transplanted mammary carcinoma bearing rats with intratumoral or i.v. injections utilizing either BGG or *Corynebacterium parvum*. They obtained the most significant results in animals treated with repeated intratumoral injections of *C. parvum*.

Likewise, Fisher et al.[14], found that the intratumoral inoculation of *C. parvum* more effectively inhibited grafted tumors than did its use by any other route.

Although the advantage of the intratumoral route versus other routes had already been observed, the reasons for this discrepancy were not yet clearly understood. The present histological findings clearly demonstrate that intratumoral injection provokes a much more widespread response of the RES than systemic treatment and thus provides a morphological basis for further studies on the action of immunostimulating agents.

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## Visualisation of lectin binding sites on the surface of human platelets using lectins adsorbed to gold granules[1]

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*Summary.* Washed human platelets have been incubated with the lectins WGA, ConA and $RCA_1$, adsorbed to different-sized gold particles. Plasma membrane receptors for each lectin were then located by scanning and transmission electron microscopy.

The presence of platelet functional defects in patients with congenital platelet disorders associated with specific abnormalities of different membrane glycoproteins[2,3] strongly suggests a role for the membrane glycoproteins in the physiologic mechanisms of platelet aggregation and adhesion. Lectins are proteins with different specificities for the sugar residues commonly found in glycoproteins and glycolipids. Certain lectins are able to activate platelets, stimulating both the release of the contents of intracellular storage organelles[4,5] and platelet aggregation[4,6]. Among such lectins are wheat germ agglutinin (WGA), concanavalin A (ConA) and *Ricinus communis* lectin ($RCA_1$). Horisberger and Rosset[7] have developed a procedure for the localization of cell surface glycoconjugates by scanning and transmission electron microscopy using lectins adsorbed to gold granules. In the present study we have used this procedure to investigate the distribution of WGA, ConA and $RCA_1$ receptors on the human platelet surface. This approach was thought potentially useful as the small size of the blood platelet (2–4 µm in diameter) makes conventional fluorescence microscope procedures using fluorescein-labelled lectins difficult to perform, and relatively little is known about the organisation of the individual components of the platelet glycocalyx.

*Material and methods.* 1. Preparation of fixed, washed platelet suspensions. Blood (9 vol.) was taken from adult human donors directly into the acid-citrate-dextrose anticoagulant of Caen et al.[8]. Prostaglandin $E_1$ ($PGE_1$) (100 nM) was added to each of the solutions used during the washed platelet isolation, which was performed using a modification of the procedure of Levy-Toledano et al.[9]. A stock solution of Metrizamide (Nyegaard, Oslo, Norway) in water at 300 mosm was diluted with 0.02 M sodium phosphate buffer pH 5.7, containing 0.139 M NaCl and 0.01 M glucose to give solutions of 25% and 10% w/v Metrizamide respectively. Step gradients were prepared by overlayering 1 ml 25% Metrizamide with 2 ml 10% Metrizamide in 10-ml plastic tubes. Each interface was slightly stirred using a looped steel wire. An aliquot of platelet-rich plasma ($1.5–2.0 \times 10^9$ platelets) was then added to each tube. Centrifugation was performed at $2000 \times g$ for 15 min at room temperature. The platelets, which sedimented to the 10%/25% Metrizamide interface, were resuspended in 0.02 M sodium phosphate buffer, pH 7.4, containing 0.139 M NaCl and 0.01 M glucose at $0.5 - 1.0 \times 10^9$ platelets $ml^{-1}$. Glutaraldehyde (4% w/v in pH 7.4 buffer) was added in an equal volume and the platelet suspension incubated at room temperature for 2 h. The fixed platelets were sedimented, resuspended in 0.01 M tris buffer, pH 7.4, containing 0.15 M NaCl, and washed twice.

2. Marking of platelets with lectin-labelled gold granules. Gold (Au) granules of different sizes (5, 12, 32 and 50 nm) were prepared as described by Horisberger and coworkers[7,10]. The code following each Au-marker in the text refers to the average diameter of the particles. The lectins used were WGA (Pharmacia Fine Chemicals), ConA (Miles laboratories) and $RCA_1$ (mol.wt 120,000) (Sigma, type II). WGA and $RCA_1$ were cross-linked to bovine serum albumin prior to the labelling of the granules[7]. The gold particles were labelled by incubation at 25 °C in the

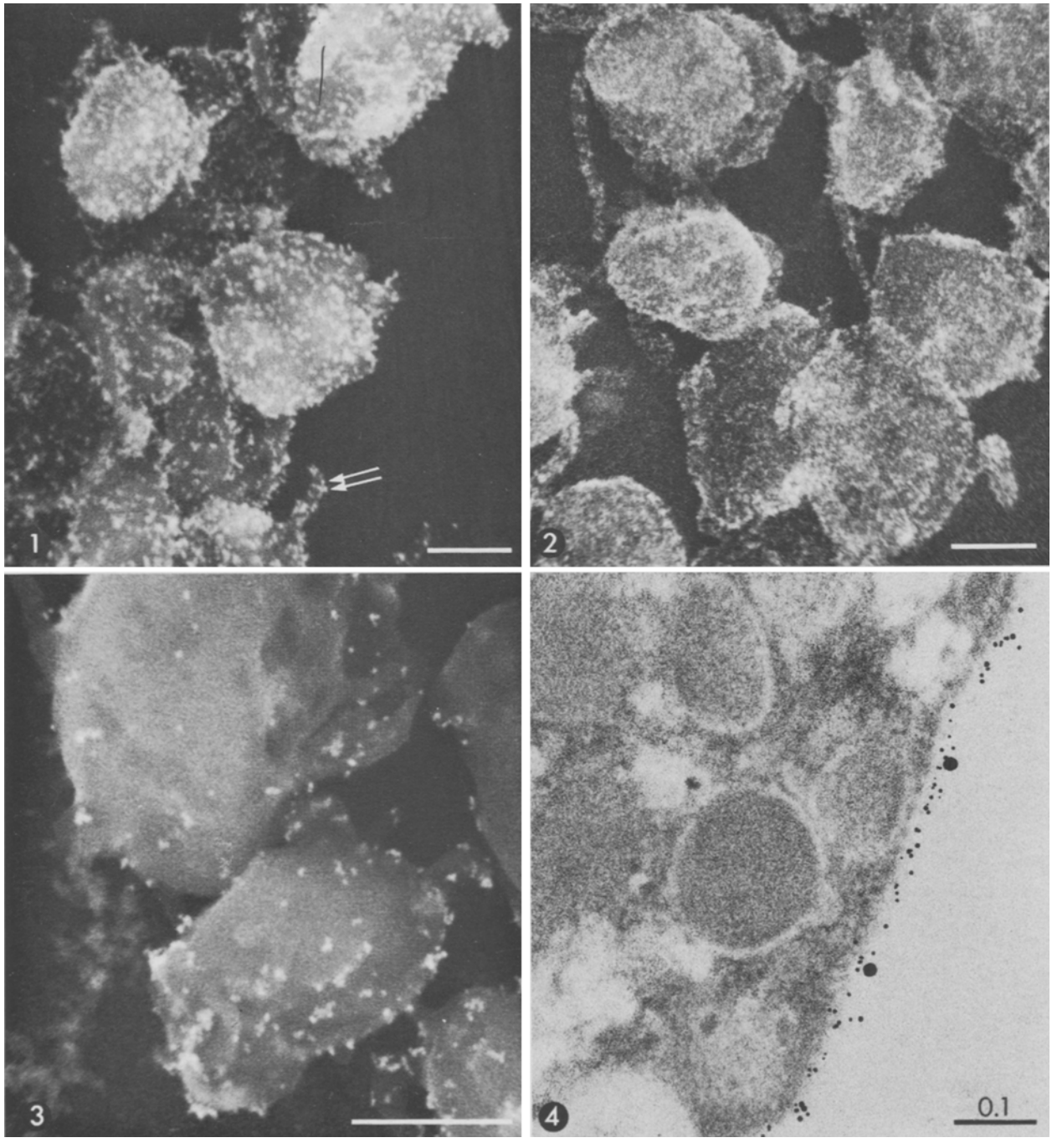


Fig. 1. Scanning electron microscopy of washed human platelets treated with WGA-$Au_{50}$ particles. The bound particles are visualised as white spots on the platelet surface. Arrows point to the presence of particles on platelet pseudopods. × 13000.
Fig. 2. Washed platelets treated with WGA-$Au_{32}$ particles. × 13000.
Fig. 3. Washed platelets treated with $RCA_1$-$Au_{32}$ particles. × 25000.
Fig. 4. Transmission electron microscopy of washed platelets treated successively with ConA-$Au_5$ and WGA-$Au_{12}$ particles. × 125000. Labelled platelets were examined after critical point drying with $CO_2$, in a Cambridge stereoscan (figures 1–3) as previously described[7,8]. Alternatively (figure 4) the platelets were embedded in 2% agar and incubated for 4 h at 4 °C in $OSO_4$ prior to being embedded according to Spurr[18]. Thin sections were then post-stained with 0.5% uranyl acetate and lead citrate and examined in a Philips 300 electron microscope. Platelets isolated from 4 donors have been examined, identical results were obtained for each sample.

presence of an added 10% excess of lectin according to published procedures[7,10]. The labelled granules were collected by centrifugation and resuspended at an optical density of 10 at 520–540 nm (the maximum absorption of the markers) in 0.05 M tris buffer, pH 7.4, containing 0.15 M NaCl, 0.5 mg/ml sodium azide and 0.5 mg/ml Carbowax 20-M (Union Carbide Chemicals, New York, USA). ConA-gold granules were suspended in buffer that also contained 1 mM $MnCl_2$ and 1 mM $CaCl_2$. Fixed washed platelets (0.1 ml, $2 \times 10^8$ platelets) were incubated for 6 h at 22 °C with an excess of lectin-labelled gold granules. Buffer (1 ml) was added and the platelets

sedimented by centrifugation. Controls were performed in the presence of appropriate sugar inhibitors: N-acetyl chitopentaose (10 mg/ml) for WGA, methyl α-D-mannopyranoside (50 mg/ml) or *Saccharomyces cerevisiae* mannan (5 mg/ml) for ConA and lactose (50 mg/ml) for $RCA_1$. The labelled platelets were washed twice and examined by scanning electron microscopy (SEM) or transmission electron microscopy (TEM) (see figure legends).

*Results and discussion.* In order to prevent the possible phagocytosis of the particles, and to immobilize the receptors prior to their contact with the lectins, the study was performed on washed platelets after glutaraldehyde fixation. SEM revealed that receptors for WGA were scattered in clusters over the entire platelet surface (figures 1 and 2). The platelet discoid shape was well maintained during the isolation procedure (the addition of $PGE_1$ aided this), however, it is interesting to note that particles (and therefore WGA receptors) were also observed on pseudopods protruding from the occasional activated platelet. Nonspecific adsorption of particles to the platelet surface was negligible. Little binding of WGA was observed in the presence of N-acetyl-chitopentaose (10 mg/ml), a potent inhibitor of WGA[11]. The number of WGA-Au particles bound increased as the particle size was reduced (compare figures 1 and 2), however, an appreciable number of particles were bound even with the largest particle size tested ($Au_{50}$). In contrast little marking was observed using $RCA_1$-labelled granules, no matter what size of Au particles were used (figure 3). The binding which did occur was inhibited by the presence of lactose (50 mg/ml). A low number of terminal D-galactose residues on the normal human platelet surface was suggested.

No binding was located by SEM using ConA-labelled particles of average size 50, 32 or 12 nm, despite the fact that normal human platelets contain a relatively large number of ConA binding sites ($5\text{-}6\times10^5$/platelet)[5]. In contrast, abundant labelling was observed by TEM using ConA-$Au_5$ particles (figure 4). The ConA receptors appeared evenly dispersed on the platelet surface although occasionally particles were observed to be in lines or clusters. The binding of ConA-$Au_5$ was only weakly inhibited by methyl-α-D-mannopyranoside (50 mg/ml) but was inhibited by yeast mannan (5 mg/ml) suggesting that the ConA was binding to oligosaccharides with a higher affinity for ConA than the monosaccharide. The platelets illustrated in figure 4 were also labelled with WGA-$Au_{12}$. This double-marking procedure clearly shows that there are different distributions of ConA and WGA receptors on the platelet surface. Furthermore, the lack of binding of $ConA_{12-50}$ particles suggested that the ConA binding sites were present in cryptic sites inaccessible to the larger sized particles. In contrast, a large proportion of the WGA receptors were accessible to WGA-$Au_{50}$ particles and were therefore presumably freely exposed at the platelet surface. Similar differences in the accessibility of lectin receptors to different-sized labelled gold particles have been observed for rat hepatocytes[12] and human erythrocytes[13].

A number of studies have begun to identify those platelet surface components with binding sites for individual lectins[14-17]. Affinity chromatography of detergent-solubilized platelet membranes has shown that ConA bound a 100,000 mol.wt glycoprotein which was shown to be a major component of the platelet surface[14]. In contrast WGA affinity columns bound 2 different glycoproteins of mol.wt 150,000 and 210,000 respectively[15]. Our results show that lectin-labelled gold granules may be a particularly useful tool with which to investigate the distribution of lectin receptors on the surface of normal and abnormal platelets. Furthermore, identification of the membrane glycoproteins and glycolipids which bind the individual lectins may lead to the mapping of these components of the platelet surface.

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## Phospholipase C-induced neural tube defects in the mouse embryo[1]

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*Summary.* Mouse embryo neurulae were exposed in vitro to phospholipase C to examine the role of carbohydrate-rich extracellular material (ECM) during neurulation. Exposure of embryos to this agent for 12 h resulted in failure of closure of the neural tube. Ultrastructural examination revealed an absence of ECM from regions of the neural tube which failed to close.

Cell sorting and tissue interactions involved in the complex process of morphogenesis probably depend on the recognition of specific surface characteristics[3]. Thus, changes in the nature of the extracellular material (ECM) present at the cell surface, or alterations in the morphology of the cell surface itself at critical periods of development, may well determine the nature and timing of cell-cell and tissue interactions.