vent protein crosslinking can give information on the nature of the links intervening in the formation of the fertilization membrane. We shall examine here the effects of some inhibitors of collagen and elastin crosslinking on the differenciation of the fertilization membrane. In a precedent paper[3] we have examined the in vitro action of glycine ester, an inhibitor of fibrin polymerization. Glycine ethyl ester, at high concentration, prevented the hardening of the membrane in the eggs of *Strongylocentrotus purpuratus*. The membrane did not stabilize and was dissolved by a sulfhydryl reagent, the mercaptoethanol. We have studied other substances much more active than glycine ethyl ester, such as penicillamine, isoniazid, benzhydrazide, benzylhydrazine. The effects of semicarbazide were also studied.

All of these experiments were made with the eggs of the sea urchin *Paracentrotus lividus*. The unfertilized eggs were incubated for 5 min in the solution of chemicals in sea water. The fertilization took place in the solution. DL-penicillamine, isoniazid (isonicotinic acid hydrazide), benzylhydrazine dihydrochloride, benzhydrazide and semicarbazide hydrochloride were dissolved in sea water and, if necessary, the pH was adjusted to 8.2, corresponding to the pH of normal sea water.

The control eggs developed a fertilization membrane which was dissolved by mercaptoethanol (30%) only during the few min following its formation. After this point, the membrane was no longer dissolved by mercaptoethanol.

The eggs treated in the presence of penicillamine ($1\times10^{-2}M$, $2\times10^{-3}M$ and $1\times10^{-3}M$) produced a membrane as quickly and as high as in the control eggs. The membranes appeared thinner in the treated eggs observed with light microscope and in dark field. The membranes were dissolved by mercaptoethanol from their formation until several hours later. In a high percentage of eggs, the fertilization membranes dissolved after a few hours of contact with penicillamine.

With isoniazid ($1\times10^{-2}M$ and $2\times10^{-3}M$) fertilization membranes were produced regularly. They were thinner than normal membranes. They were dissolved by mercaptoethanol until several hours after their formation. With ($1\times10^{-3}M$) only 50% of the membrane can be dissolved by mercaptoethanol several hours after their formation. The membranes extended considerably without dissolving. In contrast to the eggs treated by penicillamine, no spontaneous dissolution of the membranes was observed with isoniazid.

Similar results were obtained with benzylhydrazine and benzhydrazide ($1\times10^{-2}M$, $2\times10^{-3}M$ and $1\times10^{-3}M$). Semicarbazide hydrochloride is active at higher concentrations. The formation of the fertilization membrane is elicited at the concentration (5×10^{-2}-M and $2\times10^{-2}M$). The thin membranes obtained were dissolved by mercaptoethanol until several hours after their formation.

The normal fertilization membrane is dissolved by mercaptoethanol during the short stage of assembly. This result suggested that the formation of S-S bonds intervene in this phase of the differenciation of the membrane. The resistance of the membrane to the dissolving action of mercaptoethanol during the hardening stage appears to be due to the formation of new bonds. These new bonds should be responsible of the hardening of the membrane. The formation of these new bonds can be prevented by penicillamine, isoniazid and other reagents studied here. In consequence the fertilization membrane preserved its sensibility to the dissolving action of mercaptoethanol.

The effects of these chemicals on collagen and elastin crosslinking have suggested the role of aldehydes derived from the oxidation of lysine side chain in the intermolecular crosslinking. Various mechanisms have been proposed: the aldol condensation of the δ-semi aldehydes derived from hydroxylysine and lysine[4,5], the formation of a Schiff base between an aldehyde derived from the oxidation of lysine side chain and the ε-NH_2 of a lysine residue[4,6,7]. Penicillamine[8], iproniazide[9], semicarbazide[10] have been shown to alter the stability of collagen fibres, probably by decreasing intermolecular bonding. The inhibition of crosslinking by penicillamine should involve a reversible interaction with the aldehydes present in tropocollagen[8]. Isoniazid, semicarbazide and other hydrazides can react with aldehydes. A number of these chemicals inhibited amino oxidases[11]. The reduttion of lysine oxidation in these conditions should decrease the intermolecular crosslinking.

On the basis of the effects of the chemicals studied on the stability of collagen and elastin on the one hand, and on the fertilization membrane on the other hand, it appears that some similar crosslinking may be involved in the stabilization of the fertilization membrane in the sea urchin egg.

Résumé. La stabilization de la membrane de fécondation de l'œuf de l'Oursin *Paracentrotus lividus* est inhibée par la pénicillamine, l'isoniazide, les hydrazines benzylique et benzoïque et la semicarbazide. Les effets de ces substances sont interprétés sur la base de leur action inhibitrice sur la formation de certaines liaisons transversales intermoléculaires. Ces liaisons pourraient impliquer, comme chez le collagène et l'élastine, des groupes aldéhydes associés entre eux par condensation de type aldol ou avec de groupes aminés avec formation de base de Schiff.

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Electron Microscopy of a Mucopolysaccharide Cell Coat in Sponges

Developmental biology has been greatly influenced by the results which have arisen from the study of cellular aggregation in Sponges[1]. Analysis of this phenomenon has led initially to the conclusion that the chemistry of the Sponge cell surface must be responsible for the explanation of this mechanism, and subsequently that mucopolysaccharides, normally present at the cell surface, appear to dominate this aspect of Sponge cell behaviour[1-3].

With cytochemical methods now available in electron microscopy, this biochemical evidence is given strong morphological support. Thus, in several Sponges, LUFT's ruthenium red method[4] reveals an electron dense layer, 200 Å thick, closely associated with the outer leaflet of the plasma membrane in all cell types (Figures 1 and 2). According to LUFT[5], this deposit corresponds specifically to an acid mucopolysaccharide-rich matrix. However, the poor penetration of the stain makes this reaction useful only for Sponges with a light mesenchyme (e.g. *Haliclona*), whereas staining by phosphotungstic acid at low pH on ultra-thin sections of material fixed in glutaraldehyde and embedded in glycol methacrylate[6,7] provides reliable evidence of an extraneous cell coat, even in Sponges with large amounts of mesoglea (e.g. *Chondrilla, Tethya, Hippospongia*). The stained outline of the pinocytotic vesicles is then particularly obvious (Figure 3). Although phosphotungstic acid in strongly acidic solutions is considered as an electron stain for complex carbohydrates[8], its histochemical specificity is still in question[9]. Therefore, the aldehyde groups, appearing in polysaccharide chains following periodic acid oxidation, have been detected by thiocarbohydrazide revealed by the addition of silver proteinate[10]. This reaction stains the cell coat heavily and furthermore makes visible other polysaccharide structures, such as glycogen (Figure 4).

The technical procedures employed do not permit us to see the unit particles that predominantly comprise the surface component involved in cell aggregation, at least using high-speed centrifugation as a method of isolation[1,11].

However, with ruthenium red staining, the macrophage cell coat of the mouse shows a mosaic appearance[12]. In the light of the above results, the outer material of the plasma membrane must contain acidic groups and complex carbohydrates. On the basis of chemical analysis in Sponges[2,13], the latter may be glycoproteins, and it would thus be reasonable to think that they carry acidic groups in the form of sialic acid residues. After sialidase treatment, staining by ruthenium red decreases but is not completely removed (unpublished observations). Hence, glycoproteins and acid mucopolysaccharides may both exist. We can therefore ascertain that Sponge cells possess a mucopoly-

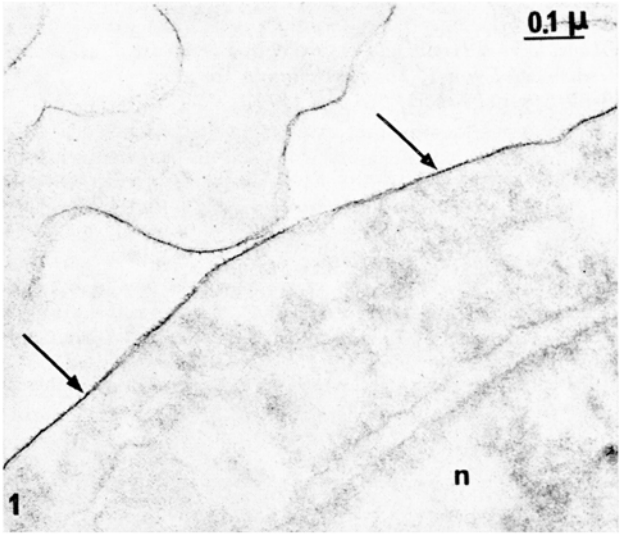

Fig. 1. *Haliclona elegans* Bow. Ruthenium red staining. Arrows indicate the cell coat. n, nucleus.

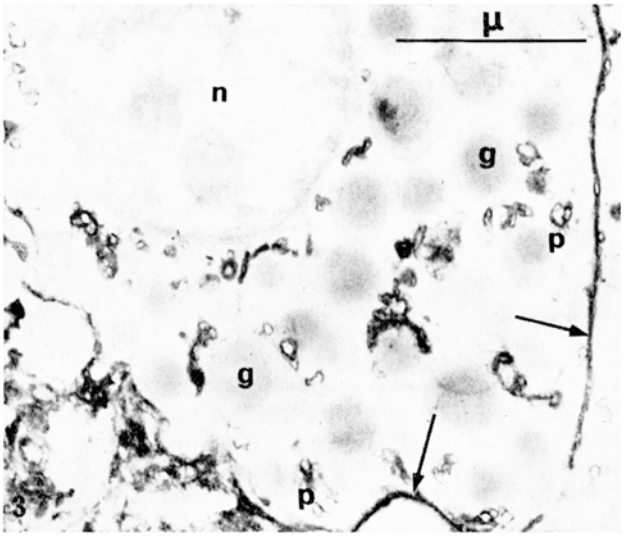

Fig. 3. *Chondrilla nucula* O.S. PTA staining, n, nucleus; g, cell granules; p, pinocytotic vesicles; arrows, cell coat.

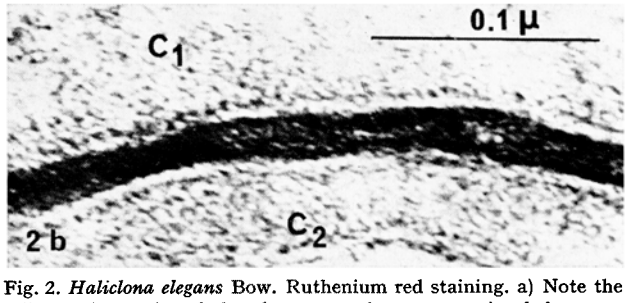

Fig. 2. *Haliclona elegans* Bow. Ruthenium red staining. a) Note the cell coat (arrows) and the plasma membrane appearing below as a lighter line. b) Note the dense ruthenium red deposit between 2 adjacent cells C1 and C2.

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saccharide moiety on their surface, as do the Sponge collagen fibers[14] and the spongin[15]. As pointed out by MOSCONA[1], the interactions between the cell surface and the extracellular components appear of particular interest in the organization of cells into developmental patterns.

Besides its supposed aggregation properties, this mucopolysaccharide layer could assume a large range of functions. It is well known that sialic acid or acid mucopolysaccharides carry a high density of negative charges. Such an anionic distribution in the immediate vicinity of cells can act as an ion exchange system and effect the rates of diffusion of charged substances[16], thus controlling the metabolic and electrical[17] pathways of the cells. Modifications of this environment can cause distortions of cell interactions as is the case with malignant and some non-malignant cells[18]. Therefore, one could expect that the properties of the cell coat would not be the same in motile cells such as archeocytes, in tightly packed cells such as choanocytes or in surface pinacocytes. It has also been suggested that the mucopolysaccharide cell coat plays an active part in membranar aspects of collagen fibrillogenesis[19,20].

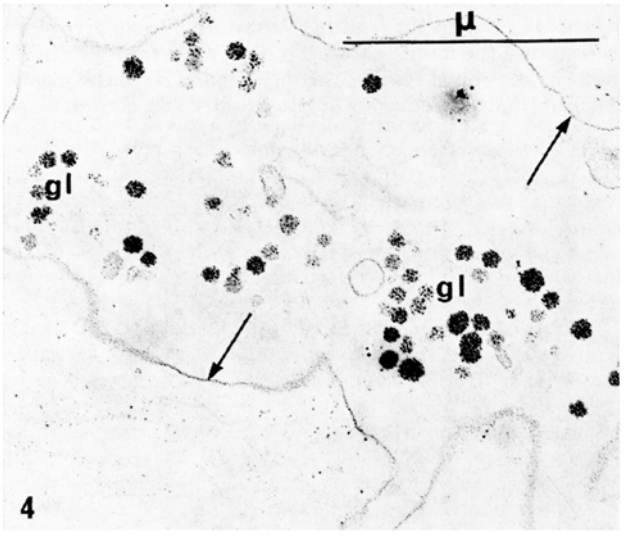

Fig. 4. *Hippospongia communis* Lmk. Silver proteinate staining. Arrows indicate the cell coat. gl, glycogen.

Résumé. A l'aide de diverses techniques (rouge de ruthénium, APT, acide périodique-TCH-protéinate d'argent), un revêtement de nature mucopolysaccharidique est mis en évidence à la périphérie des cellules d'Eponges. Le rôle et la signification de cette structure sont analysés.

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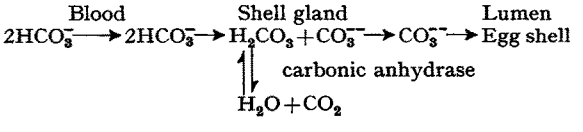
Uptake of Metabolic CO_2 by the Otoliths of the Chick Embryo

The otoliths of the chick embryo consist of calcium carbonate in the form of calcite[1], and of an organic matrix made up of a protein of non-collagenous nature and of a complex mucous substance, the structure of which contains various carboxylic and sulphuric radicals[2,3]. Earlier studies have shown that when chick embryos are treated immediately after the beginning of their morphogenesis, i.e. after 4 days of incubation, with carbonic anhydrase inhibitors (acetazolamide, dichlorophenamide, etoxizolamide, neptazane)[4,5], injected into the white of the egg, the formation of the otoliths is inhibited in a large proportion of the embryos treated.

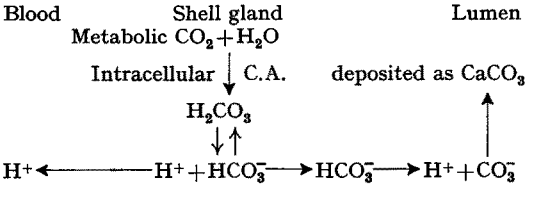
Carbonic anhydrase is demonstrable histochemically in the 5-day-old embryo and is confined to the epithelium of the endolymphatic sac. Following the administration of the above-mentioned inhibitors, the carbonic anhydrase is not detectable[6].

These findings indicate that carbonic anhydrase plays an important part in the morphogenesis of the otoconia of the chick embryo. The morphogenesis of the otoliths of the chick embryo appears similar to the formation of the shell of the chicken egg[7]. It has been demonstrated that carbonic anhydrase is present in the oviduct of hens, and that the development of the shell is inhibited when these animals are treated with specific inhibitors of carbonic anhydrase[8,9].

The most widely accepted hypotheses concerning the deposition of calcium carbonate in the egg shell are those of GUTOWSKA and MITCHELL[10]:



and of DIAMANTSTEIN[11] in which the shell glands derive the carbonate ions from their own metabolic production of CO_2



The latter theory is supported by the presence of carbonic anhydrase in the gland cells, with the pH values of the venous blood observed during the formation of the shell[12,13], and with the fluctuations of bicarbonate in the blood during this process. This theory postulates that