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.

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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).

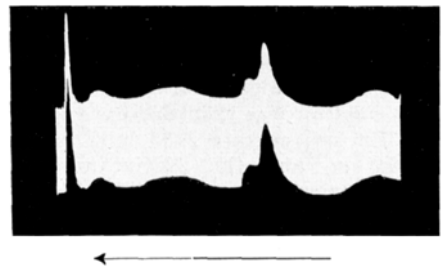

Fig. 1.–Electrophoresis pattern (ascending) after 120 min, of the original hog gastric mucosa vitamin B_{12}-binding preparation (1% [w/v] soln., at 6 V/cm, phosphate, pH 6, I = 0·2).

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.

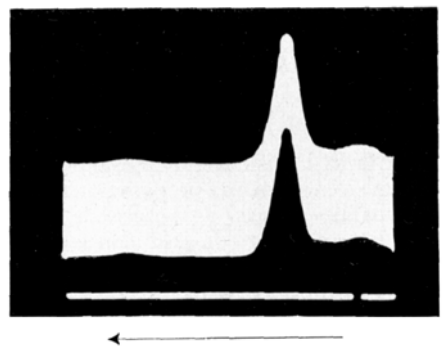

Fig. 2.–Electrophoresis pattern (ascending) after 120 min, of the almost pure hog gastric mucosa vitamin B_{12}-binding preparation (1% [w/v] soln., at 6 V/cm, phosphate, pH 6, I = 0·2).

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

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centrifugation, thrice triturated with ethanol to remove excess phenol and dried with ether. The highest binding power resulted in the fraction precipitated at 20–30% ethanol concentration (10·869 μg/mg). This fraction was redissolved in phenol-water solution and the ethanol fractional precipitation repeated: the activity resulted at the same ethanol levels, but only a slight increase in binding power was observed (11·961 μg/mg). This fraction was then dissolved in water (2% w/v), at 0°, Barium acetate (3% w/v) was added to assist flocculation, and the precipitates formed by fractional addition of ethanol collected by centrifugation, redissolved in water and treated with Na_2SO_4[1]: a precipitate was obtained, discarded by centrifugation, and the supernatant was dialysed 48 h against distilled water at 0–4° and dried in vacuo from the frozen state. The highest binding power resulted in the fraction precipitated at the 50–60% ethanol level. Electrophoretic examination shows that the material is not grossly inhomogeneous (only traces of a component with a slow and another with a high mobility seem to be present as impurities).

The collected physical and chemical data on this almost pure preparation of vitamin B_{12}-binding factor are: total nitrogen 8·2%, uronic acids nearly absent, hexosamines 17·1%, reducing sugars 18·3%, non-glucosamine polysaccharides 21·6%, binding power on vitamin B_{12} 22·123 μg/mg. Electrophoretic mobility (ascending) (Fig. 2) is about $-2{\cdot}3 \times 10^{-5}$ $cm^2s^{-1}V^{-1}$.

With inhibition agglutination tests (anti-A and anti-H sera), only a very slight activity at the concentration of 1 mg/ml is present.

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Riassunto

Vengono riferiti i risultati dell'esame con elettroforesi continua su carta di una preparazione dalla mucosa gastrica di maiale dotata di potere di legame sulla vitamina B_{12} e l'isolamento da essa con metodi di estrazione e di precipitazione frazionata del fattore responsabile di tale legame.

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Nuclear Uptake of Methionine-S^{35} in the Newt Embryo

The incorporation of glycine-2-C^{14} predominantly into the nuclei of the dorso-axial structures and induced neural plate of the amphibian embryo has been described in a previous paper, and interpreted as resulting from a higher nuclear metabolism of those regions of the embryo[1]. It should be of interest to experiment with another amino-acid, such as methionine, which might be expected to enter into rather more localized metabolic processes than glycine.

In a new experimental series, embryos of *Triturus alpestris* were kept at room temperature in solutions of DL-methionine-S^{35}, with original activities of 37·2 or 40·9 μc/ml. The incubations were all finished before the tracer had decayed for a month. The solutions also contained sodium sulphadiazine (May & Baker) to preserve asepsis. As the ratio of methionine to sulphadiazine was approximately 1·4:1, any antagonism between them, as

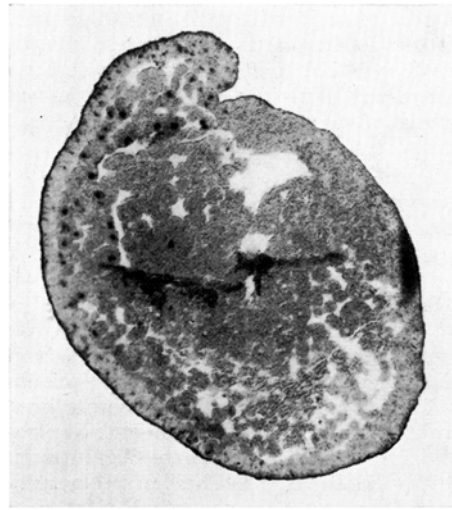

Fig. 1.–Autoradiograph of a mid-gastrula (× 40).

described elsewhere[1], would probably have been very slight. Early blastulae or older embryos were slit open to facilitate the penetration of the tracer, and neural-fold stages onwards were transversally transected. After culture in the tracer solution, the embryos were rinsed three times in a similar solution made with non-radioactive methionine. The technique for preparation of the autoradiographs has been given before[2], but in the present experiments the exposure of the film was sometimes prolonged up to two months. When the last autoradiographs were developed the tracer had decayed for 3·5 months.

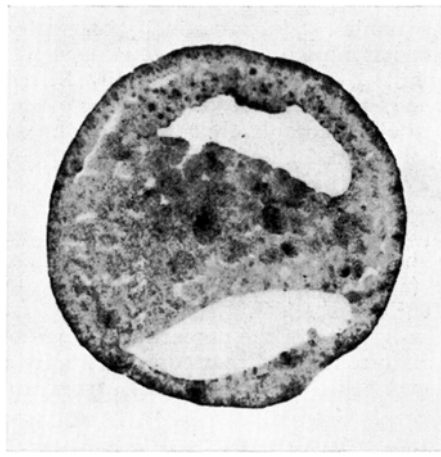

Fig. 2.–Autoradiograph of a neural-plate embryo (× 40).

Embryos kept for 24 h in the tracer solution, from cleavage until the first appearance of the blastopore, show a marked nuclear activity in the animal half, which can be sometimes noticed particularly in the nuclei of the

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