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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−65.9% in the inactivated samples of sera. Sera of Hp 2–2 phenotype: We found a loss of peroxydase activity ranging from −49.1 to −73.5 % in the inactivated samples of sera.

The difference of the peroxydase activity in native and inactivated sera seems to be dependent upon the Hp phenotype of the sera. Therefore it is not probable that a third heat-sensitive-substance, e.g. the complement, is involved in the formation of the complex, or that a yet unknown heat-sensitive factor is required for exerting the peroxydase activity of the complex. The assumption seems obvious that it is the Hp molecule that is altered by the action of heat. At present we have no satisfactory explanation for this phenomenon.

It has to be considered, however, that the haptoglobins of Hp 1–1 phenotype may differ in their chemical composition. CONNELL et al.[3] proved that the Hp 1–1 phenotype is divided into 3 subtypes which are controlled by the 2 genes: Hp^{1F} and Hp^{1S}. The α-chains of the Hp molecules, determined by these 2 genes, differ from each other in only one amino acid. It may be supposed that the decrease in peroxydase activity appearing as a consequence of inactivation can be attributed to this minor chemical dissimilarity. SMITHIES et al.[4] pointed out, and later NAUCE and SMITHIES[5] proved conclusively that the Hp^2 gene is a result of a partial gene duplication, and the consequence of the non-homologous crossing over of Hp^{1F} and Hp^{1S}. This would mean that complete heat-resistant α-chains could only occur purely in the subtype of some Hp 1–1 homozygote – Hp 1F–1F or Hp 1S–1S. Depending on the subtype of Hp^1 gene, certain sera of Hp 2–1 phenotype might partly contain complete heat-resistant α-chains. Sera of Hp 2–2 phenotype could no longer contain complete, heat-resistant α-chains. In accordance with this, we found more distinct loss of peroxydase activity in sera of Hp 2–1 phenotypes, and an even more distinct loss in sera of Hp 2–2 phenotypes. This is only a hypothesis, and further studies on subtypes of haptoglobins are required to solve the problem.

Zusammenfassung. Nachweis, dass in nativen und inaktivierten identischen Serumproben, gemessen an der Peroxydaseaktivität des Haptoglobin-Hämoglobin-Komplexes, die Hitzeresistenz der Haptoglobin-Typen in der Reihenfolge Hp 1–1, Hp 2–1, Hp 2–2 abnimmt.

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Antibodies to Venous Tissue in Phlebothrombosis

The increasing number of surgical interventions by the use of homologous venous grafts in the arterial system suggested the significance of studies on the antigenicity of venous tissue[1]. In addition, in a certain number of cases, phlebothrombosis is a chronic disease of a cyclic course, characterized by the alternation of active and inactive phases. This course exhibits similarity with allergic events. Based on the assumption that allergic and autoimmune processes may play a role in the pathology of phlebothrombosis, we attempted to demonstrate antibodies against venous tissue in the sera of patients with phlebothrombosis.

Material and methods. Patients. Sera of 35 patients with phlebothrombosis of the limb and of 22 control subjects were investigated. The patients showed different clinical features predisposing to the development of the phlebothrombosis. The venous obstruction was not a consequence of surgical intervention. The severity of the classical symptoms depended on the size and the length of the veins involved.

Antigen. An extract was prepared from human vena cava tissue with 1 *M* calcium chloride-*Tris*-citrate buffer, pH 7.5 (CTC-extract), according to ROBERT et al.[2–4]. The CTC-extract contained water-soluble proteins (partly of plasmatic origin), and proteoglycans.

Immunologic tests. a) Linear immunodiffusion (Oudin)-method. Agar (1% w/v) was dissolved in 0.15 *M* phosphate buffer containing 0.85 saline (pH 7.0). The antigen concentration was 2 mg protein/ml. b) Passive haemagglutination test. The CTC-extract was coated with tannine-treated human red cells of blood group O.

Results and discussion. Using the Oudin-test, precipitation was obtained in the case of 1:16–1:32 serum dilution. Passive haemagglutination test was accepted as positive when titres showed 1:32–1:64 values. As seen in the Table, the presence of auto-antibodies against the CTC-extract of human veins could be established in 70% of the cases so far investigated. Both tests gave negative results in the control subjects.

The question whether the presence of vena-autoantibodies in phlebothrombosis is the cause or the consequence of the pathological process cannot be answered as yet, similarly to several other autoimmune diseases. Probably our further investigations on the specificity of these autoantibodies will give more information on this subject.

Immunological tests with vein CTC-extract

Methods / Patients	Lincar immunodiff. (Oudin)			Passive haemagglutination			Total		
	n	pos	neg	*n*	pos	neg	*n*	pos	neg
Phlebothrombosis	24	19	5	11	7	4	35	26	9
Controls	22	0	22	22	0	22	44	0	44

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