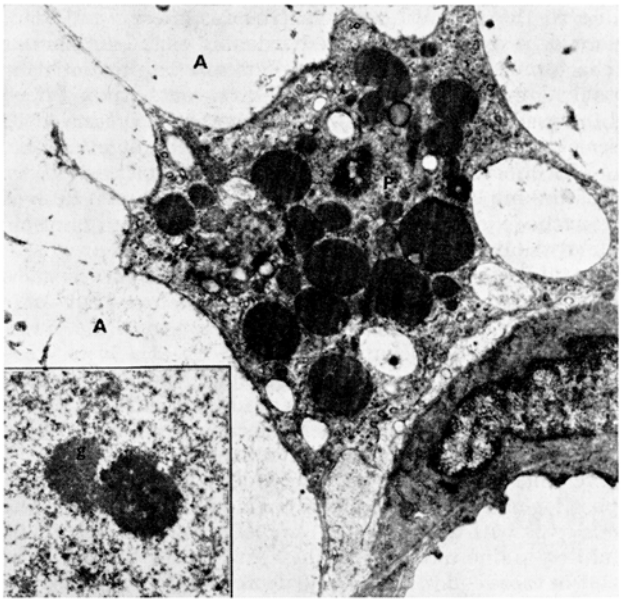

Fig. 1. Pericyte (P) surrounded by oedematous astrocytic processes (A). ×7 870. Inset: Separation of the fibrillar (f) and granular (g) components of the nucleus of a neuron. ×8 000.

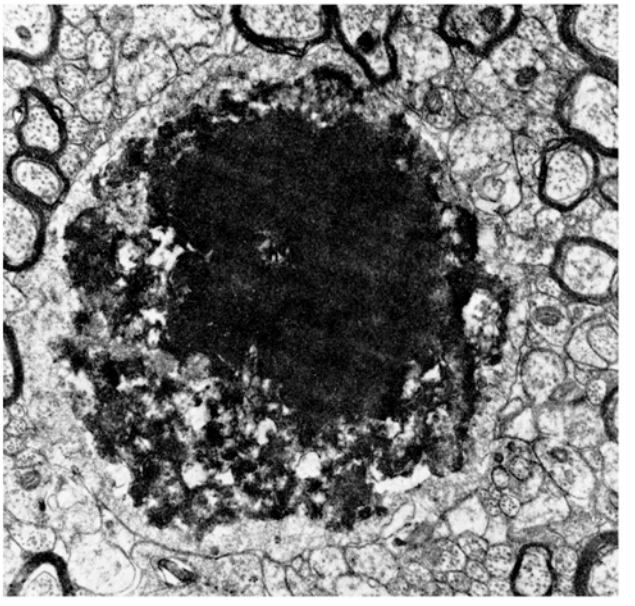

Fig. 2. Cell necrosis 12 h after the injection. The dead cell with pyknotic nucleus (n) is surrounded by unaffected cell processes. ×14 000.

have frequently been classified as oligodendrogliomas[13]. It cannot be excluded, however, that ENU may preferentially cause necrosis of proliferating cells known to persist postnatally in the subependymal layer[14].

All types of glial cells displayed an increased population of dense bodies after 24 h, including the formation of autophagic vacuoles. The degenerate cell organelles found in astrocytic processes were sometimes surrounded by concentric layers of thin astrocytic lamellae. Microglial cells had numerous dense bodies of variable size and complexity; these often occupied a large proportion of the cell, displaying granular and/or lamellar structure.

The neurons of the regions examined showed no consistent changes throughout the experiment with the exception of irregularity of the nuclear profiles and of nucleolar segregation (Figure 1, Inset). Such separation of the nucleolar fibrillar and granular components is regularly seen in carcinogen-treated rat liver[15].

To summarize, ENU causes extensive degenerative changes in all types of glial cells, while neurons react less obviously to the toxic stimulus.

Résumé. Une forte dose de composé carcinogène N-éthyle-N-nitrosourée (ENU) a été injectée à des rats Wistar mâles adultes: des changements ultrastructuraux des cerveaux furent observés. La dégénérescence et la nécrose des oligodendrocytes, l'oedème des astrocytes et l'accroissement du nombre des corps denses dans les cellules gliales ont été les réactions du cerveau à ce stimulus chimique.

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Effects of Various Inhibitors of Protein Cross-linking on the Formation of Fertilization Membrane in Sea Urchin Egg

At fertilization, sea urchin eggs produced a membrane. A few min after its formation, the fertilization membrane exhibited an increase of its mechanical resistance[1] and of its stability towards various chemicals. Runnström[2] distinguished 2 stages in the differenciation of the fertilization membrane: the assembly stage and the solidification or hardening stage. It is suggested that the 2 stages correspond to the formation of various types of binding necessary for the cohesion of the membrane. The increase of the stability of the fertilization membrane should involve new types of links. Two ways of studying this problem appear experimentally possible: in the first place, the study of effects of various chemical reagents on the membrane at different stages of its formation. The knowledge of the properties of reagents can help to investigate the nature of the links involved in the structure of the membrane. In the second place the use of agents able to pre-

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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].