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.

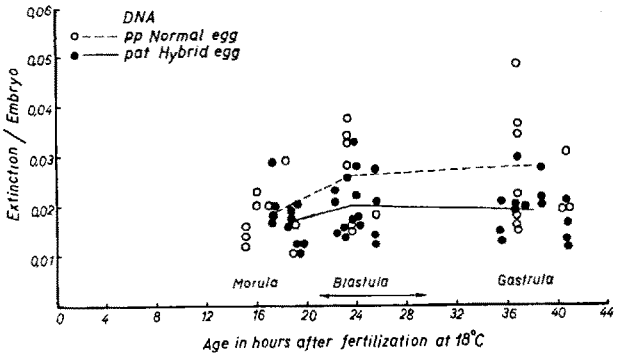

Fig. 2.—Content of DNA during the early development of *Triton palmatus* and the lethal hybrid *Triton palmatus* ♀ × *Salamandra atra* ♂.

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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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).
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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).

tenoids as when grown in the light. SPOEHR and MILNER[1] report that increasing the *R* value (degree of reduction) of the culture medium reduces considerably the chlorophyll and, to a lesser extent, the carotenoid synthesis.

The experiments reported here were carried out to determine quantitatively and qualitatively the carotenoids synthesised by *Ch. vulgaris* when grown under comparable conditions in the light and dark.

The alga, obtained from the Type-culture collection of the Botany School, Cambridge, was cultured statically at 25° in 100 ml conical flasks containing 25 ml of the following medium:– KNO_3 0·025 g, $MgSO_4$. $7H_2O$, 0·027 g, KH_2PO_4, 0·02 g, glucose 1·0 g, peptone 0·5 g, and water 100 ml. Inoculation was with 1 ml of a 5–7 day old culture grown in the light on the same medium. The cultures were kept in a thermostatically controlled water bath, either in a tin (dark) or continually exposed at a distance of 20–30 cm to 2 × 80 W. fluorescent "daylight" tubes.

The cultures were harvested and the pigments extracted using a method devised by GOODWIN and JAMIKORN (1954). Using the usual criteria of identification[2], the following carotenoids were found in both light and dark cultures:– β-carotene, lutein, violaxanthin (in traces), and neoxanthin. The relative amounts of these pigments present were the same in light and dark cultures namely β-carotene 10%, lutein and violaxanthin 75–80%, and neoxanthin 10–15%. These results indicate a very close pigment relationship between our *Ch. vulgaris* and the *Ch. pyrenoidosa* examined by STRAIN[3], the only difference being that no ε-carotene could be found in the former. Furthermore, these observations also add evidence to support the generalization[4] that although members of the Chlorophyceae tend to resemble green leaves in their carotenoid distribution, their xanthophyll mixture is much less complicated than that of green leaves, which contains at least 15 components[5].

The quantitative results given in the accompanying table show that under our experimental conditions growth is complete in both light and dark cultures after 14 days and that the amount of growth in the dark is only about 1/6 that obtained in illuminated cultures. Furthermore, although pigment production is of the same order in illuminated and non-illuminated young cultures, in old cultures there is relatively much more pigment in dark than in light cultures. The difference is quite obvious visually, and must also involve chlorophyll synthesis, old light cultures being a very faint green whilst old dark cultures are dark green. In some experiments (not recorded here), this difference was obvious even after 21 days. The carotenoid concentration continues to increase in the fully grown dark cultures, and eventually becomes higher than the maximal values obtained in light cultures. The reason for these results is not known, but it is probably that the continous illumination destroys the pigments in the fully grown cultures. The concentrations recorded in the Table, for both the total carotenoids and β-carotene, fall within the limits recorded for a number of fresh water Chlorophyceae[1].

Action of diphenylamine. TURIAN[2] found that diphenylamine inhibited carotenoid synthesis in *Mycobacterium phlei*, and this has been further demonstrated in *Phycomyces blakesleeanus*[3] and *Rhodospirillum rubrum*[4]. Experiments with this inhibitor showed that *Ch. vulgaris* was very sensitive to it, growth being completely inhibited at a concentration of 1/280,000. This extreme sensitivity to diphenylamine has also recently been noted in another green alga *Haematococcus pluvialis*[5]. On the other hand, the bacteria and fungi just listed, grow normally on a concentration of 1/35,000–1/70,000 although pigment synthesis is considerably inhibited.

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Zusammenfassung

Wird *Chlorella vulgaris* in einem Medium, das Glukose, Pepton und Mineralsalze enthält, gezüchtet, so synthetisiert sie sowohl in Licht wie im Dunkeln β-Carotin, Lutein, Violaxanthin und Neoxanthin. Das Wachstum im Dunkeln beträgt jedoch nur 1/6 von demjenigen im Licht. Die Carotinoide verschwinden aus alten Lichtkulturen, werden aber in alten Dunkelkulturen weiter synthetisiert. Diphenylamin hemmt das Wachstum dieses Organismus bei einer Konzentration von 1/280 000 vollständig.

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Carotenoid synthesis by *Ch. vulgaris*.
(Amounts produced in 25 ml medium in 100 ml conical flasks: Temperature 25 °.)

Light Cultures				Dark Cultures				
Age of Culture (days)	Dry weight mg	Total Amount (μg)	Carotenoids Conc. (mg/100 g dry weight)	β-Carotene as % of total pigments	Dry weight (mg)	Total Amount (μg)	Carotenoids Conc. (mg/100 g dry weight)	β-Carotene as % of total pigments
7	21·0	10·7	51	8·5	4·7	7·0	15	13·8
14	57·3	60·0	105	10·4	11·7	8·9	76	6·4
21	58·3	65·2	112	6·7	10·5	21·4	204	11·8
28	68·7	52·1	76	10·0	9·4	17·4	185	10·7