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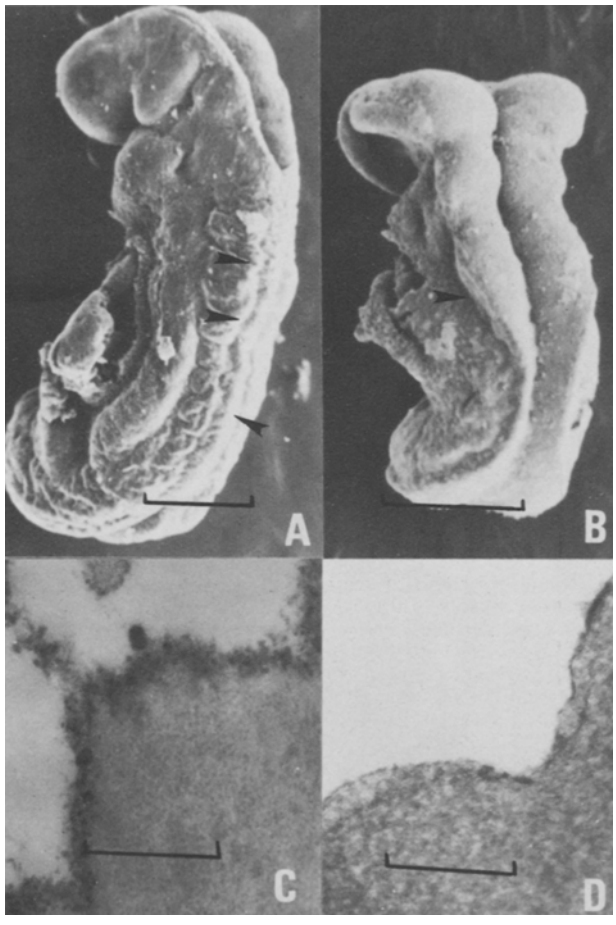
Summary. Mouse embryo neurulae were exposed in vitro to phospholipase C to examine the role of carbohydrate-rich extracellular material (ECM) during neurulation. Exposure of embryos to this agent for 12 h resulted in failure of closure of the neural tube. Ultrastructural examination revealed an absence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



A SEM photomicrograph of a 10–15 somite control embryo showing the extent of neural tube closure after 12 h in vitro. The neural tube is closed in all but the most cephalic and caudal regions. Note the presence of somites (arrowed), and the first branchial arch (1). Scale bar = 200 µm. *B* Postero-lateral view of an embryo isolated after exposure for 12 h (0–12-h period) to phospholipase C. The neural tube has failed to close throughout its entire length, and the neural folds are everted, especially in the upper 'spinal' region (arrowed). Scale bar = 200 µm. *C* TEM photomicrograph of lanthanum staining material lining the neural groove in the upper 'spinal' region of a control embryo. Scale bar = 0.5 µm. *D* Photomicrograph of a cross-section of the neuroepithelium near the cell surface in a similar region to that illustrated in figure C. This embryo was exposed to phospholipase C for the 1st 12 h in culture, and isolated and stained immediately following. Note that the lanthanum positive material is almost completely absent. Scale bar = 0.5 µm.

Carbohydrate-rich material is present at the cell surface during the morphogenesis of a number of tissue types. ECM has been demonstrated on apposing cell surfaces prior to palatal shelf fusion[4–7], preceeding fusion of the nasal processes[8] and on the apical surface of the neural folds prior to neural tube closure in the chick[9], amphibian[10] and mouse[11] embryos.

Despite the temporal correlation between the deposition of the ECM and epithelial fusion, the role of this material in the fusion process itself is unclear. In the present study ECM was removed from the apical surfaces of the neural folds with phospholipase C to examine the effect of this treatment on the course of neurulation in the mouse.

Methods. Random-bred CFLP (Anglia labs) female mice were mated to males of the same strain, and the morning of finding a vaginal plug designated the 1st day of pregnancy. Embryos were removed from the uterus early on the morning of the 9th day, and explanted into roller bottles[12] containing 1×10^{-3} mg/ml phospholipase C (Sigma Chemical Company, P7633) and 2 ml of heat inactivated rat serum prepared as described by Steele and New[13]. 0.1 ml of distilled water alone was added to control serum.

Embryos were exposed to this agent for a total of 12 h, either immediately after explantation (0–12-h culture period) or after 24 h (24–36-h culture period). The bottles were gassed with 5% CO_2 in air for 10 min at 12-h intervals throughout the 36-h culture period.

At the end of the culture period, embryos were washed in phosphate buffered saline, fixed at room temperature in 2% glutaraldehyde solution, containing 3% sucrose and 1% alcian blue 8GX (G.T. Gurr, London), for 2 h. Embryos were then rinsed in 0.1 M sodium cacodylate buffer, and post-fixed for 30 min in 5% osmium tetroxide containing 10% sucrose and 1% lanthanum nitrate (pH 7.2)[14]. Embryos were rinsed again in buffer, dehydrated through a graded alcohol series and embedded in resin[15]. Thin sections were cut and examined unstained in a Philips 300 electron microscope. Additional embryos were fixed for 2 h at room temperature in 2% glutaraldehyde (without alcian blue) and dehydrated through a graded alcohol series. These were then critical point dried, sputter coated and subsequently viewed and photographed in a Cambridge S600 scanning electron microscope.

Results. When embryos were exposed to phospholipase C for the first 12 h after explantation, the neural folds were observed to elevate normally, but failed to fuse in the midline in the majority (15/16) of these embryos (figure, C). When phospholipase was introduced 24 h after the beginning of the culture period, at a stage just prior to fusion of the neural folds in the cephalic region, approximation of the folds occurred, but in all 12 embryos treated in this way, the neural folds failed to fuse. In all other respects, overall embryonic development did not appear to be impaired compared to controls.

Ultrastructural examination of controls revealed a dense deposition of lanthanum-positive material on the surface of the neuroepithelium, particularly over the prospective fusion zone (figure, C). In phospholipase-treated embryos, no lanthanum staining material was observed (figure, D), although fragments of staining material were occasionally observed along the neural groove.

Discussion. The ultrastructural observations presented here confirm Sadler's[11] light microscopical study of ECM accumulation along the neural groove of the mouse embryo. It seems likely that this material plays an important role during neurulation, as its removal with phospholipase C inhibited neural tube closure. Similar observations have been made by Lee and his colleagues[9,16,17], who exposed chick neurulae to concanavalin A, and found it to inhibit neural tube closure. These authors suggested that concanavalin A was probably bound to the ECM rendering the cell surface too 'rigid' to participate in the changes required for neural tube closure.

Rice and Moran[18] using electron probe microanalysis of amphibian neurulae pretreated with lanthanum, demonstrated the presence of both phosphorus and bound lanthanum along the neural groove. They suggest that the lanthanum in the stain had replaced calcium in the ECM to render it electron dense, and further suggested that the ECM may act as a storehouse for bound calcium. The phosphorus observed in the ECM is probably a normal component of the cell membrane phospholipid matrix[19], and the phospholipase employed in the present study may have acted at the membrane lipid bilayer.

It appears that the ECM may be required in maintaining apposition of the neural folds prior to normal neural tube

closure. Further investigations into its biochemical nature may shed light on its role in cell-cell interaction during morphogenesis.

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Influences of the submaxillary gland of male mice on the immune response to sheep red blood cells

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Summary. Removal of the submaxillary gland (SMG) from male but not female mice caused a suppressed immune response to sheep red blood cells. Administration of a SMG saline extract from male mice to SMG-ectomized males restored the suppressed response to control levels. This suggests that the male mouse SMG contains a factor(s), possibly of an endocrine nature, capable of influencing cells involved in an immune response.

Although the submaxillary gland (SMG) is generally regarded as an exocrine organ, some work has alluded to a possible endocrine function[1,2]. As reported previously, removal of the SMG enhanced the delayed type hypersensitivity response in male mice, and administration of saline extracts from male mouse SMG resulted in a lowering of the response in SMG-ectomized males to the level seen in normal males[3]. This suggests that the SMG of male mice contains an endocrine factor (or factors) which can suppress one kind of cell-mediated immune response. In this study we report on the consequences of removal of mouse SMG on antibody formation as measured by plaque forming cell (PFC) response to sheep red blood cells (SRBC).

Materials and methods. ICR-strain male and female mice were fed an ordinary laboratory diet and water ad libitum. The SMG was removed at the age of 6 weeks under

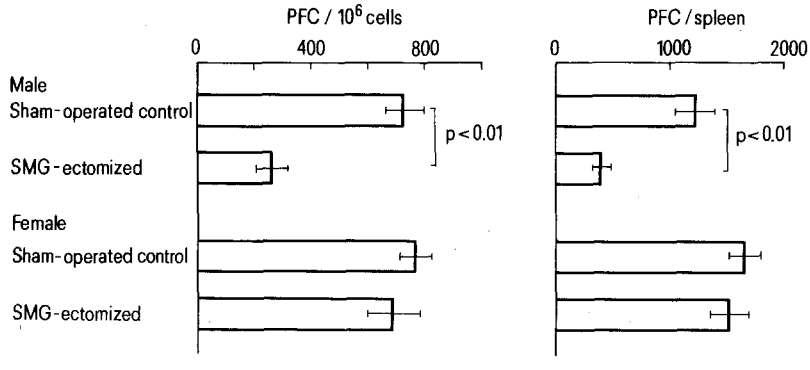

Fig. 1, Following the SMG-ectomy, male mice show a suppressed immune response to SRBC at 8 weeks after surgery. Each column shows the mean ± SE of 8 animals.

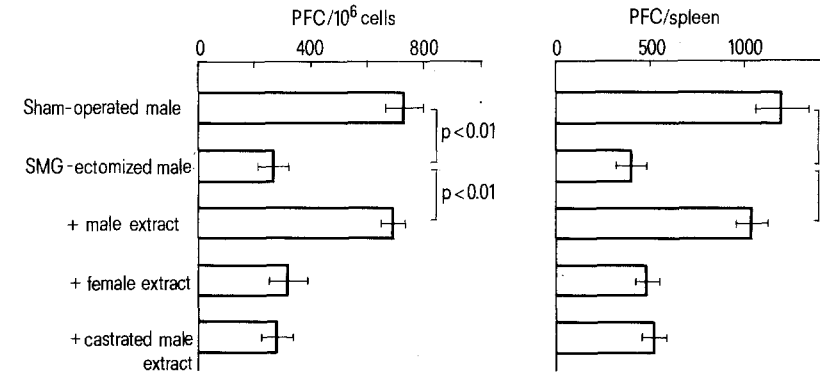

Fig. 2. Saline extract of the SMG of male mice normalize the suppressed PFC response in SMG-ectomized animals. But those of females and castrated males do not affect it. Each column shows the mean ± SE of 8 animals.