TEORELL[1] haben nachgewiesen, dass *in vitro* kleinste Proteinmengen die Anfärbung der NS. maskieren können (Versuche mit Malachitgrün, Methylviolett u.a.). Entsprechende eigene Versuche mit GC. haben gezeigt, dass selbst bei relativ hohen Konzentrationen von Eiweisskörpern (2,5% RNS. mit 5% Albumin) die Anfärbung der RNS. nicht gestört wird. Das stark positiv geladene GC.-Molekül hat eine weitaus stärkere Affinität zur RNS. als die Eiweisskörper und verdrängt vielleicht sogar die Eiweisskörper aus ihrer Bindung mit der Nukleinsäure, wie dies zum Beispiel von Lanthan bekannt ist. Im histologischen Schnitt scheinen ähnliche Verhältnisse zu bestehen. Es ist zudem anzunehmen, dass die Bindung zwischen Eiweisskörpern und Nukleinsäure durch die Fixation so weit gelockert wird, dass die Phosphorsäuregruppe der NS. frei wird und dem Farbstoffmolekül zur Verfügung steht.

Die Frage des spezifischen Charakters der GC.-Färbung und der quantitativen Verbindung mit Desoxyribonukleinsäure soll in weiteren Untersuchungen geklärt werden.

W. SANDRITTER, H. DIEFENBACH und F. KRANTZ

Aus dem Senckenbergischen Pathologischen Institut der Universität Frankfurt am Main, den 11. Januar 1954.

Summary

It is shown that the dye gallocyaninchromalum combines quantitatively with ribonucleic acid. 1 molecule gallocyanin is equivalent to approximately 15 phosphorus atoms. The quantitative combination *in vitro* is not disturbed by proteins.

[1] E. und G. HAMMARSTEN und T. TEORELL, Acta Med. Scand. *68*, 219 (1928).

Observations on the Nucleic Acids During the Development of the Lethal Hybrid *Triton palmatus* ♀ × *Salamandra atra* ♂[1]

The view that the nucleic acid content is closely related to the morphogenetic activity has drawn support from cytochemical and microphotometrical studies during the development of various organisms. Recently evidence has been cited that the developmental failure in amphibian lethal hybrids is primarily due to an interruption in the synthesis of nucleic acid[2]. Like most of the interspecific crosses between anurans[3], the hybrids obtained by fertilizing *Triton palmatus* eggs with *Salamandra atra* sperms are lethal and their development is arrested at the late blastula or the early gastrula[4]. By means of the FEULGEN reaction and UNNA's methyl green-pyronin mixture, approaches were made by BALTZER and SCHÖNMANN[5] to estimate the nucleic acid content in the embryonic cells of such lethals. Their results showed that the hybrid cell is poor in desoxyribonucleic acid (DNA) while its amount of ribonucleic acid (RNA) is normal[6]. Obviously a more accurate method is needed in order to give a definite answer to this problem. Therefore further efforts have been made by subjecting the lethal hybrid *T. palmatus* ♀ × *S. atra* ♂ to microphotometrical analysis. The present report deals with quantitative measurements of RNA and DNA at different developmental stages of both hybrids and controls.

Eggs taken out of the oviduct of *T. palmatus* females, which had been treated previously with gonadotropic hormone, were partly inseminated with *S. atra* sperms (*pat* hybrid) and partly with sperms of the same species (*pp* control). Both types of eggs were kept at 18°C until the desired stages were reached. For assaying the nucleic acid content, the microphotometrical method of OGUR and ROSEN[1] was used. Briefly, each egg was fixed in 96% ethyl alcohol and then treated twice with 10% trichloracetic acid for at least half an hour in the refrigerator. After washing again in alcohol it was treated with tetrahydrofuran under reflux for 4 h. The extraction of RNA was made in 3 cm³ of 5% perchloric acid at room temperature for 20 h and that of DNA at 70°C for 40 min. The nucleic acid content in the egg extract was finally determined by the BECKMAN spectrophotometer at 220–280 mμ.

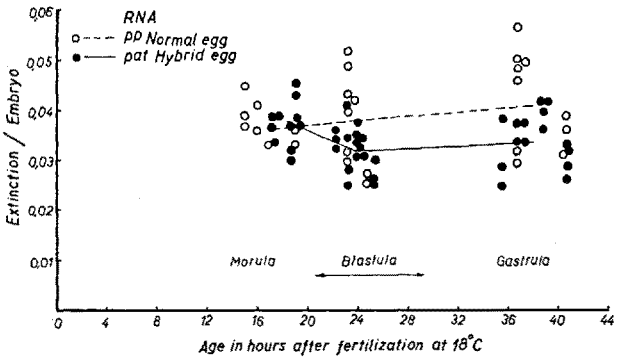

Fig. 1.—Content of RNA during the early development of *Triton palmatus* and the lethal hybrid *Triton palmatus* ♀ × *Salamandra atra* ♂.

Very recently the method of OGUR and ROSEN for the determination of nucleic acid in amphibian eggs was critisized by SZE[2]. According to him the value given by this method is too high, probably due to the presence of other disturbing substances. We have standardized the method with a solution of pure nucleic acid (Hoffmann-La Roche, Basel). A concentration of 54 γ/cm³ gave an extinction of 1·39 units. The RNA content in the early gastrula of the controls has a value of 4·78 γ/embryo. Taking the size of the egg into consideration, this value is essentially in accordance with that obtained by STEINERT[3] in *Rana esculenta*, but apparently too high compared with the DNA content given by SZE[4] for *Rana pipiens*. However, in this work the hybrid and the control eggs were measured under identical conditions and the technical error inherent in the method can be considered as insignificant.

The extinction values at 260 mμ are presented graphically in Figures 1 and 2. We shall consider first the changes of RNA and DNA in the *pp* controls. From the morula to the early gastrula there is a hardly detectable increase of RNA. This is in agreement with the observations by STEINERT[3] in anurans. According to

[1] This work was supported by a grant from the Karl-Hescheler-Stiftung.

[2] J. BRACHET, Symp. Soc. Exp. Biol. *6*, 173 (1952).

[3] J. BRACHET, Ann. Soc. Zool. Belg. *75*, 49 (1944). – J. A. MOORE, J. Exp. Zool. *101*, 173 (1946).

[4] W. SCHÖNMANN, Roux' Arch. *138*, 345 (1938).

[5] F. BALTZER and W. SCHÖNMANN, Rev. suisse Zool. *58*, 459 (1951).

[6] F. BALTZER, Symp. Soc. Exp. Biol. *6*, 230 (1952).

[1] M. OGUR and G. ROSEN, Arch. Biochem. *25*, 262 (1950).

[2] L. C. SZE, J. Exp. Zool. *122*, 577 (1953).

[3] M. STEINERT, Bull. Soc. Chim. biol. *33*, 549 (1951).

[4] L. C. SZE, J. Exp. Zool. *122*, 577 (1953).

him, the increase of RNA does not become significant until gastrulation. The picture is, however, different for DNA. The amount of this substance increases rather rapidly from the late cleavage to the middle blastula stage, and afterwards it rises only slightly until the early gastrula. In their study of the DNA content in *Rana temporaria* eggs, HOFF-JORGENSEN and ZEUTHEN[1] found that the synthesis of this acid begins at the late blastula stage and continues at a lower rate during gastrulation. According to the present result, it seems that the synthesis of DNA begins earlier in *Triton palmatus* than in *Rana temporaria*. The early increase of DNA is understandable because, as pointed by BRACHET[2], there is a steady increase of the cell nuclei during segmentation and the FEULGEN reaction becomes more intense at later developmental stages. KUTSKY[3] and SZE[4] also reported that there is a steady increase of DNA during the development of *Rana pipiens*. The latter author further demonstrated that the increase of the cell number is higher before gastrulation. This fact affords an explanation of why the DNA content increases from the morula to the blastula.

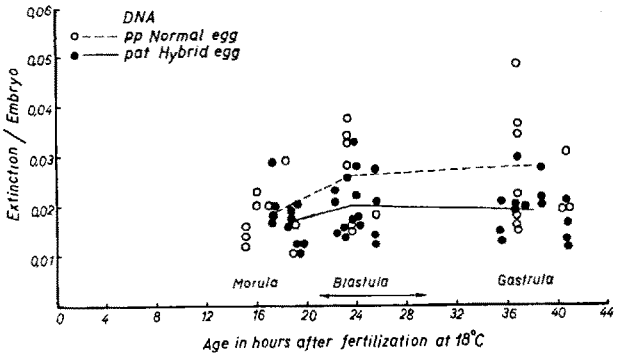

Fig. 2.—Content of DNA during the early development of *Triton palmatus* and the lethal hybrid *Triton palmatus* ♀ × *Salamandra atra* ♂.

Let us now turn to the nucleic acid contents in the *pat* hybrid. The microphotometrical measurements revealed that at 18 h after fertilization the syntheses of RNA and DNA are equally efficient in the two types of embryos. Hereafter the average values of the hybrids become apparently lower than those of the controls[5]. It should be mentioned that STEINERT[6] recorded a similar drop of the RNA content at the later development of the lethal hybrid *Rana esculanta* ♀ × *Rana temporaria* ♂. However the tendency of the RNA reduction in the present case obviously occurs earlier than that in the anuran hybrid. The curve in Figure 2 also indicates that in the *pat* hybrid there is a slight increase of DNA up to 24 h, although it never reaches the amount of this substance in the controls. In other words, the trend of the curve indicates that the DNA synthesis in the lethal hybrid is reduced but by no means entirely stopped.

Cytologically, the lethal hybrid first shows abnormal mitoses at the late blastula stage (critical phase, ca. 23–29 h after fertilization[7]). We have seen that it is exactly at this period that the synthesis of nucleic acid becomes effectively blocked. During segmentation, so far as the cytological picture shows, the lethal hybrid has a normal development. Its slight increase of DNA at the early development is therefore understandable.

The drop of the nucleic acid content is apparently only one of the many damaging effects in the lethal hybrid. For instance there have been recorded several studies showing that the rate of oxygen uptake is lower in the lethal hybrid than in the control[1]. Since our knowledge concerning the mechanism of nucleic acid synthesis is still highly hypothetical, it would be unwise in this place to discuss at length the ultimate cause which leads to the nucleic acid reduction in such lethal embryos. BRACHET[2] has put forward the idea that the introduction of a foreign sperm into the egg causes a breakdown of the synthesis of cytoplasmic ribonucleoprotein which is normally under the control of the nucleus. In order to clarify the situation, more critical studies of the nucleo-cytoplasmic relationship in such hybrid materials are badly needed.

The author is indebted to Prof. Dr. E. HADORN for his interest in this investigation and to Mr. C. ZELLER for his advice concerning the technical part.

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Institute of Zoology and Comparative Anatomy, University of Zurich, January 18, 1954.

Zusammenfassung

Der Gehalt an Ribonukleinsäure und Desoxyribonukleinsäure der normalen Tritoneier (*T.palmatus*) und der letalen Bastardeier (*T.palmatus* ♀ × *S.atra* ♂) wurde im Morula-, Blastula- und Gastrulastadium mikrophotometriert. Bei normalen Eiern wurde keine wesentliche Änderung des Ribonukleinsäuregehaltes festgestellt. Hingegen nahm die Konzentration der Desoxyribonukleinsäure von der späten Morula bis zur mittleren Blastula deutlich zu und zeigte dann bis zum Gastrulastadium nur geringe Zunahme.

Während der Furchung ist der Nukleinsäurengehalt der Bastardeier normal. Die mittlere Blastula (kritische Phase: etwa 24 h bei 18°C) ist aber gegenüber den Kontrollkeimen bedeutend ärmer an beiden Nukleinsäuren. Eine leichte Zunahme der Desoxyribonukleinsäure vor der kritischen Phase, ohne jedoch die Konzentration eines normalen Eies zu erreichen, wurde beobachtet. Die entwicklungsphysiologische Deutung der vorliegenden Befunde wird diskutiert.

[1] F. HOFF-JORGENSEN and E. ZEUTHEN, Nature *169*, 245 (1952).
[2] J. BRACHET, Symp. Soc. Exp. Biol. *6*, 173 (1952).
[3] P. B. KUTSKY, J. Exp. Zool. *115*, 429 (1950).
[4] L. C. SZE, J. Exp. Zool. *122*, 577 (1953).
[5] The differences in RNA between control and hybrid eggs are statistically significant at both blastula and gastrula stages. The same is true for DNA at the gastrula stage.
[6] M. STEINERT, Bull. Soc. Chim. biol. *33*, 549 (1951).
[7] W. SCHÖNMANN, Roux' Arch. *138*, 345 (1938).

Some Observations on Carotenoid Synthesis by the Alga *Chlorella vulgaris*

Very little is known about the occurrence or biosynthesis of carotenoids in *Chlorella* spp. STRAIN[1] states that *Ch. pyrenoidosa* contains α-, β-, and ε-carotenes, lutein, violaxanthin and neoxanthin, and MYERS[2] found that *Ch. vulgaris* when grown in the dark produced the same chlorophylls and probably the same mixture of caro-

[1] H. H. STRAIN in *Manual of Phycology* (Waltham Chronica Botanica Co., 1952).
[2] J. MYERS, Plant Physiology *15*, 575 (1940).