the eggs. The relative percentage of free xanthophylls is highest in the eggs, then in the ovaries and lastly in the testicles. Xanthophyll-esters are present only in the ovaries and in the eggs. They have been considered as the metabolically less active form of xanthophylls and by analogy with vitamin A as a sort of storage of carotenoids[1]. The ratio of the total xanthophylls to carotenes + echinenone increases in the following order: testicles < ovaries < eggs.

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Zusammenfassung

Die relativen Mengen der Karotinoide in den Eiern, Ovarien und Hoden von *Paracentrotus lividus* sind verschieden. Qualitative Unterschiede werden in den freien Xanthophyllen gefunden.

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Analysis of the *in vitro* Reaction between Jelly-Coat and a Cytoplasmic Component (Antifertilizin[1]) of Sea-Urchin Eggs

The ultracentrifugal isolation from eggs of *Arbacia punctulata* of a cytoplasmic component possessing the properties of Lillie's antifertilizin, has recently been reported[2]. Precipitation of the jelly-coat which surrounds the unfertilized eggs, and prevention of sperm agglutination ordinarily induced by egg-water, are properties of this component. The chemical composition of the active fraction was not investigated, attention being drawn only to its association with the pigment of the egg[2].

The reaction between jelly-coat and "antifertilizin" can be studied conveniently *in vitro*, since mixing egg extract and a solution of jelly-coat results in precipitation. Advantage was taken of this property for further study of the nature and significance of the reaction. Qualitative results of this investigation are reported here; the quantitative aspects will be discussed elsewhere.

Eggs of *Paracentrotus lividus*, *Psammechinus microtuberculatus*, *Sphaerechinus granularis* and *Arbacia lixula* were obtained by shaking the gonads in sea-water or, in the case of the latter two species, also by spontaneous shedding that follows cutting of the shell. The eggs were filtered through cheese-cloth to free them from tissue debris, and washed repeatedly in sea-water. The following operations were carried out in a cold room at 3°C. The jelly-coat solution was prepared by first treating the eggs with acid sea-water and then precipitating the jelly-coat substance by addition of 1·5 volumes of 95% alcohol[3]. After standing 30 minutes, the precipitate was centrifuged down, redissolved in about 6 volumes of distilled water and dialysed against distilled or sea-water. Egg extract was prepared by homogenising the jelly-free eggs in 0·25 *M* sucrose in a Potter-Elvehjem glass-homogenizer. The homogenate was centrifuged at 18,000 × *g* (in a refrigerated centrifuge) and the supernatant centrifuged once more with a distilled water layer on top to rid it of the free fats[1]. The final homogenate was then dialysed against distilled water. The reaction between jelly-coat solution and egg extract was done at room temperature in small test tubes.

The addition of a jelly-coat solution (in distilled or in sea-water) to an egg extract (dialysed against distilled water) of the same species results, as already mentioned, in the formation of a precipitate. Progressively increasing the amount of added jelly-coat solution yields an endpoint, beyond which further addition of jelly-coat solution no longer causes any more precipitate to appear. At this endpoint the U.V. analysis of the supernatant showed a shift in the absorption maximum to 275 mμ from 260 mμ in the original egg extract. The *N*/*P* ratio in the delipidated precipitate was about 12. While this suggests that nucleoproteins are involved in the precipitation reaction, the possibility of other substances in the egg homogenate cannot be excluded from this reaction with the jelly-coat solution; in fact, it has already been reported that lysozyme and thrombin can give a precipitation reaction with jelly-coat solutions[2]. Preliminary experiments have also shown that incubation of the egg extract with crystalline ribonuclease causes a decrease of about 1/3 in the *N* and *P* contents of the precipitate as compared with those obtained when the untreated homogenate is added to the same jelly-coat solution.

Cross-reactions between egg extracts and jelly-coat solutions of the different species have also been studied and the results are given in the accompanying Table. Jelly-coat solutions of *Arbacia*, *Sphaerechinus*, and *Psammechinus* give a strong reaction with egg extracts of all the species investigated, while jelly-coat solutions of *Paracentrotus* consistently failed to react with egg extracts of *Arbacia* and gave a weak reaction with those of *Paracentrotus* itself and of *Psammechinus*.

Jelly-coat solutions	Egg extracts			
	Arbacia	*Paracentrotus*	*Sphaerechinus*	*Psammechinus*
Arbacia	+ + +	+ + +	+ + +	+ +
Arbacia + sp.	—	—	—	—
Arbacia U.V.	—	not tried	—	—
Paracentrotus	—	+	+ + +	—
Paracentrotus + sp.	—	—	—	—
Paracentrotus U.V.	—	—	—	—
Sphaerechinus	+ + +	+ + +	+ + +	+ + +
Sphaerechinus + sp.	—	+	+	—
Sphaerechinus U.V.	—	—	—	—
Psammechinus	+ + +	+ + +	+ + +	+ + +
Psammechinus + sp.	—	—	—	—

+ sp.: jelly-coat solution treated with sperm; U.V.: jelly-coat solution irradiated with ultraviolet rays for 2 hours.

Jelly-coat solutions from all species, except *Sphaerechinus*, when "saturated" with excess of fresh sperm, proved to have lost completely their ability to react with egg extracts; the jelly of *Sphaerechinus* retains, however, some reactivity after such treatment.

For this experiment the following procedure was used. Spontaneously shed sperm was sharply centrifuged in the cold and, after removal of the supernatant sperma-

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liquor, was thoroughly mixed with jelly-coat solution. After about one minute the suspension was centrifuged and the supernatant tested for its sperm-agglutinating ability. In case this was still present, additional sperm was added and the operation repeated until the jelly-coat solution was inactive against spermatozoa.

It is suggested that those groups in the jelly-coat which agglutinate spermatozoa are the ones responsible for the reaction with "antifertilizin". We are trying to identify these groups. It is known that U.V. irradiation abolishes the sperm-agglutinating ability of jelly-coat solutions[1]. We have found that U.V. irradiation abolishes the precipitation reaction between jelly-coat solution and "antifertilizin", and this may be a further indication of similar or identical groups involved in both reactions.

Evidence has been given for the presence in the cortical layer of the sea-urchin egg of a glycoprotein similar to or identical with the jelly-coat substance[2]. It has also been suggested that this glycoprotein may act as an inhibitor whose removal may be the first step in the activation of the egg[3]. Furthermore the cortical layer of the *Arbacia* egg has been shown to contain also ribonucleic acid[4].

The results presented in this paper give evidence of the formation of complexes between jelly-coat substance and cytoplasmic nucleoproteins. A similar complex may well be present in the cortical layer of the unfertilized sea-urchin egg, the fertilizing spermatozoon being instrumental in breaking it down and thereby liberating the nucleoprotein.

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Zusammenfassung

Bei der Reaktion zwischen einer Lösung von Gallerthüllensubstanz und Extrakt von Seeigeleiern *in vitro* werden u.a. Nukleoproteine ausgefällt. Die Reaktion wird durch Behandlung der Gallerthüllensubstanz mit Spermatozoen oder durch Ultraviolettbestrahlung aufgehoben.

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Action of Capsular Polysaccharide and a Polygalacturonate on the Development and the Virulence of Pneumococcus Type III

It is well known that the virulence of pneumococcus is especially due to the presence of a capsular polysaccharide, specific for every type (S.S.S.). In the case of pneumococcus III, the particular virulence is, according to WOOD and SMITH[1], related to the presence of an especial "slime layer" of the capsula which interfers notably with surface phagocytosis. The slime layer can be made visible by means of fresh staining with methylene blue which stains it metachromatically. This metachromasia is caused, according to REEVES and GOEBEL[1], by a high molecular polymer of 4 β glucuronosidoglucose.

We have known for some time that some strains of virulent pneumococcus, if cultivated on ordinary media, lose both the capsula and the virulence, passing from the form "S" to the form "R". Among the several ways of restoring the lost virulence, we may mention the simultaneous injection of cultures of capsulated and virulent pneumococci killed by heat (GRIFFITH). This procedure leads to a new virulence and also to the transformation of the type. The substance which provokes this transformation seems, according to the studies of MAC CARTHY AVERY and MAC LEOD[2], to be a desoxyribonucleid acid.

A certain action upon the degree of virulence of the germ seems also to be exercised by the same S.S.S. MORRIS and KERR[3] have in researches on immunity noted an augmentation of the pathogenic action on mice of pneumococcus III sensibilized with the homologous S.S.S.

In these preliminary experiments, I have made virulent some pneumococcus III strains by addition of a small quantity of S.S.S. III to the culture media. I have made use of three strains of pneumococcus III, of different origin, which were already virulent for the mouse, I have indicated them respectively by the abbrevations: BG42; ML47; TR49. The BG42 strain had been preserved by passing through broth T; the ML47 strain by drying *in vacuo*; the TR49 on desiccated spleen of a infected mouse. All these strains were found by biological test to have lost their virulence completely; and they showed on selective media (blood-agar, serum-agar, etc.) colonies of pneumococcus in the "R" form which when inoculated intraperitoneally into small white mice, even in large quantity, never caused the death of animal.

Nor was it possible to restore the virulence of the strains after several passing in mice: while the injection together with the Proteus which provoked a putrid peritonitis resulting in the death of the animals, did not allow me to separate a pneumococcus which was virulent in successive inoculations.

However the colonies, which have developed on culture media in the presence of blood or serum, have shown themselves to be of "R" form and not made up of encapsulated elements.

The addition of 0·5 mg of S.S.S. III[4] to every tube of broth with addition of ascitic fluid made it possible starting from the original strains, to obtain the pneumococci in capsulate form. Inoculation into mice confirmed the virulence with a high percentage of deaths from septicemia. In some case I also had the production of inflammatory pulmonary lesion with hepatization.

In order to control the behaviour of the capsular slime layer of the virulent pneumococcus III, I used fresh staining with alkaline methylene blue up to 1%. With such a method the pneumococci without capsules are immediately stained an intense blue, due to rapid diffusion of the stain into the body of the microbe, while the slime-covered organisms take the stain more slowly and feebly, making it possible to assume a more or less evident metachromasia. Generally a greater degree of metachromasia corresponds to a higher virulence of the microbe.

In order to ensure if the virulence which I obtained with addition to culture medium of S.S.S. III, should

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