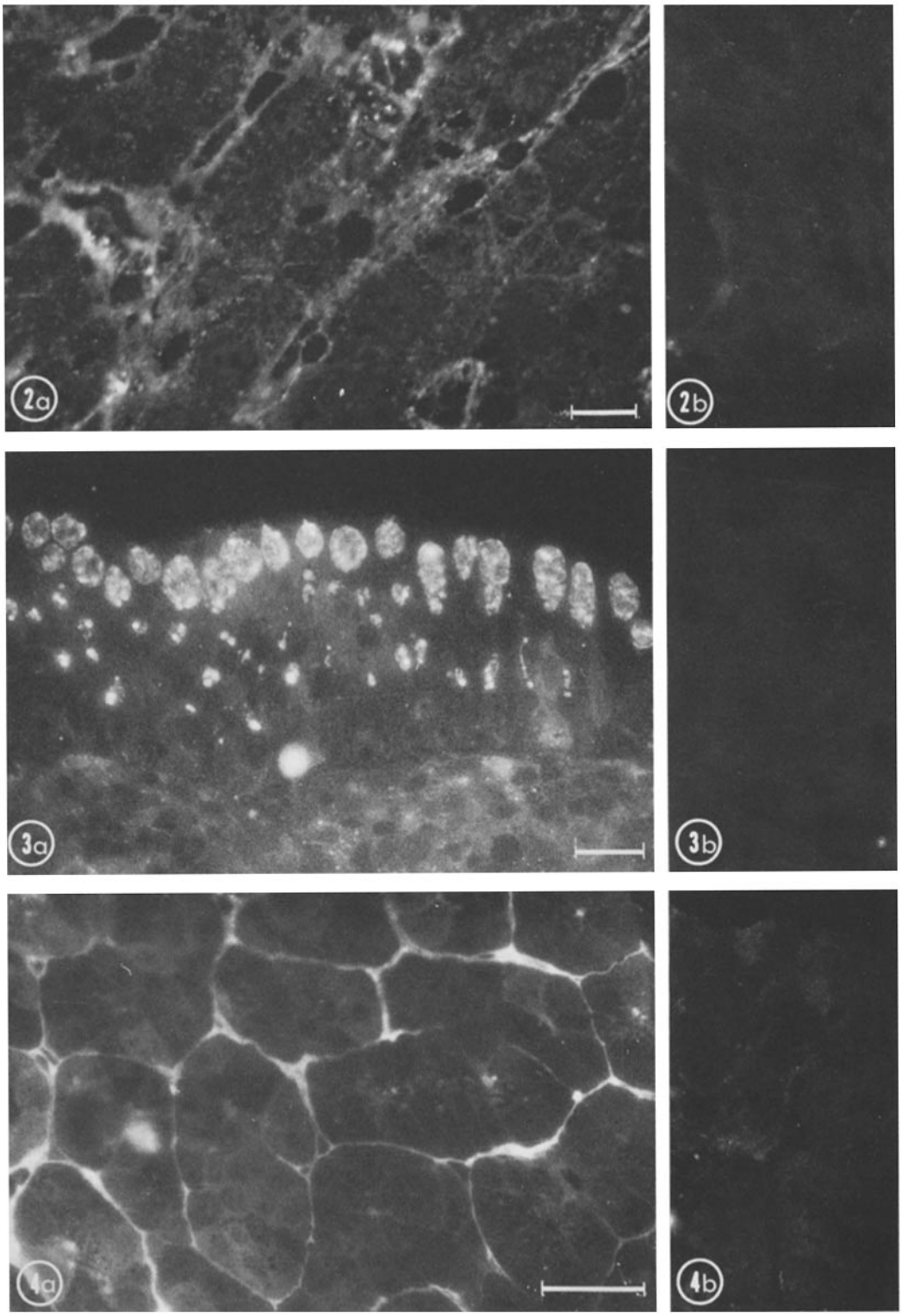
FIGURE 1 Interaction of immune gamma globulin with adult tissue extracts and purified liver lectin. The center well contained 10 μl of immune gamma globulin solution. The labeled wells contained 10 μl of concentrated extract from liver (*L*), pancreas (*P*), or intestine (*I*). The unlabeled wells contained 10 μl (5 μg) of purified liver lectin. Nonimmune gamma globulin showed no reaction with this antigen.

are all immunologically indistinguishable. Gamma globulin derived from the antiserum also was a potent inhibitor of the lectin activity from all the adult tissues that were tested, confirming their immunological similarity (Table I). Identical or higher concentrations of gamma globulin from nonimmune serum had no effect on lectin activity.

Lectin Localization

Sections from adult liver, pancreas, and intestine were studied by indirect immunofluorescence and immunoelectron microscopy. In all cases, sections reacted with nonimmune rabbit gamma globulin showed very faint and diffuse labeling. In addition, immune gamma globulin was absorbed by reaction with lectin coupled to polyacrylamide beads until hemagglutination inhibitory activity had been reduced by >90%. This absorbed gamma globulin also gave very weak, diffuse fluorescence (Figs. 2*b*, 3*b*, and 4*b*). In some adsorption experiments with intestine and pancreas, we sequentially adsorbed antiserum and correlated loss of hemagglutination inhibition activity with staining intensity. Staining was detectably diminished when 75% of hemagglutination inhibition activity was adsorbed and declined markedly with further adsorption. This experiment argues strongly against the possibility that the antibody that we are studying by immunofluorescence is directed against a trace antigenic contaminant in the purified lectin preparation. Were this so, it is very unlikely that this trace contaminant, too scarce to be detected by gel electrophoresis, would be an efficient adsorbant of the antibody that stains the tissue.

In the adult liver, strong fluorescence was concentrated in the lining of the hepatic sinusoids (Fig. 2*a*). Most hepatic parenchymal cells showed little or no specific fluorescence, but some hepatocytes were faintly positive (Fig. 2*a*). Determination of the exact localization of sinusoidal labeling was not possible by fluorescence microscopy. For this reason, we made and examined electron micrographs after immunoferritin staining. We found a high concentration of ferritin in the Kupffer cells. However, substantial endogenous ferritin is normally found in these cells and was observed in our controls. Therefore, it is difficult to be sure that the immunoferritin staining was significant. We believe that it is significant by exclusion because (*a*) studies with other tissues show that the antigen survives the fixation procedure that we used for electron microscopy (see below), and (*b*) the remaining components of the



FIGURES 2–4 Indirect immunofluorescent localization of endogenous lectin in liver (Fig. 2), intestine (Fig. 3), and pancreas (Fig. 4). Sections were stained with antilectin gamma globulin (2*a*, 3*a*, and 4*a*) or an identical amount of gamma globulin obtained by absorption of the antilectin gamma globulin with the purified antigen (2*b*, 3*b*, 4*b*). The sections were then stained with rhodamine-labeled goat anti-rabbit IgG. Calibration bars, 10 μm.

sinusoid lining, including the extracellular space of Disse and the endothelial cell bodies, appeared relatively free of label. We conclude that it is likely that the concentration of lectin in the lining of the hepatic sinusoids is associated to a large extent with Kupffer cells.

In the intestine, immunofluorescent staining again showed discrete localization, in this case within the secretory granules of the mucus-secreting goblet cells (Fig. 3 *a*). Little or no labeling was found along the luminal surface, within the absorptive epithelial cells, or within the basement membrane (Fig. 3 *a*). This discrete localization within the mucus-secretory granules was confirmed by immunoferritin labeling (not shown).

The pancreatic acini provided another type of specialized secretory cells for examination. However, the results were quite different. Bright fluorescence was confined to the extracellular space between pancreatic acini (Fig. 4 *a*). No significant fluorescence was seen within the exocrine cells, the zymogen granules, the centroacinar lumen, or between adjacent exocrine cells. Immunoferritin staining clearly demonstrated the dense labeling in the extracellular matrix between the plasma membranes of the acinar cells (Fig. 5 *a*) and confirmed the sparsity of intracellular staining (Fig. 5 *b*).

DISCUSSION

The major inference from this study is that this lectin does not appear to play the same role in different tissues. Previous studies suggested a role in the differentiation of muscle cells and neurons (6, 11), two cell types that undergo terminal differentiation and persist without replacement throughout the life of the animal. Present studies show localization to intestinal goblet cells and cells lining the liver sinusoids of the adult. These have no obvious functional relationship to immature neurons or muscle cells. Furthermore, in pancreas the distribution is completely different; here the lectin is localized extracellularly. The only relationship that this may have to its location in other tissues is that a fraction of the lectin in myoblasts and immature neurons was shown to have an extracellular location—i.e., was associated with the cell surface (6, 12).

Taking our observations at face value, it might be concluded that the function of the lectin is quite different in each of the tissues examined. In liver, it is in an ideal position to perform clearance functions like those described by Ashwell and Morell (1). They found a lectin in mammalian liver which they believe binds partially degraded plasma glycoproteins that have lost their terminal sialic acid residues, leading to their cellular uptake and degradation. For this function, the location of our lectin in the lining of the hepatic sinusoids seems more appropriate than the location of theirs in the hepatic parenchymal cells (7). It should be noted that a similar carbohydrate-binding protein, also believed to have a clearance function, has been found in chicken liver (8); but, this protein is clearly different from our lectin because it has a different subunit mol wt (26,000, compared to 15,000 in our case) and a different sugar specificity (*N*-acetyl-D-glucosamine compared to lactose in our case).

If we had to guess at the function of the lectin in goblet cells, we might suggest a function in mucus secretion. Perhaps the lectin serves to bind the glycoproteins in mucus, providing an overall structure for the secreted material. Such a function might prove to be related to the function of the lectin in the extracellular spaces of the pancreas. Here, too, it could play some role in binding extracellular glycoproteins, in this case to organize some form of connective tissue matrix.

We conclude from these studies that this lectin functions in different ways in different tissues. However, the possibility that there is some underlying common basis for all that we observed cannot be excluded. It also remains possible that the lectins found here and in past studies are not precisely identical. Previously, we showed that the lectins from embryonic muscle, brain, and liver, and from chick liver, appear identical by physicochemical and immunological criteria (10). In the present studies, lectins from pancreas, intestine, and liver give lines of identity in immunodiffusion. Immunological identity was also found when we compared the purified lectins from embryonic muscle and adult liver using an antibody raised against purified muscle lectin. The lectins are also very similar in that their hemagglutination activities are all inhibited by lactose and by antibodies directed to adult liver lectin or to embryonic muscle lectin. Nevertheless, the possibility that there is some structural and functional difference in these proteins has not been absolutely excluded.

It is notable that a lectin similar to this chicken lectin has been identified in bovine tissues (5) and in the electric organ of the eel (13). At the moment,

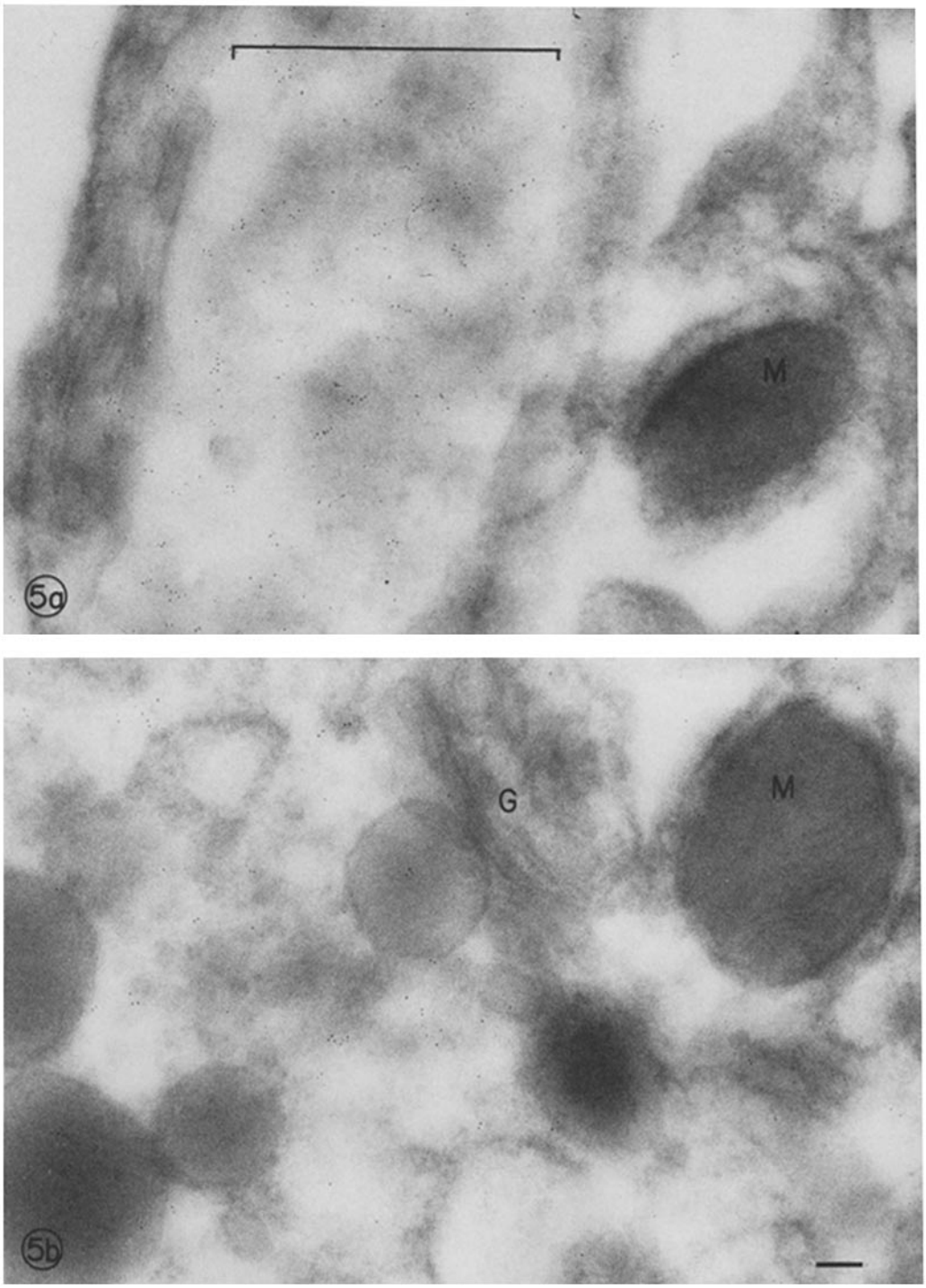


FIGURE 5 Electron micrographs of ultrathin frozen sections of adult chicken pancreas, stained with antilectin gamma globulin followed by ferritin-labeled goat anti-rabbit IgG. Extracellular space between acinar cells (bracket) shows heavy labeling (Fig. 5 *a*). Relatively sparse intracellular labeling (Fig. 5 *b*). *M*, mitochondrion; *G*, Golgi apparatus. Calibration bar, 0.1 μm.

the function of these proteins remains no more or less obscure than that of the long-known lectins from plants.

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