oder weniger hellgrüne Keimblätter auftreten, erscheinen nach der Quellung in Aureomycin- und in Achromycinlösungen (oder in einem Gemisch beider) je nach Menge des Tetracyclins und Dauer der Einwirkung *alle Grade* der Bleichung. Zum Teil beruht dieser Unterschied darauf, dass Streptomycin (wie auch Neomycin) weniger keimungshemmend ist als Aureomycin, so dass man letzteren Chlorophylldefekt nur in einem später eintretenden, aber weniger fortgeschrittenen Keimungsstadium beobachten kann. In diesem Zusammenhang mag noch erwähnt werden, dass Aureomycin *in vitro* die Succinodehydrogenase hemmt (blockiert).

Andere Arten von Chlorophyllhemmungen als die hier besprochenen sind früher von uns[1] erwähnt worden.

Über die Biochemie der Tetracycline, besonders des Aureomycins, findet man in der Literatur wertvolle Angaben. Die toxischen Wirkungen, welche Aureomycin im tierischen Stoffwechsel ausübt, konnten durch Versuche von LOOMIS sowie von METER, OLESEN und WILLIAMS[2] auf die Hemmung der oxydativen Phosphorylierung zurückgeführt werden. Dieselbe wird durch einen Enzymkomplex in den Mitochondrien vermittelt. Von wesentlichem Interesse ist der Nachweis (ROINE[3]), dass Aureomycin nach *peroraler* Eingabe einen erheblichen Einfluss auf die Bakterienflora des Darmes ausübt. Der Gehalt des Aureomycins im Serum nach oraler Eingabe wird durch Zitronensäure, Brenztraubensäure oder andere stark erhöht[4]. Zahlreichen Angaben zufolge ist Aureomycin ein spezifisches Mittel zur *Virus*-Bekämpfung. Diese und andere Ergebnisse haben uns veranlasst, den Einfluss des Aureomycins auf die frischen Tumorzellen des Yoshida-Ascites-Tumors zu untersuchen.

Von diesen an anderer Stelle demnächst mitzuteilenden Versuchen sei hier nur folgendes erwähnt:

0,3–0,5 ml Ascites unserer Yoshida-Ascites-Tumorratten wurden mit dem gleichen Volumen von Aureomycinlösungen verschiedener Konzentration (pH 7) 22 h bei 0° inkubiert und dann den Versuchsratten intraperitoneal eingespritzt. Wenn der verwendete Ascites 150000–200000 Tumorzellen je Kubikmillimeter enthielt, so waren zur Inkubation rund 4 mg Aureomycin erforderlich, um die Tumorentwicklung zu verhindern. Die tumorhemmende Wirkung des Achromycins war wesentlich schwächer, diejenige des Neomycins war nicht sicher nachweisbar.

H. v. EULER und M. L. STEIN

Institut für organisch-chemische Forschung der Universität Stockholm, den 10. November 1954.

Summary

EULER (1947) and M. L. STEIN (1953) had observed that germinating seeds of barley, rye, wheat and other grasses are colourless when exposed to solutions containing more than 0·2% of streptomycin or dihydrostreptomycin. These plants are not inhibited in growth. The chlorophyll already formed is not destroyed by streptomycin.

The present authors describe the bleaching effect on germinating seeds of chlortetracycline and tetracycline. These antibiotics inhibit or reduce the formation of chlorophyll in germinating seeds but the authors could not obtain this inhibition without toxic effects (reduced growth). Contrary to streptomycin, the tetracyclines are not able to precipitate nucleic acids and nucleoproteins.

[1] H. v. EULER, Z. physiol. Chem. *295*, 411 (1953). – Siehe auch C. L. HAMNER und H. B. TUKEY, Bot. Gazz. *112*, 525 (1952).

[2] J. C. METER, J. J. OLESEN und J. H. WILLIAMS, Science *113*, 273 (1951). – Proc. exp. Biol. Med. *81*, 215 (1952). – H. A. LARDY, J. Biol. Chem. *195*, 215 und 225 (1952).

[3] P. ROINE und T. ETTALA, Nature *169*, 1014 (1952).

[4] H. J. EISNER *et al.*, Pharm. exp. Therap. *108*, 442 (1951).

On the Irreversible Agglutination of the Sperm of the Sea-Urchins Caused by the Sperm Extract

Antifertilizin, a substance neutralizing the sperm agglutinating effect of egg-water of the sea-urchin, was extracted from the sperm first by FRANK[1]. Later TYLER and O'MELVENY[2] described an acid soluble substance, which is extracted by acidulated sea-water. As to the action of antifertilizin in neutralizing fertilizin, agglutinating the eggs, dissolving the gelatinous coat of the egg, paralyzing the sperm and activating the unfertilized egg, this has been previously reported (TYLER; WICKLUND[3]).

In the present experiment a new method of extraction of antifertilizin was used. The materials used were the sea-urchins *Paracentrotus lividus* and *Arbacia lixula*. About 10 cm³ of sperm suspension in sea-water were fixed by adding 50 cm³ of acetone. The solid fraction was collected on filter paper, and was then suspended in 20 cm³ of distilled water by vigorous shaking. The suspension was acidulated by adding 5 cm³ of N/10 HCl, and gently heated by boiling and after cooling filtered. The filtrate thus obtained was brought to pH 8 before use.

The sperm extract of both species of sea-urchins show a remarkable power to agglutinate the sperm of both species irreversibly. The sperm cells move actively in dilute solutions of the sperm extracts at the beginning and then form spherical masses. The mode of agglutination at the beginning is nearly the same as that caused by egg-water of the same species. Within 10 min the sperm cells form clots of irreversible agglutination. The sperm extracts of *Paracentrotus* and *Arbacia* cause the irreversible agglutination on the sperm of *Paracentrotus* up to 40 and 20 times dilution with sea-water respectively. The agglutination of the sperm of *Arbacia* is also observable in the presence of excess of $NaHCO_3$. After mixing equal volumes of sperm extract and M/2 $NaHCO_3$, the mixture is diluted with sea-water. The above mentioned extracts of *Paracentrotus* and of *Arbacia* show irreversible agglutination of the sperm of *Arbacia* up to 16 and 64 dilutions respectively. The agglutination is not observable in the control (mixture of bicarbonate and sea-water).

The eggs agglutinate instantaneously by sticking to each other forming a precipitation membrane on the surface of the jelly-coat, when they are put into the neutralized extracts. The sperm extract of *Paracentrotus* shows the ability of egg agglutination up to 40 and 160 dilutions to the eggs of *Paracentrotus* and of *Arbacia* respectively, and that of *Arbacia* is effective up to 20 and 80 dilutions to the eggs of those species respectively. The egg of *Arbacia* is therefore more sensitive to the extracts.

The extracts also show the capacity to neutralize the fertilizin, the sperm agglutinin of egg-water. The egg-

[1] J. A. FRANK, Biol. Bull. *76*, 199 (1939).

[2] A. TYLER and K. O. MELVENY, Biol. Bull. *81*, 364 (1941).

[3] A. TYLER, Physiol. Rev. *28*, 180 (1948). – E. WICKLUND, Ark. Zool. [*A*] *40*, Nr. 5 (1948).

water of more than 1000 units for the homologous sperm is completely inactivated by one tenth volume of the sperm extract of pH 8.

These facts show that the extracts are of the nature of the antifertilizin in the egg agglutination and in the neutralization of the fertilizin. Accordingly, the method of extracting sperm used in the present experiments gives a higher yield of antifertilizin than do the methods previously used. The writer believes that the irreversible agglutination of the sperm by the sperm extract is one of the characters of the antifertilizin.

ISAO MOTOMURA

Stazione Zoologica, Napoli, and Biological Institute, Tohoku University, Sendai, Japan, October 20, 1954.

Zusammenfassung

Eine neue Methode der Extrahierung von «Antifertilizin» aus den Samenzellen von Seeigeln, *Paracentrotus lividus* und *Arbacia lixula*, wurde beschrieben. Der Extrakt zeigte folgende Wirkungen: Neutralisierung des Fertilizins, nicht umkehrbare Agglutinierung der Samenfäden und der Eier bei beiden Seeigelarten.

Observations on Vitamin B_{12}-Binding Factor from Hog Gastric Mucosa

In a previous paper[1] the results have been presented of the analysis by means of continuous paper electrophoresis of a vitamin B_{12}-binding preparation obtained from hog gastric mucosa by a modification of the method of GLASS and coworkers[2].

The collected analytical figures of this preparation are: total nitrogen 7·8% (micro-Kjeldhal), uronic acids (as glicuronic acid)[3] 6·2%, hexosamines (as glucosamine hydrochloride)[4] 15·9%, reducing sugars (as glucose)[5] 16·3%, non glucosamine polysaccharides (as equimolar mixture of galactose and mannose)[6] 19·5%, binding power on vitamin B_{12} (as vitamin B_{12} bound by 1 mg of the preparation in presence of an equal concentration of free vitamin B_{12}, cup plate method with *E. coli* 113/3)[7] 0·904 μg/mg, titres as A and H group substances (greatest dilution completely inhibiting the agglutination of A_1 and O red blood cells respectively by human anti-A and eel anti-H sera in amounts of 3–4 completely agglutinating doses)[8] $1:1{\cdot}6 \times 10^6$ and $1:4 \times 10^5$.

Electrophoretic examination (Tiselius apparatus) (Fig. 1) showed the presence of five components with mobilities (ascending) of $-2{\cdot}0$, $-2{\cdot}7$, $-5{\cdot}5$, $-7{\cdot}4$, and $-8{\cdot}6 \times 10^{-5}\ cm^2\ s^{-1}\ V^{-1}$.

The heterogeneity of the preparation suggested an attempt to analyze the different components by means of continuous paper electrophoresis (Durrum apparatus[9] soln. 2% w/v, phosphate, pH 6·4, I = 0·03, 580–600 V, 0·011–0·012 A, temp. 0–1°, *t* 48 h, Macherey, Nagel&Co., no. 214 paper, 35 × 35 cm sheets).

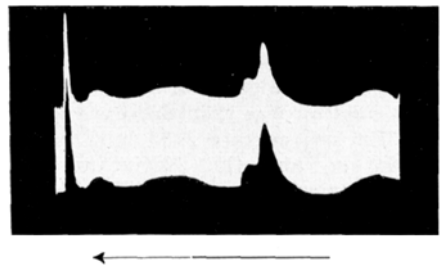

Fig. 1.–Electrophoresis pattern (ascending) after 120 min, of the original hog gastric mucosa vitamin B_{12}-binding preparation (1% [w/v] soln., at 6 V/cm, phosphate, pH 6, I = 0·2).

Colour reactions on the paper itself (bromophenol blue[1], toluidine blue[2] and Schiff[3]) and chemical, serological and microbiological assays on the different fractions collected, made it possible to distinguish only two groups of components.

One of these two groups had a low mobility, strongly absorbed u.v. light, positively reacted to Schiff, was stained by bromophenol blue and showed high values of non-glucosamine polysaccharides, hexosamines, reducing sugars, group substances A and H and binding power on vitamin B_{12}; the other group had a high mobility, showed fluorescence in u.v. light, metachromasia with toluidine blue and a high value of uronic acids.

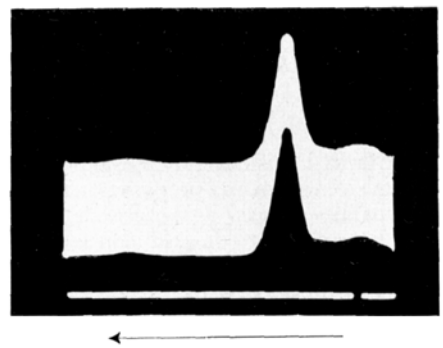

Fig. 2.–Electrophoresis pattern (ascending) after 120 min, of the almost pure hog gastric mucosa vitamin B_{12}-binding preparation (1% [w/v] soln., at 6 V/cm, phosphate, pH 6, I = 0·2).

As it was not possible to separate completely by continuous paper electrophoresis the components of the binding preparation under analysis, an attempt was made to purify the binding factor by the methods of extraction and fractional precipitation already used for the isolation of different blood group substances[4].

The original vitamin B_{12}-binding preparation was treated (5% w/v) with phenol-water solution (90% w/v) and the phenol insoluble residue discarded: to the phenol supernatant fluid, ethanol was fractionally added as a 1:1 phenol-ethanol mixture. The fractions precipitated at different ethanol levels were collected by

[1] A. CRESSERI, Boll. Soc. It. Biol. Sper. *30*, 718 (1954).
[2] G. B. J. GLASS, L. J. BOYD, M. A. RUBINSTEIN, and C. S. SVIGALS, Science *115*, 101 (1952).
[3] Z. DISCHE, J. Biol. Chem. *167*, 189 (1947).
[4] L. A. ELSON, W. T. J. MORGAN, Biochem. J. *27*, 1824 (1933).
[5] E. J. KING, *Microanalysis in medical biochemistry* (London, Churchill, 1947).
[6] M. M. GRAFF, E. M. GREENSPAN, I. R. LEHMAN, J. J. HOLECHECK, J. Lab. Clin. Med. *37*, 736 (1951).
[7] A. CRESSERI, Atti dell'XI° Congr. Soc. It. Ematologia, Roma, *300* (1953).
[8] W. T. J. MORGAN and H. K. KING, Biochem. J. *37*, 640 (1943).
[9] E. L. DURRUM, J. Amer. Chem. Soc. *73*, 4875 (1951).

[1] H. G. KUNKEL and A. TISELIUS, J. Gen. Physiol. *35*, 89 (1951).
[2] K. G. RIENITS, Biochem. J. *53*, 79 (1953).
[3] F. M. ANTONINI, F. GRANDONICO, and G. PIVA, Lo Sperimentale *103*, 1 (1953).
[4] W. T. J. MORGAN and H. K. KING, Biochem. J. *37*, 640 (1943). – E. F. ANNISON and W. T. J. MORGAN, Biochem. J. *52*, 247 (1952).