Location of lectin receptors on rat hepatocytes by transmission and scanning electron microscopy[1]

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Summary. Rexeptors for various lectins have been located on isolated hepatocytes by transmission and scanning electron microscopy, using gold markers of variable sizes. Quantitative data indicated that binding of some lectin markers depended upon their sizes.

Recently we reported a new technique for the location of cell surface glycoproteins by scanning electron microscopy (SEM) using, as markers, gold granules labelled with lectins[2,3]. This method has now been applied to the location by SEM of lectin receptors on isolated rat hepatocytes. Concanavalin A (ConA) and wheat germ agglutinin (WGA) receptors were also simultaneously located by transmission electron microscopy (TEM) using gold markers of different sizes. Quantitative data on these binding studies are also reported.

A few report have already appeared on the binding of lectins, such as ConA, WGA, and *Ricinus communis* lectins conjugated or not to fluorescein or ferritin using isolated hepatocytes[4,5] or isolated rat liver cell membranes[4,6–8]. However, this is the first report on the location and the simultaneous location of lectin receptors on hepatocytes by SEM and TEM, respectively.

Experimental. Preparation and labelling of gold granules. Gold granules of different sizes were obtained as described previously[2,3], either by reduction of gold chloride with white phosphorus (Au_5) or by reduction of 0.01% $HAuCl_4$ (100 ml) with 1% sodium citrate (4 ml, Au_{17}; 2 ml, Au_{26}; 1.5 ml, Au_{32}; 1 ml, Au_{50}; 0.75 ml, Au_{64}; 0.6 ml, Au_{75}). The code following each Au-marker refers to the average diameter of the granules in nm. The granules were labelled as reported before[2] with ConA (Miles Laboratories), soya bean agglutinin (SBA) and WGA (Pharmacia Fine Chemicals), peanut agglutinin (PNA) and *Ulex europaeus* lectin (Anti-H) (P. L. Biochemicals, Milwaukee, USA) and *Ricinus communis* lectin (RCA_I) (Sigma, type II). Prior to labelling of gold granules, WGA and RCA_I were cross-linked to BSA[2]. The labelled granules were collected by centrifugation[2] and suspended to an absorbance of 10 at λ_{max} in 0.05 M Tris 0.15 M NaCl, pH 7.2 containing 0.5 mg/ml sodium azide and Carbowax 20-M (Union Carbide Chemicals, Co. New York, USA) (buffer A). ConA gold markers were suspended in buffer A made 0.001 M in $MnCl_2$ and $CaCl_2$.

Marking of hepatocytes. Rat hepatocytes were isolated by the procedure of Seglen[9]. Their viability, estimated by the Trypan blue method[9], was 92%. The cells were immediately fixed for 15 h at 4°C in 0.05 M Tris 0.15 M NaCl containing 2.5% glutaraldehyde. They were extensively washed and suspended in buffer A.

Rat hepatocytes (0.1 ml, 2×10^5 cells) were incubated for 6 h at 22°C with an excess of lectin-labelled god markers (0.1–0.2 ml). Buffer A was added (1.2 ml) and the cells were sedimented at 700 g for 15 sec. The absorbance was measured in the supernatant and the number of bound Au_{32-50} granules was calculated as reported previously[2]. Controls were performed in the presence of: N-acetyl chitopentaose[10] (1.5 mg/ml) for WGA, methly-α-D-mannopyranoside (40 mg/ml) for ConA, N-acetyl-D-galactosamine (5 mg/ml) for SBA, D-galactose (5 mg/ml) for RCA_I, N-acetyl-chitopentaose (1.5 mg/ml) and/or L-fucose (10 mg/ml) for anti-H lectin. Spectrophotometric measurements or TEM and SEM observations indicated that non-specific absorption was practically nil. The marked cells were washed with buffer A and examined by TEM and SEM.

Results. In this study, glutaraldehyde fixation was used to prevent lectin receptor movement on the surface of the cell membrane. This fixative has been reported to have little or no effect on the binding of ConA and WGA to cell membranes[11,12]. The data of the table indicated that the binding of some lectin markers only occurred when the marker size was below a certain limit. While RCA_I-Au_{32} was bound by the hepatocyte

Binding of lectin-labelled gold markers to rat hepatocytes

Marker	Competitive lectin	Granules bound/cell	Lectin specificity
PNA-Au_{50}		No binding	D-galactose[14]
SBA-Au_{17}		Low binding	N-acetyl-D-galactosamine[15]
SBA-Au_{26-75}		No binding	
ConA-Au_{50}		16,300	α-D-glucose, α-D-mannose and related saccharide groups[16]
ConA-Au_{64}		No binding	
Anti-H-Au_{50}*		7400	L-fucose[17]
Anti-H-Au_{50}**		9000	N-acetyl-chitobiose[17]
WGA-Au_{32}		290,000	N-acetyl-D-glucosamine containing oligosaccharides[18,19]
WGA-Au_{32}	ConA**	260,000	
WGA-Au_{50}		44,000	
WGA-Au_{50}	SBA***	32,000	
RCA_I-Au_{32}		49,500	D-galactose[20]
RCA_I-Au_{32}	ConA***	121,000	
RCA_I-Au_{50}		Low binding	

* The cells were marked in the presence of N-acetyl-chitopentaose. ** The cells were marked in the presence of L-fucose. *** The cells were incubated with ConA or SBA (1 mg/ml), washed and marked with the gold markers.

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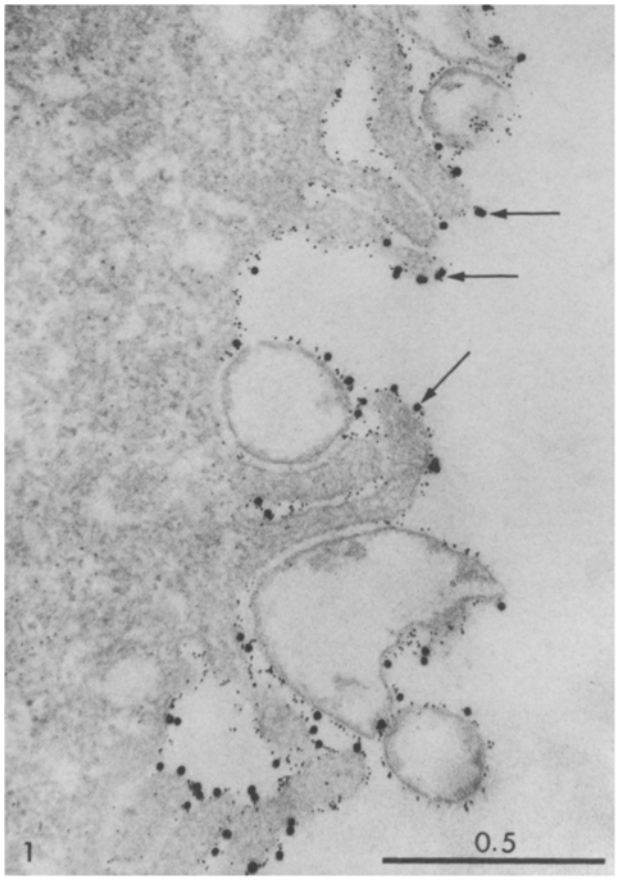

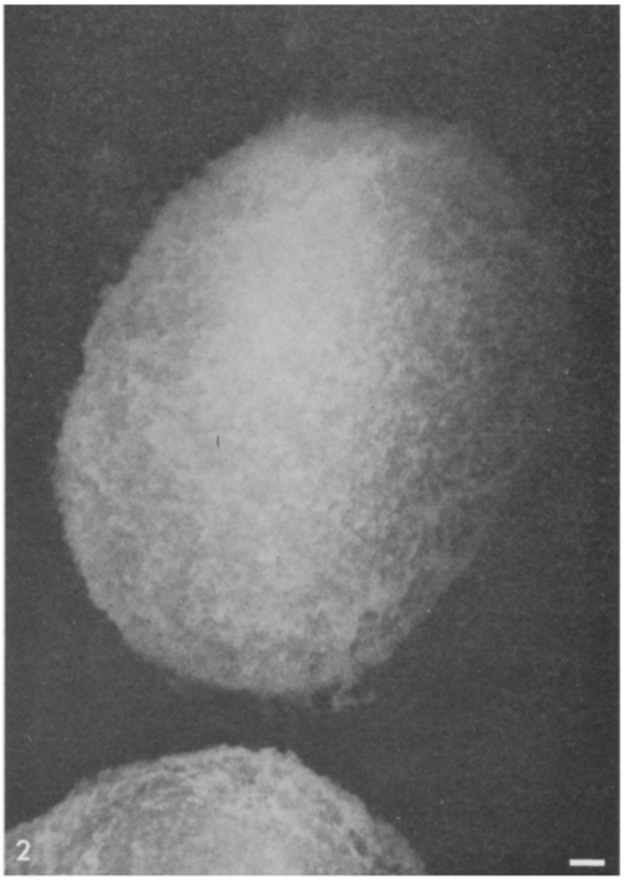

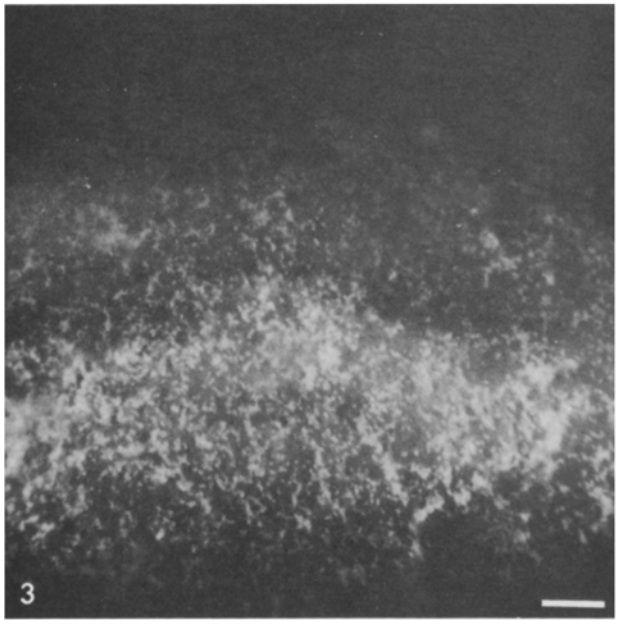

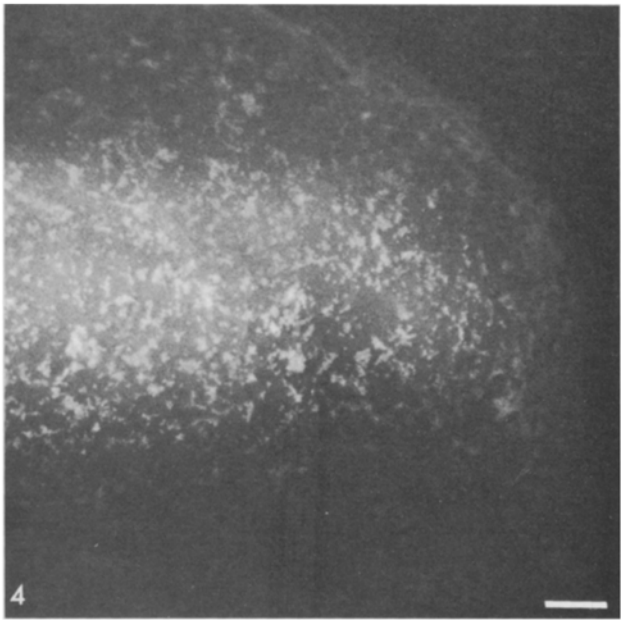

Marked hepatocytes were deposited on SEM aluminium stubs and dehydrated on the stubs by increasing the concentration of ethanol. They were then subjected to the critical point method with CO_2 and examined without metal coating in a Cambridge S 4-10 Stereoscan at an accelerating voltage of 30 kV

Fig. 1. Simultaneous location of WGA and ConA receptors *on rat hepatocyte membrane*. Cells were successively incubated with WGA-Au_{17} and ConA-Au_5. The marked cells were fixed for 10 min in 0.1 M phosphate, pH 7.0 containing 2% glutaraldehyde and embedded in 2% agar. They were fixed for 4 h at 4 °C in Os_2 and embedded according to Spurr[21]. Thin sections were post-stained with 0.5% uranyl acetate and lead citrate and examined in a *Philips 300* electron microscope. *The cell surface is continuously marked with* ConA-Au_5, while areas where cells are in contact with each other, are preferentially marked with WGA-Au_{17} (arrow). No significant difference was found when the cells were marked in the reverse order. ×60,000.

Fig. 2–4. Location of WGA and ConA receptors on isolated rat hepatocytes by SEM.

Fig. 2. Hepatocytes marked with WGA-Au_{75}. ×4500.

Fig. 3. Hepatocytes marked with WGA-Au_{50}. At this magnification, clusters of granules can be clearly distinguished. ×8500.

Fig. 4. Hepatocytes marked with Con-Au_{50}. The marker is also distributed in clusters but the density is smaler than that of WGA-Au_{50}. ×8500.

cell surface, RCA_I-Au_{50} reacted very poorly. Similar results were obtained with human erythrocytes using RCA_I and SBA gold markers. However, the large size ConA-Au_{50} was bound by hepatocytes (but not ConA-Au_{64}) contrary to erythrocytes where the ConA marker size had to be below 13 nm in size to obtain a binding reaction (unpublished observations). These differences are attributed to the more or less narrow spacing between the brushes of ektoprotein or glycoprotein of the cell surface[13] which does not allow the marker to reach its receptor.

Although WGA and anti-H lectins in the presence of L-fucose have similar specificities, the cells bound 5 times less anti-H-Au_{50} than WGA-Au_{50}. This could indicate that these lectins do not share a common receptor. SBA competed very little with WGA-Au_{50} which suggested that their receptors are not close to each other. Since RCA_I is a glycoprotein[20] and is precipitated by ConA, binding of RCA_I-Au_{32} increased when the cells were prelabelled with ConA. It has been claimed that ConA and WGA bind to separate sites on liver cell membranes[7]. This was confirmed by competitive experiment with WGA-Au_{32}, whose binding was inhibited only by 10% when the cells were saturated with ConA (table).

Rat hepatocytes were successively marked with WGA-Au_{17} and ConA-Au_5 (figure 1). As reported by Virtanen and Wartiovaara using ConA conjugated with ferritin[4], the cells had a microvillous surface continuously marked with ConA-Au_5. However, the cells were marked with WGA-Au_{17} preferentially where they were in contact with each other. No significant difference was found when the cells were labelled in the reverse order.

Hepatocytes marked with WGA-Au_{75}, WGA-Au_{50} and ConA-Au_{50} were examined by SEM (figures 2–4). The markers were distributed in clusters, the density of WGA-Au_{50} being greater than that of ConA-Au_{50} (figures 3 and 4) in agreement with the data of the table. Calculations on micrographs indicated that 35,000 WGA-Au_{50} granules were bound per cell. This figure, lower than that estimated by spectrophotometric measurements (44,000), is explained by a small loss of particules during the preparation of the specimen for SEM. A similar distribution of the markers-Au_{50} was found with the other lectins by SEM examination, the density of marking corresponding to the data of the table.

In conclusion, lectin-labelled gold markers having different sizes are useful for determining simultaneously the distribution of 2 lectin receptors but also for estimating the spacing between the glycoprotein brushes. While spacing must be below 32, 50 and 64 nm before the receptors can bind SBA-, RCA_I and ConA-Au markers, respectively, all sizes of WGA-Au markers bind to the cell surface. This indicates that WGA-receptors extend from the cell surface and are not masked by other glycoproteins.

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