(b) Antiserum against 12-hour stages: as (a).
(c) Antiserum against unfertilized eggs: as (a).

Each preparation was titrated with extracts and standard solutions of unfertilized eggs, 4-, 12-, 48-, and Li-treated 48-hour stages. The reaction was negative in all these cases.

Discussion

The results of the experiments indicate that one or several molecular species of new specificity occur in 0·9% saline extracts from 48-hour old *Paracentrotus* larvæ. This new type of specificity is serologically fairly distinct from the "egg" antigens which are also present in abundant amounts. These new antigens are not present at all, or perhaps in only undetectable quantities, in the corresponding extracts from unfertilized eggs and earlier developmental stages. In addition, it appears that egg extracts do not contain any precipitin producing antigens besides those in the extracts from the later stages up to 48-hours development. A strong synthetic activity, giving rise to new extractable antigens of probably protein nature, thus seems to start after the blastula stage is passed and the gastrulation is initiated. As will be remembered, suspensions of the entire sea-urchin material were used for immunization. Thus if the new antigens had been present in appreciable amounts from the beginning, but in some fraction not extractable by 0·9% saline, rabbit antibodies against them might have been obtained after injecting earlier stages (The possibility of competition of antigens must however still be kept in mind.). In this connection, it may be of interest to note that the extractability of protein-nitrogen is greater in unfertilized eggs than in later stages. The protein-nitrogen content of 0·9% saline extracts from 48-hour old *Paracentrotus* plutei is thus 19% of the total nitrogen, whereas the corresponding value from unfertilized eggs is 27% (GUSTAFSON, unpublished). The still speculative question of whether single molecular structures or single molecules of the "new" type already exist in the unfertilized egg cannot of course be answered on the basis of an investigation of this kind.

Li-treated 48-hour stages were used in order to determine if the very strong effect this treatment has on the formation of the organs, on the respiration, etc., would also manifest itself in inhibiting the synthesis of certain antigenic cell compounds. Morphologically, these 48-hour stages are distinguished by a pronounced vegetalization; they chiefly consist of a small ectodermic vesicle, attached to a strongly increased exo-gastrulated entoderm. The results of our absorption experiments however show that no difference between the antigen-constitution of extracts from Li-treated and normal 48-hour stages seems to exist.

A further investigation of the qualitative and quantitative serological properties of our solutions could not be performed owing to lack of sea-urchin material. This was also the chief hindrance for a further study of the functional and structural significance of the antigens described above. Such experiments, which might be of great interest, will be undertaken later.

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Wenner-Gren's Institute for Experimental Biology, University of Stockholm (Sweden), September 11, 1948.

Zusammenfassung

Eier des Seeigels *Paracentrotus lividus* wurden in unbefruchtetem Zustand und in verschiedenen Entwicklungsstadien gefroren, vakuumgetrocknet, fein zerrieben und mit 0,9%iger Kochsalzlösung extrahiert. Diese Produkte wurden als Suspensionen Kaninchen injiziert und damit Immunsera erhalten, in denen spezifische Antikörper mit Hilfe der Präcipitinreaktion nachzuweisen waren. Als Antigenlösungen dienten klare, dialysierte Zentrifugate der Extrakte.

Mit spezifischer Adsorption konnte folgendes festgestellt werden: In den Extrakten von 48 Stunden alten Larven (Plutei) ist eine Antigenfraktion vorhanden, die in früheren Stadien noch fehlt. Sie wird offenbar von der Seeigelzelle erst nach vollendetem Blastulastadium und nach begonnener Gastrulation in größerer Menge gebildet. Extrakte von 48 Stunden alten Larven, die durch Lithiumbehandlung stark vegetativiert waren, unterschieden sich in ihrer serologischen Konstitution anscheinend nicht von denen normaler Plutei.

The Effect of Streptomycin on Tissue Cultures

Streptomycin isolated by WAKSMAN[1] from *Actinomyces griseus* is next to penicillin the most important of the antibiotics thus far investigated. Like penicillin it is administered in large doses and therefore it is of some interest to investigate its direct influence on the cells. Experiments with different preparations of penicillin showed toxic action on mitosis which was due presumably to impurities present in the preparation. (Pure penicillin G was practically harmless for cells[2].) HEILMAN[3] tested several preparations of streptomycin on cultures of rabbit's spleen and found a very low toxicity for wandering cells as well as for fibroblasts. She measured only the growth of the cultures and did not take into account the direct influence on mitosis. BARSKI[4] found no effect in the case of 50–100 S-units/cc of streptomycin in tissue explants of rat's lung.

In our experiments the action of streptomycin was tested on the explants from the heart, aorta, and frontal bone of the chick embryo. The tissue was cultivated by the usual hanging drop method in a medium composed of one drop of fowl-plasma and of one drop of chick-embryo extract. In one series of cultures streptomycin was added directly to the medium. The growth of cultures was measured and after 48 hours the tissue was fixed and stained with Ehrlich hematoxylin. In another series the normal growing cultures were opened after 24 hours' incubation and the hollow in the slide was filled with the solution of streptomycin so that the cells were in direct contact with the solution. After being waxed they were returned to the incubator. After 10 hours' contact the cultures were fixed and stained. Streptomycin used in our experiments was a pure crystalline sample: Streptomycin (sulphate) Cutter Laboratory, California U.S.A. The solutions were diluted with Tyrode and kept in the refrigerator at +2°C. A control with Tyrode was run for each series of streptomycin cultures. In the experiment 180 cultures were investigated and 10,200 mitosis were examined. The mitosis were analysed according to v. MÖLLENDORF[5] and the following results were obtained:

No toxic influence could be seen when 25 S-units/cc

[1] S. WAKSMAN, E. BUGIE und A. SCHATZ, Proc. Staff Meet. Mayo Clin. *19*, 537 (1944).
[2] O. BUCHER, Schweiz. med. Wschr. *76*, 290 (1946); *76*, 375 (1946); *77*, 171 (1947); *77*, 849 (1947).
[3] E. HEILMAN, Proc. Soc. exp. Biol. *60*, 365 (1945).
[4] E. BARSKI, Ann. Inst. Pasteur *74*, 1 (1948).
[5] W. v. MÖLLENDORF, Arch. exp. Zellforsch. *21*, 1 (1937); Z. Zellforsch. *27*, 301 (1937).

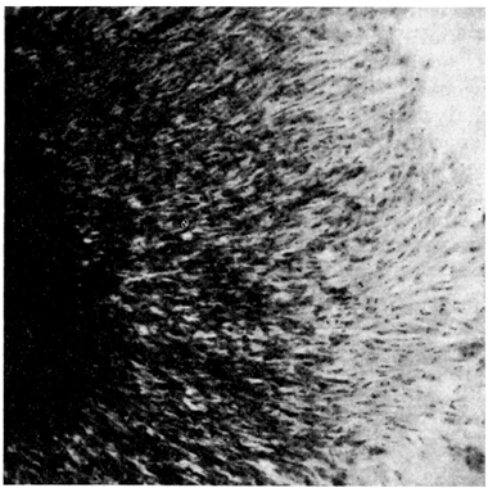


Fig. 1. – Heart fibroblasts from the chick embryo in the presence of 2,500 S-units/cc. The culture looks normal. Hematoxylin.

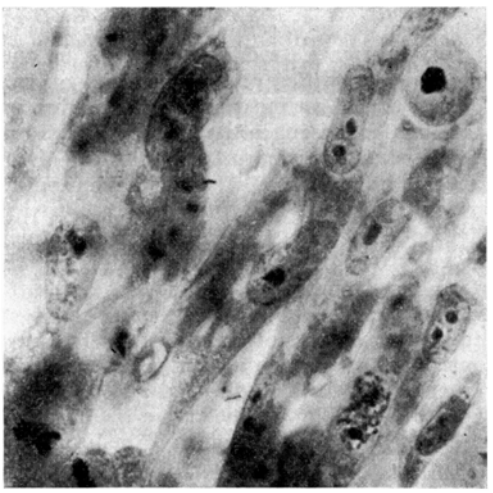
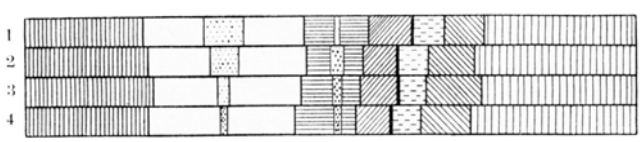


Fig. 2. – A detail from the same culture. At the right a metaphase with pyknotic chromosomes. Hematoxylin.

were used. The concentration of streptomycin 2,500 units/cc caused a small shift of the anaphase to the right (Fig. 3). The concentration of 25,000 S-units/cc was toxic and completely arrested the growth. At higher concentrations some anomalies were observed in the dividing chromosomes. At a concentration of 250 and 2,500 S-units/cc the number of pyknotic chromosomes in the early metaphase increased as compared with the control (Fig. 3). Rhexis of the chromosomes could be observed in the second stage of the metaphase. It increased with the concentration of streptomycin. A sudden decrease taking place at the concentration of 2,500 S-units/cc could be explained by the simultaneous high degree of pyknosis so that only those chromosomes which lay far enough from the others could be observed.



1 2·500 S-units/cc
2 250 S-units/cc
3 25 S-units/cc
4 Tyrode

prophase
mataphase I
metaphase II
anaphase
telophase I
telophase II
reconstruction
pyknosis in metaphase I
rhexis in metaphase II

Fig. 3.

The described influence on mitosis is not characteristic for streptomycin only, rhexis or pyknosis being common pathological phenomena. The majority of those substances which do not destroy the cell instantly, damage mitosis in its most sensitive stage, i.e. in its metaphase.

To emphasize the low toxicity of streptomycin we are quoting the toxic concentration of some other substances which influence and damage the chromosomes during the cell-division in some special way:—The effective concentration of colchicin is 10^{-8}/cc, of trypaflavin 10^{-7}/cc, of chelidonin 10^{-6}/cc, of narcotin $2{\cdot}5 \cdot 10^{-5}$/cc, of œstradiol $5 \cdot 10^{-5}$/cc[1]. The concentration of streptomycin which provokes pathological mitosis is $2{\cdot}5 \cdot 10^{-3}$/cc.

The age of the solution of streptomycin was of no importance. Solutions of two months' standing had the same effect as fresh ones.

We are continuing these experiments with other antibiotics and are trying by the combination of these to obtain sterile cultures from aseptic material.

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Department of Animal Physiology, University of Prague, June 6, 1948.

[1] H. Lettré, Hoppe Seylers Z. physiol. Chemie *278*, 201 (1943); *281*, 133 (1944); Naturwiss. *30*, 184 (1942).

Die bakteriostatische Wirkung von Chalkon, Flavanon, Flavon und Flavonol

Im Rahmen unserer chemotherapeutischen Studien konnten wir einen deutlichen wachstumshemmenden Einfluß des Buteins (3′,4′,2,4-Tetraoxychalkon) auf *Staphylococcus aureus* nachweisen. Es wurde daraufhin eine größere Zahl von Oxychalkonen hergestellt und auf ihre antibakterielle Wirkung hin geprüft. Einige dieser Verbindungen waren noch in einer Verdünnung von 1:640 000 bei *St. aureus* wirksam[1]. Inzwischen war bereits von D. H. Marrian, P. B. Russell und A. R. Todd[2] über p-Aminoderivate des Chalkons berichtet worden. Diese Verbindungen wirkten jedoch nur schwach gegenüber *St. aureus* und *Streptococcus haemolyticus*.

Einige Flavone wurden von H. S. Mahal[3] bei *E. coli* und *B. typhosa* untersucht; eine Wachstumshemmung zeigte sich nicht. Dagegen konnten J. Naghski, M. J. Copley und J. F. Couch[4] einen bakteriostatischen Effekt des natürlich vorkommenden Flavonolderivats Quercetin bei *St. aureus*, *B. abortus* und *Aerobacterium polymyxa* feststellen. Nach A. A. Andersen und J. A. Berry[5] wird auch das Wachstum von *Cl. botulinum* durch Quercetin beeinträchtigt. Kürzlich wurden von F. Blank und R. Suter[6] einige natürlich vorkommende Anthocyane und Flavonole untersucht, wobei jedoch keine bakteriostatische Wirkung dieser Stoffe gegenüber verschiedenen Kokken, *Bacillus coli* und *B. paratyphi* gefunden werden konnte.

[1] E. Schraufstätter und S. Deutsch, Z. Naturf. *3b*, 163 (1948).
[2] P. B. Russell und R. A. Todd, J. Chem. Soc. London 1419 (1947).
[3] H. S. Mahal, Proc. Ind. Acad. Sci. Sect. B. *5*, 186 (1937); C *1*, 1397 (1938).
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[5] A. A. Andersen und J. A. Berry, Science *106*, 644 (1947).
[6] F. Blank und R. Suter, Exper. *4*, 72 (1948).