digitating pattern straight actin filaments from adjacent I-bands appear to be discontinuous and to alternate in the region of the Z-disc (Figure 2d). The fuzz image is the most common and the Z-disc does not exhibit any clearly definable pattern and appears diffuse (Figure 2e). Tilting longitudinally oriented specimens (rotation of the specimen about the longitudinal axis of myofibrils) through the $\pm 60°$ obtainable with the electron microscope equipped with a goniometer stage revealed that considerable changes occur in the appearance of the Z-disc. With a starting image of a church steeple pattern (Figure 3a) a tilt of 15° produced little change apart from a less clear image (Figure 3b). With a tilt of 30° and 45° the disc was converted into a fuzz image (Figure 3c and d) which in turn emerged as a straight line at the maximum tilt of 60° (Figure 3e). Tilting the section through the 60° in the manner described above also produced a decrease in the width of the myofibrils, approximation of actin filaments, and an enhanced contrast of the specimen (Figure 3). Tilting the same Z-disc at right angles to the previous series showed that the initial image of a church steeple pattern gradually changed to the interdigitating pattern without any obvious intermediate fuzz stage (Figure 4). Tilting transversely cut sections of the myofibrils at the level of the Z-disc failed to show any significant alteration of the image no matter in what direction they were tilted.

By projecting the perspex model (Figure 1) representing 2, 3 or 4 rows of actin filaments we have found a high degree of correlation between the patterns produced by altering the orientation of the model and by tilting the specimens. However, the degree of tilting required to produce changes in pattern did not correspond precisely in the 2 systems.

The above results provide evidence for the first time that some of the varying images of the Z-disc that are seen with the electron microscope are accounted for by the orientation of the specimens. They also suggest that the three dimensional structure of the Z-disc is essentially similar to that proposed by KNAPPEIS and CARLSEN[1] but probably with a configuration less rigidly ordered than has been previously indicated. The results clearly show that the fuzzy appearance of the Z-disc, often interpreted as an indication of the presence of a diffuse matrix permeating the Z-disc filaments[10, 2] may be due to the orientation of the specimen which results in the non-alignment of the filaments. Our findings together with those of previous authors, however, offer no explanation for the unusually broad Z-line found in the cat myocardium[8] or in the cases of nemaline myopathy[9].

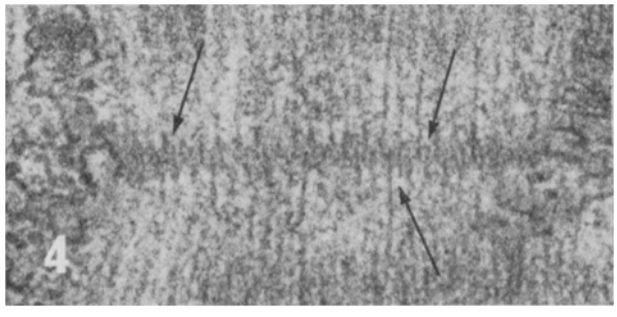

Fig. 4. The same Z-disc as in Figure 3, this time tilted (rotated) about its long axis, which is at right angles to the previous figure. Interdigitating filaments are seen particularly where arrowed. × 90.000.

Résumé. A l'aide du microscope électronique muni d'un goniomètre, la structure tri-dimensionnelle du disque Z de la fibre musculaire striée a été analysée. Les résultats obtenus sont comparés avec un modèle construit en perspex et avec les images conventionnelles du disque Z.

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Changes in the Differentiation of the Sea Urchin Larva by Action of a Detergent upon the Unsegmented Egg

The normal development of sea urchin eggs depends on the balance between two different trends governing respectively the differentiation of ectodermal and entomesodermal parts of the larvae. This balance can be displaced by treating the eggs with certain chemicals. In the animalization, for example, the differentiation of ectoderm is favoured at the expense of the entomesoderm. The mode of action of animalizing agents is not known. An action on the cell surface has been suggested[1–3].

Detergents represent an interesting class of chemicals able to react with the cell membrane, disturbing its structure and removing proteins fixed on the surface of membrane. Earlier observations with sea urchin eggs have shown interesting effects of detergents on the differentiation of larvae. An anionic detergent, lauryl sulfate, was shown to be able to produce radialization of larvae[4]. Radialization corresponds to a weak animalization. According to RUNNSTRÖM[5], this detergent increased the frequency of animalization in eggs pretreated before fertilization with thiocyanate. RUNNSTRÖM made the interesting observation that the detergent was most effective if the eggs were exposed to its action very early after fertilization. Chemicals with a high surface activity like salts of bile acids were shown to be effective animalizing agents[6].

In this paper we shall try to elucidate the mode of action of a detergent and to obtain information about the

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processes controlling the balance between ectodermal and entomesodermal differentiation.

Material and methods. The experiments were performed with the sea urchin, *Paracentrotus lividus*. The unfertilized eggs were washed and their jelly coat was removed by treating them with acid sea water, according to the technique of VASSEUR[7]. The removing of the jelly coat avoids the fixation of detergent by this material and makes its elimination by washing easier. The fertilized eggs, after treatment with lauryl sulfate at 20 °C, were washed several times and transferred into culture dishes. Sulfadiazine ($1 \times 10^{-3} M$) was added to avoid bacterial development. The eggs were cultivated at 22 °C. A solution of sodium lauryl sulfate (0.02%) in sea water was prepared for each experiment and used to make the subsequent solutions.

Results. In a first series of experiments, the eggs were treated 30 sec after fertilization and kept 3 min in the 0.006% solution of lauryl sulfate. The eggs were examined immediately after their transfer into normal sea water and appeared slightly deformed. The pigment was irregularly displaced forming one or two patches. This is in contrast to the distribution of the pigment in normal eggs, where it is disposed in a band occupying the vegetative half, except for an unpigmented cap at the vegetal pole. The contours of the hyaline layer were irregular and its general aspect appeared granular. In the cultures of these eggs we found 2 types of animalized larvae. Those of the first type were hyperciliated blastula belonging to the type $^1/_2$ and $^3/_4$ according to the classification of HÖRSTADIUS[8]. These animalized larvae were devoid of archenteron. Primary mesenchyme cells were rare. No pigment cells or spicules were found. The larvae of the second type were flattened to oval or triangular forms. The apical tuft was normal. A stomodaeum was differentiated. No archenteron, mesenchyme cells, pigment cells or spicules were found. Also the general aspect of these larvae was very clear; they looked like those obtained by HÖRSTADIUS by removing micromeres and blastomeres Veg. 2, from young morula[9]. In addition, other types such as prism and small plutei, from 10 to 20%, according to the experiments, were found in the cultures.

In a second series of experiments, the eggs were treated with 0.006% lauryl sulfate for 3 min, the treatment beginning 15 min after the fertilization. These cultures gave small plutei and a few flattened larvae with stomodaeum like the larvae found in the preceding cultures.

Some experiments were performed by treating unfertilized eggs by 0.006% lauryl sulfate for 3 min, then fertilized; these eggs did not develop. A number of multinucleated eggs were observed.

The eggs fertilized into normal sea water and transfered 15 min later into 0.006% lauryl sulfate were stopped at the beginning of the segmentation. We found in these cultures unsegmented eggs mono- and multinucleated together with stages of 2, 4 and 8 blastomeres. The pigment was displaced and the hyaline layer appeared irregular and granular.

The results presented here show that lauryl sulfate exerts a strong animalizing action when the eggs are treated for a short time very early after the fertilization. The animalization was not observed when the treatment was begun 15 min after fertilization.

In these experiments, the low concentration used and the short time of treatment make possible a limited alteration of the membrane, compatible with the further development of the larvae. The possibility that lauryl sulfate solubilized a protein material located at the surface of the fertilized eggs should be considered, together with the role of this material in the differentiation of the entomesoderm.

Résumé. Le traitement de l'œuf d'oursin, quelques secondes après la fertilisation, par un détergent, le lauryl sulfate de sodium, provoque l'hyperdéveloppement des structures ectodermiques chez les larves (animalisation). Cet effet n'est plus observé lorsqu'on intervient plusieurs minutes après la fécondation. On suggère que le lauryl sulfate inactive ou solubilise un matériel, peut être de nature protéique, localisé à l'intérieur ou à la surface de la membrane plasmatique de l'œuf fécondé. Ce matériel serait responsable de la différenciation de l'entomésoderme des larves.

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The Effect of Inactivation of Sera on the Peroxydase Activity of Haptoglobin-Hemoglobin Complex

Since POLONOVSKI and JAYLE[1] discovered the haptoglobin in 1938 (Hp), we have acquired a considerable body of knowledge about the structure, physico-chemical properties, function and genetics of this glycoprotein; but we do not know much of its behaviour when exposed to heat. An observation made by chance on inactivated sera lead to the experiments described below.

We examined the peroxydase activity of the haptoglobin-hemoglobin complexes formed in the native and inactivated samples of the same sera. We tested 44 sera of Hp 1–1, 38 sera of Hp 2–1, 34 sera of Hp 2–2 phenotypes. The inactivation was carried out at 56 °C for 30 min. The complexes were formed by means of human cyanmethemoglobin: to 0.5 ml serum added 0.5 ml of 50 mg/100 ml cyanmethemoglobin solution. The peroxydase activity was measured on a guiacol substrate according to the procedure described by OWEN et al.[2] The peroxydase activity of native and inactivated samples of sera was compared. The value of peroxydase activity was expressed in extinction of the Linson-3-photometer, and the differences in extinction were given in per cent.

Sera of Hp 1–1 phenotype: in the case of 9 sera the peroxydase activity in the inactivated samples of sera was higher than in the native samples. The difference varied between + 0.9 and + 5.5%. No difference was to be seen in the case of 3 sera. We found a loss of peroxydase activity ranging from −1.1 to −39.5% in 32 of inactivated samples of sera. Sera of Hp 2–1 phenotype: We found a loss of peroxydase activity ranging from −31.4 to

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