Der Einfluß von Vitamin E auf *Daphnia longispina* O.F.M.

A. VIEHOEVER berichtet, daß Tokopherol (angereichertes Preßöl von Weizenkeimlingen) die in übervölkerten *Daphnia magna*-Kulturen stark verminderte Vermehrung innert 48 Stunden wieder anzuregen vermag[1].

Es wurde mit einer andern als der von VIEHOEVER gewählten Versuchstechnik der Einfluß von Vitamin E auf den Lebensablauf von *Daphnia longispina* O.F.M. untersucht:

Das Vitamin E wurde uns als *dl*-α-tokopherol-phosphorestersaures Na in verdankenswerter Weise von der Firma Hoffmann-La Roche & Co. zur Verfügung gestellt.

1000 Tiere, die alle einer mit *Scenedesmus quadricauda* gefütterten reinen Linie entstammen, wurden in Gruppen zu 10 in 200 cm³ Kulturwasser[2] gehalten und täglich beobachtet. Als Futter erhielten sie suspendierte Bäckerhefe, die stabilisiert worden war (Trocknung bei 40° C im Luftstrom[3]). Jeden zweiten Tag wurden die Daphnien über eine Passage von destilliertem Wasser in frisch zubereitetes Kulturwasser übergeführt. Das Zählen der Tiere erfolgte täglich, ebenso das Entfernen der toten und neugeborenen.

Die Kontrolltiere erhielten nur Bäckerhefe, die Versuchstiere zusätzlich Vitamin E (0,5 mg/200 cm³ Wasser) bei jedem Wasserwechsel. Die Temperatur schwankte während der Versuchsdauer zwischen 18 und 20° C.

Aus den für jeden Tag errechneten Lebenserwartungen der überlebenden Daphnien ergeben sich die hier interessierenden Zahlenwerte (s. Tabelle).

Aus der Tabelle ist ersichtlich, daß die Daphnien, die von Geburt an Tokopherolzusätze erhielten, eine Lebenserwartung haben, die von der der Kontrolltiere deutlich verschieden ist. Weiter kann den Zahlen entnommen werden, daß nach einer gewissen Wartezeit (im Versuch 6 Tage) ein Zusatz von Vitamin E unter den betreffenden Bedingungen keinen Einfluß mehr auf die Lebenserwartung der Versuchstiere hat.

Vergleicht man die Lebenserwartung der Jungen von Vitamin-E-Tieren mit derjenigen der Jungen von Zuchttieren (Algenfutter) bei reiner Hefeernährung, so zeigt es sich, daß kein Unterschied besteht; die dabei erhaltenen Werte stimmen mit den bei den Kontrolltieren errechneten Lebenserwartungen recht gut überein.

Die Vermehrung der Versuchstiere wie auch der Kontrolltiere war sehr klein und lag für beide Gruppen unterhalb 0,1 Junges pro Weibchen und Tag. Mit Algen gefütterte Daphnien zeigten zur selben Zeit eine etwa 12mal größere Vermehrungsrate.

Zusammenfassung. Aus den Versuchsergebnissen kann geschlossen werden, daß *dl*-α-tokopherol-phosphorestersaures Na unter den angegebenen Bedingungen einen günstigen Einfluß auf die Lebenserwartung von *D. longispina* hat, daß aber im Gegensatz zu VIEHOEVER keine Wirkung auf die Vermehrung der Versuchstiere beobachtet werden kann.

EDW. FLÜCKIGER und H. FLÜCK

Pharmakognostische Abteilung des Pharmazeutischen Instituts der ETH., Zürich, den 31. Oktober 1949.

Summary

The influence of a water-soluble vitamin E upon the duration of life and the propagation of *D. longispina* has been studied. The animals were kept in artificial culture medium[1] and lived on stabilized baker's yeast[2]. It could be shown that administration of vitamin E increases under the described conditions the average length of life in *D. longispina*, but that it has no detectable influence upon their propagation.

[1] EDW. FLÜCKIGER und H. FLÜCK, l. c.
[2] H. INGOLD, l. c.

[1] A. VIEHOEVER, Amer. J. Pharm. *110*, 297 (1938).
[2] EDW. FLÜCKIGER und H. FLÜCK, Exper. *5*, 486 (1949).
[3] H. INGOLD, *Aneuringehalt und Gärgeschwindigkeit von Hefepräparaten*, Diss. (Bern 1948).

Some Observations about the Morphology of Bacteriophage

The morphology of bacteriophage can be studied with the help of the electron microscope[1]. In his last papers

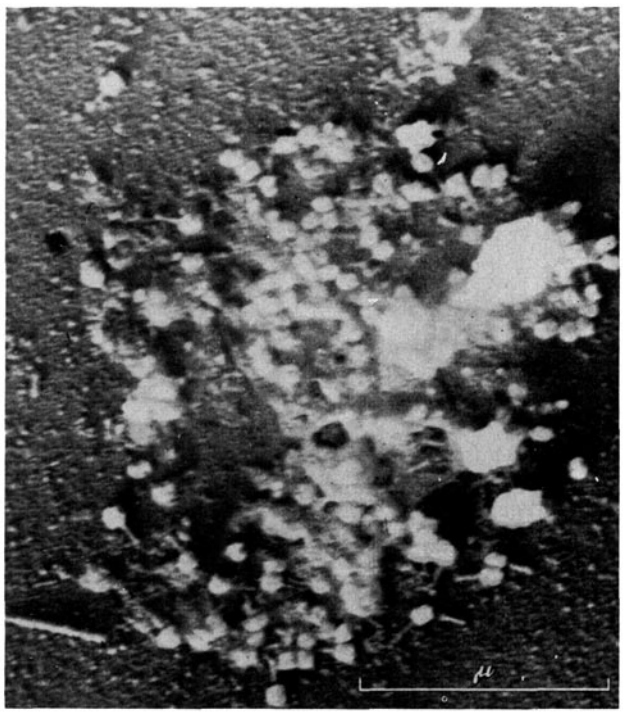

Fig. 1.

[1] H. RUSKA, Arch. Virusforschung *2*, 343 (1942). – N. KOTTMANN, Arch. Virusforschung *2*, 388 (1942). – S. E. LURIA, M. DELBRÜCK, and T. F. ANDERSON, J. Bact. *46*, 57 (1943).

Bedingung	Vitamin E vom 0. Tag an	Vitamin E vom 2. Tag an	Vit. E vom 4. Tag an	Vit. E vom 6. Tag an	Kontrolltiere	Junge aus 1. Kolonne	Junge aus Zucht
Anzahl	3·100	100	100	100	3·100	50	50
$t/2$	9,8–20,5	22 5	10,9	4,2	4,2–10,3	7,9	7,8
$t/4$	28,5–40,1	34,9	32,1	6,0	6,1–21,4	15,1	13,6
w	50 –58	55	59	17	21 –35	27	21

$t/2$ Zeit in Tagen, bis 50% der Tiere gestorben sind $t/4$ Zeit in Tagen, bis 75% der Tiere gestorben sind
w Lebensdauer des ältesten Tieres eines Versuches

WYCKOFF[1] demonstrated how the phages develop and multiply within the host cell. He studied the morphology of plaques and the development of the phages T 2, T 3, and T 4 in liquid medium. His work showed that the development of the phage is accompanied by very different modes of production. It seems that the observed phenomena are those to be expected of particles behaving as if they were minute microorganisms multiplying by division at the expense of the bacterial protoplasm. The manner in which the conversion of bacterial protoplasm to the phage particles is effected is, of course, the answer to the question how bacteriophage multiplies. No definite answer to this has yet been given.

We publish here our own observations which bear on this problem. The cultures of *Escherichia coli* were cultivated for one hour in broth, then mixed with phage and, after several hours, centrifuged, washed with saline and with distilled water. After each washing they were recentrifuged. A microdrop of the final suspension was placed on the screens and shadowed with chromium at an angle of 16°. We used phage T 2 and a wild type phage, which is probably T 3 or T 7.

(*a*) Rod-like structures. Fig. 1 shows lysis with phage T 2, and Fig. 2 a similar lysis with wild-type phage. Both these figures show peculiar rod-like structures, which on the lower part of Fig. 1 form a part of the tail. On the other photograph these structures are a little widened at the end so that it looks as if the round macromolecules of the phage would be formed from these rods. Single phage particles can be seen around the lysed bacterial cell. On Fig. 1 the tail is a little thinner than the rod. This circumstance perhaps is explained in Fig. 3, where the lysed bacterial cell is completely converted into phage particles, and where on several phage particles a cylindrical thickening is clearly visible. This gives the impression that the tail in formation is thicker than its final form. In our collection of electron micrographs we have pictures where many rod-like structures are lying here and there among the phage particles in the decomposed bacterial mass. It seems that these rods are in some relation to the formation of tail of phage T 2.

(*b*) Agglomeration of phage particles. WYCKOFF observed several times that the bacterial cell is converted into phage particles without remnant. In such a case we observed peculiar formations which are demonstrated in Fig. 4. Four particles of the phage are stuck together, and only two of them have tails. This is phage T 2, every particle of which should have a tail. Fig. 4 shows that the phage particle has a thinner region down its center. It is, of course, difficult to say whether the particle is in a state of division or fission, or whether this phenomenon is due to the drying process. We have observed that in some cases the body of the phage is crescent-shaped, especially the young forms.

(*c*) Different scattering power of the phage particles. Fig. 5 shows phage T 2. In the middle are particles which are not fully developed, on the edge of the colony are fully grown bodies. It can be clearly seen that the latter ones are bigger and scatter the electrons more than the particles in the centre of the colony. This may be a

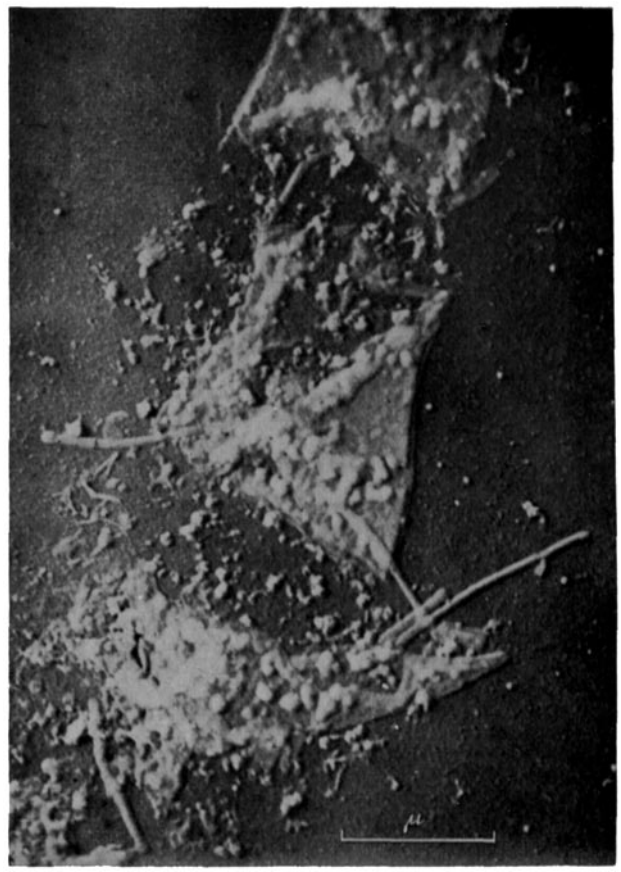

Fig. 2.

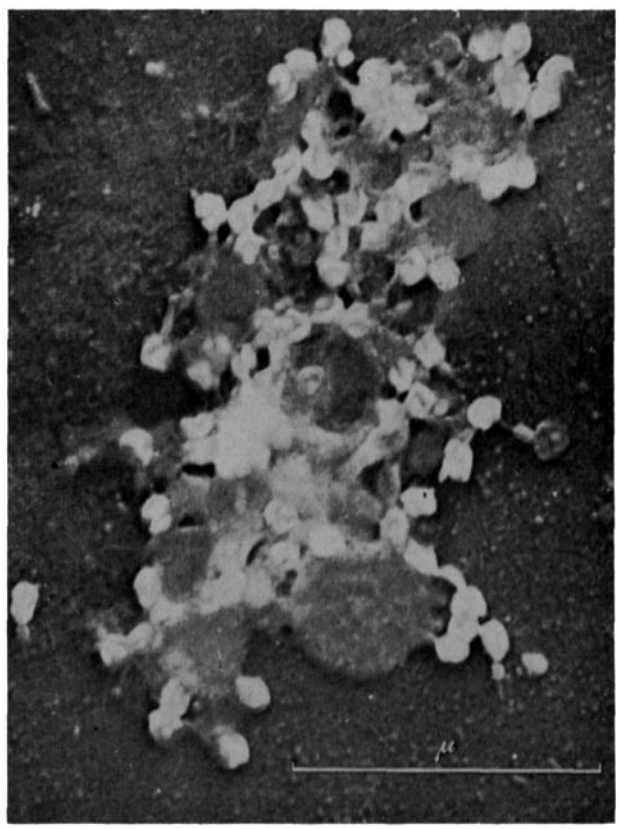

Fig. 3.

[1] R. W. G. WYCKOFF, Bioch. et biophysica acta *2*, 27 (1948); Bioch. et biophysica acta *2*, 246 (1948); Proc. Soc. Exp. Biol. Med. *71*, 144 (1949); Nature *162*, 649 (1948).

consequence of a larger water content of the undeveloped particle.

FERDINAND HERČÍK

Institute for Biology, Medical Faculty, Masaryk University Brno, Czechoslovakia, October 16, 1949.

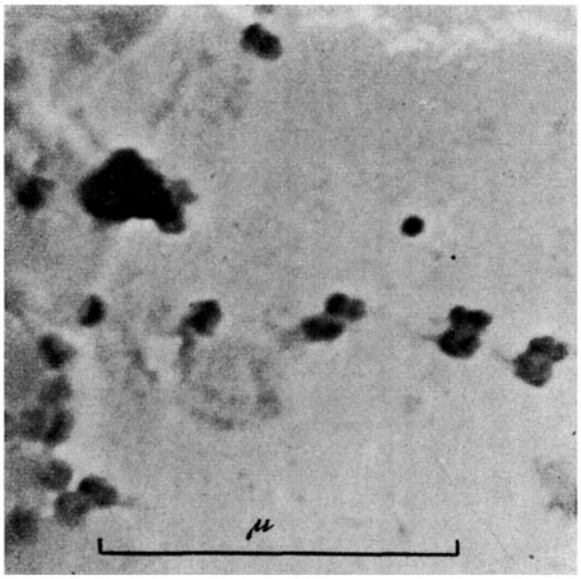

Fig. 4.

Zusammenfassung

In den lysierten Zellen von *E. coli* wurden stäbchenförmige Strukturen beobachtet, die vielleicht mit der Bildung der Geißel im Bakteriophagen T 2 in Beziehung stehen. Andere Elektronenbilder zeigen teils einen Prozeß, der der Spaltung des Bakteriophagkörpers ähnelt, teils eine verschiedene Streuung der Elektronen in jungen und in alten Bakteriophagkörpern.

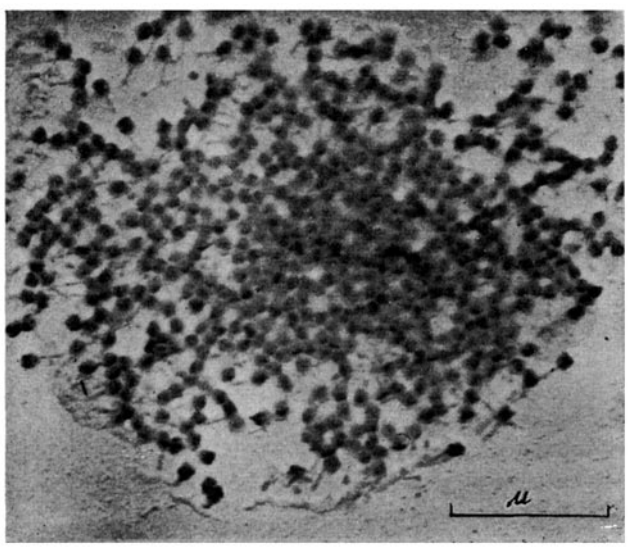

Fig. 5.

Filaments in Cultures of Bacteriophage

HERČÍK has very recently described[1] the presence of filaments in cultures of bacteriophage growing on susceptible colon bacilli. It is the purpose of the present note to confirm the existence of such filaments and to offer additional evidence of their intimate relation to the more familiar sperm-like units of bacteriophage activity.

Filaments of the general shape and size described by HERČÍK appear in more than 200 electron micrographs we have taken of products of lysis of *Escherichia coli*. With very few exceptions these objects have been seen after lysis with the even-numbered strains T 2 and T 4; the wild strain used by HERČÍK, on the other hand, seems to have more the appearance of an odd-numbered strain. The filaments under discussion always are of a constant and uniform diameter which is essentially that of the particles of phage. Sometimes they are like flat ribbons or collapsed tubes (Fig. 1), sometimes they are more substantial cylindrical objects. Occasionally they are fairly uniform but usually they are more or less completely broken up into segments which commonly (Fig. 2) have approximately the dimensions of mature phage particles. Very often a filament will have parts that are empty and other parts that have this segmented character.

It is not yet possible to define precisely the experimental conditions under which these filaments are produced. We have never found them when lysis has been carried out under optimal conditions using young and especially susceptible bacteria. Lysis can proceed at a retarded rate at temperatures below 37° C; in our experiments filaments have appeared most often in cultures which have been inoculated with a concentration of bacteriophage insufficient for immediate infection of all bacteria. If lysis is allowed to proceed in such a culture at room-temperature or in the ice-box for a period of time varying between a number of hours and several days, many filaments are sometimes produced.

These filaments do not resemble the flagellar processes that also are to be seen during the lysis of the B strain of *E. coli*. These flagella are of two sorts. One has the appearance of very delicate peritrycate flagella which develop on organisms in a several hour-old culture. They can develop in the absence of bacteriophage. The other is far thicker, of a uniform and constant diameter that is about that of the tail of a normal bacteriophage particle. A short length of such an intermediate filament appears at the right of Fig. 1. Like the more numerous large filaments that are the principal subject of this note, these tail-sized filaments have been seen only in bacteriophage-diseased cultures.

It would be rash to try to decide yet concerning the significance of these filamentous forms. They have never been seen except in bacteriophage-infected cultures and presumably, therefore, should be considered as an aspect of the bacteriophage-bacterial system. They definitely are not a step in the normal process whereby bacteriophage proliferates, whatever that may be; but one cannot ignore the possibility that they are involved in the multiplication of bacteriophage under less than optimal conditions, and perhaps, as HERČÍK's observations suggest, during the laboratory adaptation of recently isolated strains. As such a possible stage in

[1] F. HERČÍK, Exper. *6*, 64 (1950). Through the courtesy of the author, the writer has had the opportunity to see this article before publication.