suspension. The hemolytic activity of the lysin had been neutralized in the basins above the β_2-globulin region where the anti-streptolysin 0 antibodies were evidently localized after the electrophoresis (see Table).

It also seemed possible to localize hemolysins after electroseparation of a streptococcal culture filtrate by means of their lytic activity on red blood cells. The hemolysis could easily be read directly in the basins.

This method thus seems to allow an accurate determination of the localization of different antibody and toxin activity after electrophoresis. However, further work may be needed to develop the technique and to determine factors of importance for the results obtained; e. g. the amount of material separated has been reported to influence the distribution of activity determined[3,9].

The human red cells and sera were kindly supplied by Dr. L. RYTTINGER, The Blood Bank, Sahlgren Hospital, Gothenburg.

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Department of Bacteriology, University of Gothenburg (Sweden) and Department of Microbiology, University of Turku (Finland), December 30, 1959.

Résumé

On expose une méthode simple par laquelle il a été possible de localiser exactement l'activité des anticorps ou des antigènes après électrophorèse. Cette méthode a été appliquée aux hémagglutinines du sérum et du lait, aux antitoxines et hémolysines.

STUDIORUM PROGRESSUS

Methods of Obtaining and Mode of Action of Stage Ontogenins

By G. K. ROUSSEV[1]

In studying the connections between the preformed and epigenetical state of individual development, the necessity of applying new methods for this purpose[2] arises more and more.

Recently a number of authors have succeeded in producing specific disturbances at a clearly defined stage of embryonic development by making use of antimetabolites of nucleoproteid metabolism[3,4].

In 1954, I found a new biological method of obtaining bioactive substances influencing embryonic development[5]. I observed that, after conglutination of father-spermazozoa to the naked surface of the developing embryo of *Triturus cristatus*, the surrounding solution acquires new qualities. This solution stops the development of younger embryos submerged in it and at that same stage of the naked embryo; older embryos develop unhindered[6]. We supposed, that the action of the solution is due to the formation of substances which were named *stage ontogenins*, bearing a number corresponding to the number of the stage of the embryo from which they are obtained[7].

Together with G. NAIDENOVA, experiments were performed to purify the native solutions of some ontogenins – 3, 4, 9, 10. Applying different biochemical methods for isolation, it was found that the action is due to the presence of strictly defined chemical substances[8] – for example by chromatogram and UV-absorption curve, the presence of phosphorus in the ester-bond of purified ontogenin 4 indicates that the latter is structurally similar to a deoxyribosenucleotid.

We had to get more data of the mode of action of the ontogenin.

Method. We worked with *Triturus cristatus* during its period of heat. The animals were caught at their natural breeding grounds and within 36 h the eggs were artificially fertilized by Hertwig's method, keeping instruments and materials sterile. The spermatozoa, both for fertilization and later for conglutination, were obtained by massaging the abdomen to cause an ejaculation which was afterwards diluted in Holtfretter solution. The experiments were carried out in small porcelain crucibles with waxed bottoms. The crucibles were placed in a gas chamber in an oxygen atmosphere at 100% relative humidity and 17–19°C.

The gelatinous envelope and the viteline membrane of the embryos, at different stages of development were removed in such a way as to cause a slight injury. The naked embryo in this way was rinsed for 10–15 min in already used triton blood serum and transferred to a 1·5 ml Holtfretter solution containing $1^0/_{00}$ glucose. This solution contained in suspension about 50000 spermatozoa recently ejaculated by the father-triton. The spermatozoa rapidly conglutinated to the surface of the naked embryo, especially to the injured parts. Usually after 4–8 h, the embryo was taken away and the solution was decanted and filtered.

The necessity of preservation of these conditions was proved in a series of experiments in which we changed some of the components and kept the rest constant. For ontogenins to be formed, it is necessary for the descending embryo to be without gelatinous envelope and vitelline membrane. If the envelopes are not removed, the spermatozoa cannot penetrate through them and we cannot establish any changes in the surrounding solution. This gave us the possibility to place uninjured embryos with the naked or injured ones – the development of the latter served us as a quick test to estimate the action of the ontogenin obtained by the first. It was usual that in one test two uninjured embryos, one young and the other at an older stage, were placed in the solutions together with the injured one.

It was found that the state of the naked embryo is of the greatest importance and that ontogenins are separated only at definite moments of the different stages and states of these embryos. For example, while maintaining all the conditions for obtaining ontogenins, we obtained ontogenin 4 out of 20 embryos in the stage 10 min after the beginning of the second cleavage till the moment of complete normal second cleavage. 40 naked embryos, 30 and 60 min after the second cleavage did not give ontogenins. Stage 2 gave ontogenins: one 25–45 min after fertilization, one $1^1/_2$–2 h, and one 6 h after fertilization. The embryos at stage 4 h after fertilization are inactive. Only in the beginning of the first cleavage Ontogenin 3 is separated. Stage 10, at the beginning of the gastrulation, and stage 12 at the moment, when the blastopore is closed to a slit, did not give ontogenins.

[1] Medical Research Institute Sofia and Institute of Morphology at the Bulgarian Academy of Science.

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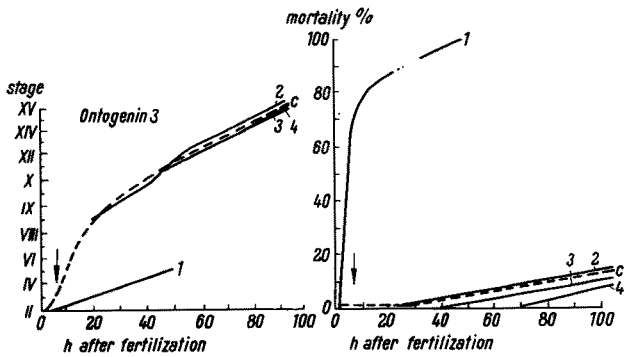
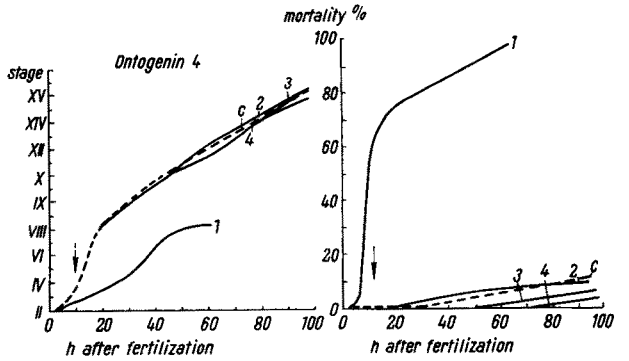

Ontogenin 3
stage
mortality %
h after fertilization
h after fertilization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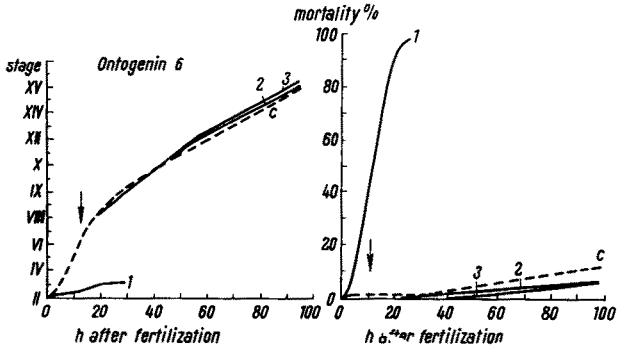
stage
mortality %
h after fertilization
h after fertilization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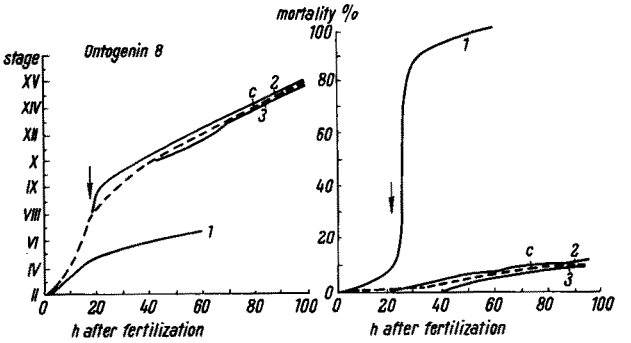
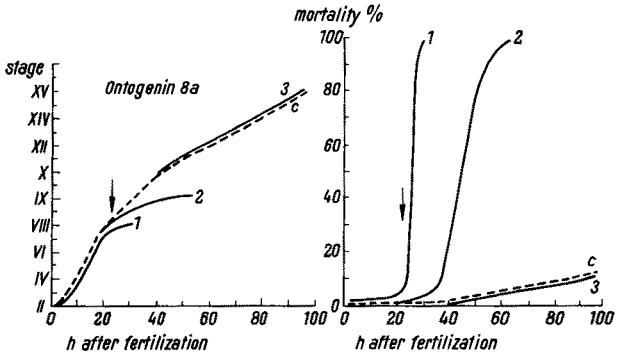
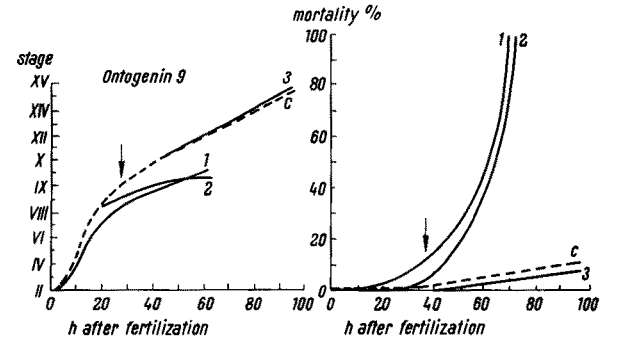
stage
mortality %
h after fertilization
h after fertilization


Ontogenin 8
stage
mortality %
h after fertilization
h after fertilization


Ontogenin 8a
stage
mortality %
h after fertilization
h after fertilization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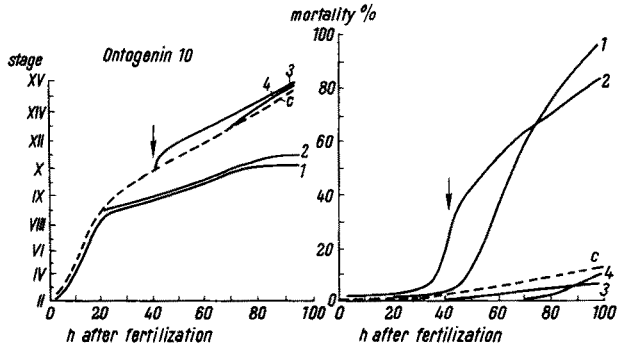
stage
mortality %
h after fertilization
h after fertilization


Ontogenin 10
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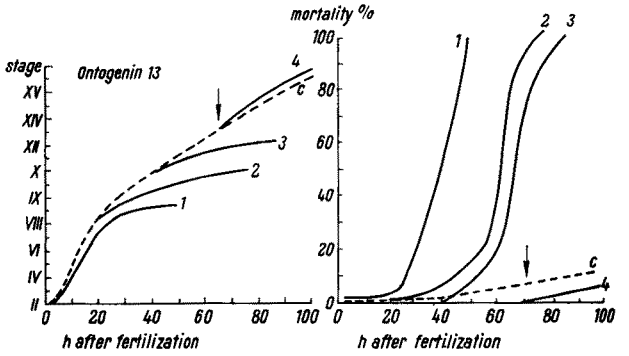
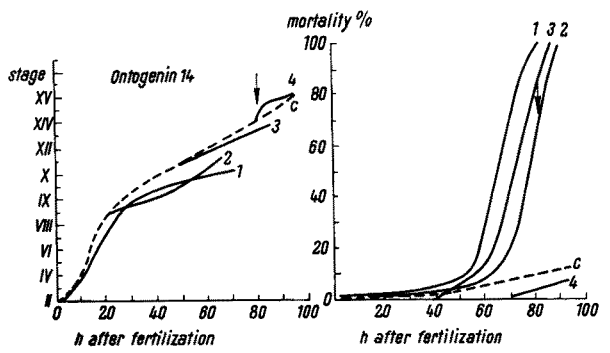

Ontogenin 13
stage
mortality %
h after fertilization
h after fertilization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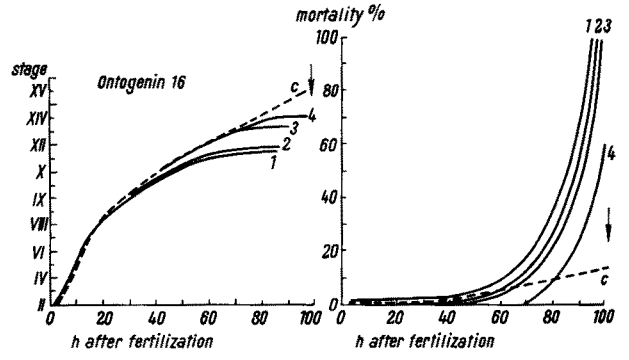
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mortality %
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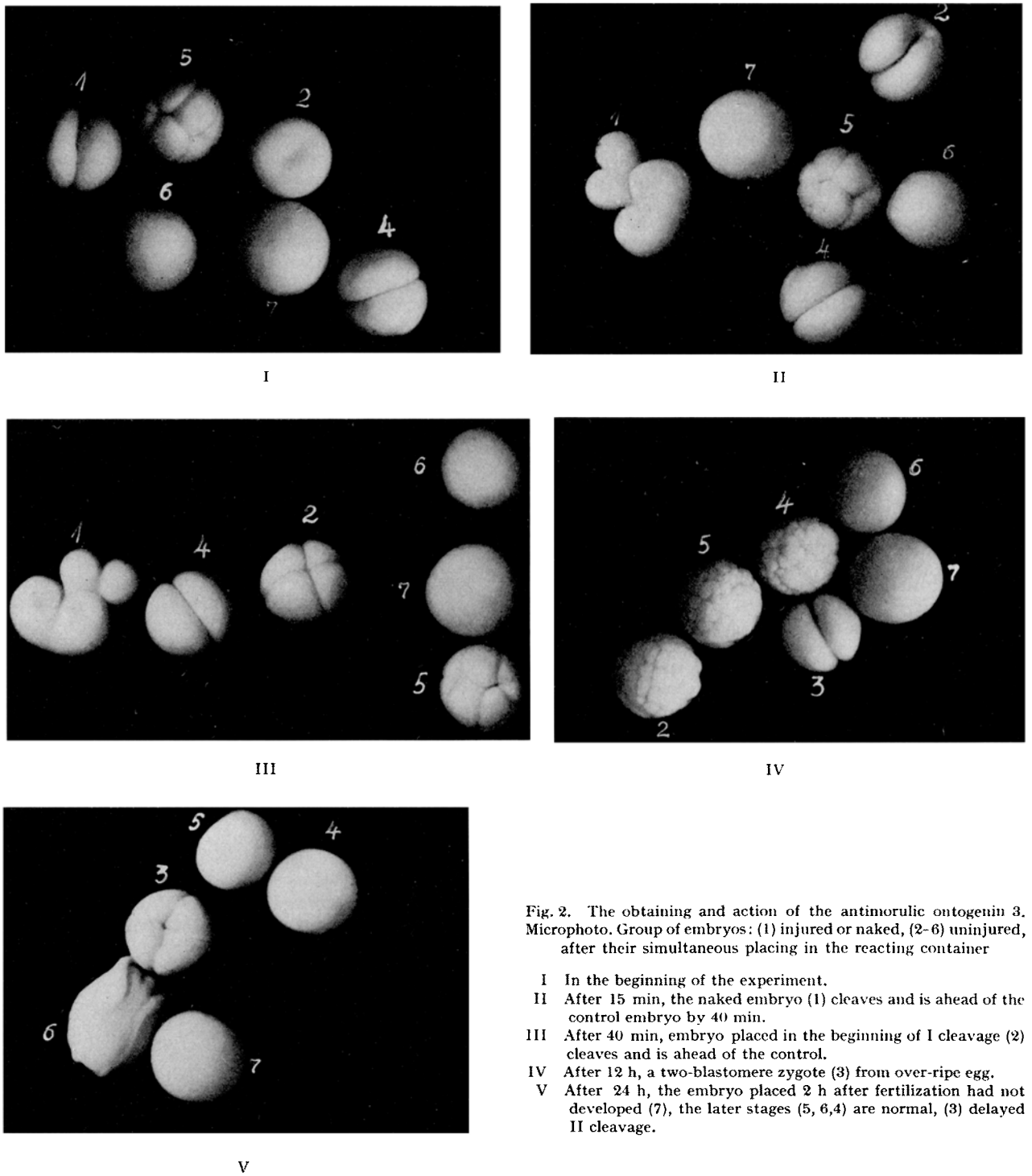

Fig. 2. The obtaining and action of the antimorulic ontogenin 3. Microphoto. Group of embryos: (1) injured or naked, (2-6) uninjured, after their simultaneous placing in the reacting container

I In the beginning of the experiment.
II After 15 min, the naked embryo (1) cleaves and is ahead of the control embryo by 40 min.
III After 40 min, embryo placed in the beginning of I cleavage (2) cleaves and is ahead of the control.
IV After 12 h, a two-blastomere zygote (3) from over-ripe egg.
V After 24 h, the embryo placed 2 h after fertilization had not developed (7), the later stages (5, 6,4) are normal, (3) delayed II cleavage.

Fig. 1. Action of the stage ontogenins, for every ontogenin:

In left: mortality % (for primary number) of the same embryos. The embryos stayed in the ontogenin-solutions 40 h, after which they were transferred to normal conditions ($t° = 19°$). The arrows show the moments of obtaining of the corresponding ontogenin. c – control – 60 embryos in Holtfretter solution with $1‰$ glucose.

In right: development of the growing embryos with vitteline membrane, put in the ontogenin solutions. 4 embryos [1–2 h after fertilization (1), one-early blastula (2), one-early gastrula (3), one-neural plate (4)] was placed together in 1·5 ml ontogenin solutions. Every ontogenin was proved in an average 30 experiments.

In another series of experiments, it was established that it is absolutely necessary for the coat of the embryos to be slightly injured.

The other conditions (father-spermatozoa, serum) are most favourable for obtaining ontogenins. It is necessary that the spermatozoa be alive, mobile, capable of fertilizing. The rinsing in the blood serum is not necessary for some stages (8,14).

We have established that an interval of 20–30 min after conglutination is necessary in order that the conglutinating solution may exert its strongest influence upon other embryos with envelopes. With constant conditions in our experiments, we obtained 22 ontogenins from the different stages of embryonic development of *Triturus cristatus*. Ontogenins 3, 4, 8, 10, and 14 were obtained utilizing spermatozoa of other individuals of *Triturus cristatus* (not the father), *Triturus vulgaris*, *Rana ridibunda*.

As with *Triturus cristatus* we obtained ontogenins in the same way from some stages of *Triturus vulgaris*[9].

After establishing the necessary conditions for obtaining ontogenins, an experiment was made to determine the ontogenetic specificity of the ontogenins obtained at different stages of the embryonic period. In 1·5 ml of every ontogenin in solution, 4 embryos were placed: one embryo 2 h after fertilization, one in the early blastula stage, one in the early gastrula stage, and one in the neural plate stage. The embryos stayed in the ontogenin solution 20 h, after which they were transferred to normal optimal conditions and were observed until the neural tube stage. For every ontogenin an average of 30 (15–40) experiments were performed. As seen from Figure 1, the growth of stages older than the naked embryos is quite normal. The younger stages, however, develop abnormally. Ordinarily the development was delayed or stopped, some characteristic disturbances being observed at every stage, such as influenced zygotes 1–6 h after fertilization, cleavage with unequal right and left halves; the grooves of the morulas regress and disappear and the blastulas have an upset animal-vegetative polarity. The gastrulas show a swelling of the blasopore. Microscopically, disturbances of mitosis, pyknosis of the nuclea, and vacuolization were discovered. If the ontogenin is obtained from an embryo of a more advanced stage of development, the quite young embryo placed in it develops normally in the beginning, but on reaching the stage of the naked embryo it perishes.

If, simultaneously with the naked embryo, we place in the solution embryos with vitelline membrane which have just begun to reach the same stage as the naked embryo, it was observed that there was an acceleration of the development of the placed or uninjured embryo, following the quicker grooving of the naked embryo (Fig. 2). If we place such an embryo in for a few hours, i. e., after the final concentration of the ontogenin has been formed, then its development is ordinarily disturbed, especially if we use embryos of over-ripe eggs, which turn out to be more sensitive towards the ontogenins (Fig. 2). We noticed a stimulating effect only with some ontogenins (3, 4, 10, 13, 14). It seems that the time of penetration of the ontogenin in the test embryo is of importance. If, for instance, the latter stays in ontogenin 3 only 15 min and after that we take it out and place it under ordinary conditions, its development is not disturbed.

The specificity of the action of the ontogenins make us think that the ontogenins represent normal specific products of a biochemical chain-process of growth. Introducing them into the embryo at the moment of their formation, we specifically stimulate the growth. On the other hand, introduction of an extreme, determining concentration into the embryos metabolism before normal and gradual formation, they cause disorders and death. This view is supported by the results of my last experiment. We homogenized the embryos exactly at the moment of development when they can separate ontogenins. We obtained dyalizable substances with the action similar to the ontogenines, which stop the development of younger embryos; older embryos develop unhindered. If the homogenized embryo is of a more advanced stage of development, the placed embryos with vitelline membrane develop normally at the beginning, after which they perish.

Text of the Remark Stage: I unfertilized egg, II gray crescent, III First cleavage, IV four cells, V eight cells, VI sixteen cells, VII 32 cells, VIII 64–256 blastomeres, VIIIa early blastula, IX middle blastula, IXa late blastula, X early gastrula, XI middle gastrula, XII late gastrula, XIII neural plate, XIV neural folds, XIVa late neural folds, XV neural folds fusing, XVI neural tube just formed.

Zusammenfassung

Der Verfasser nimmt an, dass durch Konglutination von Spermien mit Embryonen von *Triton cristatus* verschiedener Entwicklungsstadien morphogenetische Wirkstoffe entstehen, die je nach Stadium des verwendeten Embryos ganz verschiedene, aber spezifische Wirkungen haben. Es werden die wichtigsten morphogenetischen Prozesse wie Furchung, Morulabildung, Gastrulation und Neurulation selektiv beeinflusst. Diese Wirkstoffe, welche auch in der Normalentwicklung nacheinander entstehen und jedesmal die betreffende Entwicklungsphase einleiten sollen, werden als «Ontogenine» bezeichnet.

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STUDIORUM PROGRESSUS

Eine neue Umlagerung der Phenylalkane und ihre Beziehung zur Friedel-Crafts-Reaktion

Von C. D. NENITZESCU*

Wie schon seit langer Zeit bekannt[1], ist die Friedel-Crafts-Reaktion nicht selten von Umlagerungen begleitet. Diese können entweder vor der eigentlichen Substitution im Alkylierungsmittel oder im fertig gebildeten Phenylalkan stattfinden. Umlagerungen der ersten Art beruhen auf intramolekularen irreversiblen Hydridverschiebungen der intermediär auftretenden Carboniumionen, z. B. im Sinne primär → sekundär, primär → tertiär und sekundär → tertiär. Aus diesem Grunde entstehen häufig bei der Friedel-Crafts-Reaktion Gemische, in welchen das verzweigtere Phenylalkan um so reichlicher vertreten ist, je höher die Reaktionstemperatur war. Die Friedel-Crafts-Reaktion ist somit als eine kinetisch kontrollierte Reaktion aufzufassen, in welcher die ursprünglich entstandenen Carboniumionen teils unmittelbar, teils nach ihrer Umlagerung, von aromatischen Systemen abgefangen werden.

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[1] Lit. bei C. C. PRICE, Organic Reactions *3*, 2 (1946).