Versuchen in Frage kommen. Ebenfalls ist es unwahrscheinlich, dass dieser Effekt bloss auf die Wirkung fremder Proteine zurückzuführen ist, da die einzelnen Komponenten der Antigen-Antikörperreaktion sich als praktisch unwirksam auf die Förderung der Fremdkörpergranulombildung gezeigt haben. Ausserdem ist das fördernde Produkt höchstwahrscheinlich ausschliesslich im Präzipitat der Antigen-Antikörperreaktion vorhanden; es dürfte sich somit um eine hochmolekulare bzw. in einer hochmolekularen Form vorliegende Substanz handeln. Welche Substanzen hier in Frage kommen, ist auf Grund der vorliegenden Untersuchungen noch nicht zu entscheiden; wir werden später auf diese Frage zurückkommen.

R. Meier, P. Desaulles und B. Schär

*Wissenschaftliche Laboratorien der CIBA Aktiengesellschaft Basel, den 15. August 1955.*

*Summary*

It has been shown that local application of a mixture of precipitating antiserum with homologous plasma or serum promotes markedly the growth of granuloma tissue in the rat.

## Trypsin Sensitivity of Some Proteins of the Sea-Urchin Egg Before and After Fertilization An Electrophoretic Analysis

Upon fertilization the cytoplasmic proteins of the sea-urchin egg undergo a reorganization, as a result of which they acquire new properties[1].

Recently it has been found[2] that the fraction precipitated at 50% saturation of ammonium sulphate from an extract of newly fertilized eggs of the sea-urchin *Arbacia lixula* is less sensitive to the attack of trypsin than the one from unfertilized eggs.

It appeared then interesting to study this different susceptibility as this may offer an opportunity for a better understanding of the nature of the changes involved. As a preliminary study, the above fraction has been submitted to an electrophoretic analysis before and after treatment with trypsin.

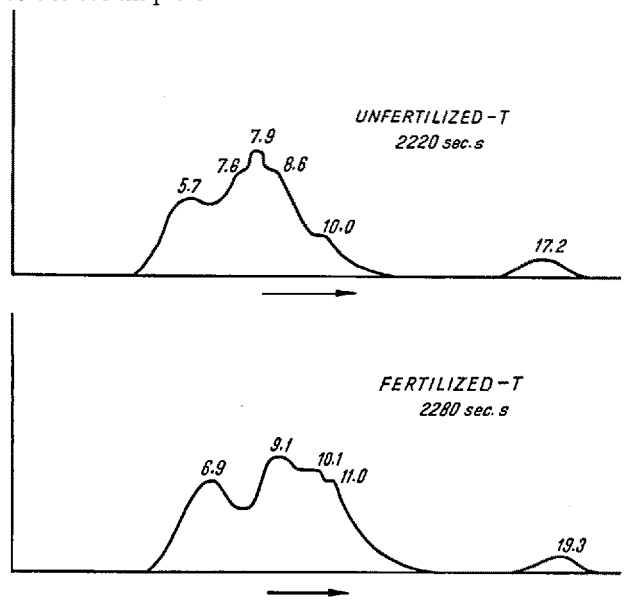
A main group of three components and a small fast component are present in the 50% fraction both from unfertilized and fertilized eggs of *Arbacia lixula*. The mobilities of the components of the fraction from fertilized eggs are somewhat higher than those of the unfertilized eggs. This point, however, will need further examination and will be discussed on a later occasion.

After a 30 min trypsin digestion, the main group is split off into five components in the fraction from unfertilized eggs whereas only four are present in the one from fertilized eggs. Here again all the components of the fraction from fertilized eggs have a higher mobility than those of the unfertilized eggs (Figure).

These results are a further evidence of the deep-lying changes the proteins of the sea-urchin egg undergo as a result of fertilization.

*Experimental.* Jelly-coat-free eggs of *Arbacia lixula* (unfertilized and about 15 min after fertilization) were homogenized in the cold with a buffered 1 M LiCl solution containing 0·2% of Versene. After centrifugation at 20,000 g in the cold for 30 min, the supernatant was precipitated with the addition of an equal amount of a saturated solution of ammonium sulphate in phosphate buffer (0·007 M, pH 7·1). The collected precipitate was redissolved in 0·5 M KCl and dialysed against 0·1 N Na-bicarbonate. 1·0 cm³ of this solution was then incubated at 32°C for 30 min with 0·1 cm³ of a 0·05% solution of Armour crystalline trypsin in 0·002 N⟷HCl. At the end of the incubation, 0·1 cm³ of 0·01 N di-isopropyl-fluorophosphate (DFP.) was added (kindly synthesized for us by Dr. Sanders, Cambridge) and after about 15 min the mixture was re-dialysed against bicarbonate as previously. The bicarbonate buffer proved quite effective for the electrophoretic analysis. Incubation and DFP. treatment were run at the same time in the controls.

For the electrophoresis, the Kern micro-apparatus was used. From the photographs of the interference diagramms the gradient curves were calculated according to the technique of Cazal and Carli[1].




Electrophoretic diagrams of the 50% fraction of unfertilized and fertilized eggs of *Arbacia* after treatment with trypsin. Descending limb. Electrophoresis in 0·1 N Bicarbonate at a Pot. grad. of 6·3 Volts/cm.

Protein concentration was determined before each electrophoretic run in order to keep concentrations as constant as possible. This determination was done according to the technique of Lowry *et al.*[2] using a solution of serum albumin as a standard.

The present investigations have been supported by grants from Consiglio Nazionale delle Ricerche and from Istituto Superiore di Sanità.

V. D'Amelio

*Laboratory of Comparative Anatomy, University of Palermo, Italy, July 10, 1955.*

*Riassunto*

La frazione precipitata al 50% di saturazione di solfato di Ammonio da estratti di uova vergini e fecondate di *Arbacia lixula* presenta all'analisi elettroforetica un gruppo principale di tre componenti. Per trattamento con tripsina questo gruppo si risolve in cinque componenti nella frazione proveniente da uova vergini ed in quattro in quella proveniente da uova fecondate.

[1] J. Runnström, Adv. Enzymol. *9*, 278 (1949). – A. Monroy, Intern. Rev. Cytology (in press).

[2] G. Giardina and A. Monroy, Exper. Cell Res. (in press).

[1] P. Cazal and G. Carli, Sem. Hop. Paris *30*, (1954).

[2] O. H. Lowry, N. J. Rosenborough, L. A. Farr, and R. J. Randall, J. biol. Chem. *193*, 265 (1951).