The first yolk granules appear when the follicle cells are about 45 μ long (stage III). This probably means that the nucleus must have a minimum degree of polyploidy before the cell is able to start its secretory activity. It means also that the epithelium consists of two types of cells between stage III and stage V, i.e. those cells which continue to divide and the non-dividing polyploid cells which secrete the products used in yolk formation. The final stage of activity is reached with maturity of the oocyte when the shell is secreted (stage VIII)[7].

Zusammenfassung. Es wird die Entwicklung der Follikelepithelzellen in den panoistischen Ovariolen von *Carausius morosus* beschrieben. Die sekretorische Zellaktivität beginnt, wenn die Zellkerne durch die Endomitose polyploid geworden sind. Amitosen treten keine auf.

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Inhibition of the Infective Activity of Phage f_2 and its Infectious RNA by Histone

Basic substances affect the infectivity of viruses. Protamine inhibits the reproduction of Mengo virus, probably by means of binding onto the cell surface (COLTER, DAVIES, and CAMPBELL[1]). Phage T_2 is inhibited by polylysin or streptomycin (SHALITIN, DANON, and KATCHALSKI[2], and COHEN[3]). Some RNA phages are inhibited by the basic antibiotics streptomycin or neomycin (BROCK[4], SCHINDLER[5], BROWNSTEIN[6], and SCHINDLER[7]).

Different effects following the association of histone with various anionic substances, essentially with nucleic acids, are under extensive investigation at present. It has been shown that histone inhibits synthesis of DNA-primed RNA (HUANG and BONNER[8], BARR and BUTLER[9], and ALFREY, LITTAU, and MIRSKY[10]) and the synthesis of DNA in vitro (HNILICA and BILLEN[11]). Histone interacts with DNA, changing its thermal denaturation profile (HUANG, BONNER, and MURRAY[8], HNILICA and BILLEN[11], and HAHN[12]), and precipitates it at a higher histone:DNA ratio. It also precipitates RNA (BUTLER and JOHNS[13]). It was shown that histone liberates ribosome-bound β-galactosidase (NEČINOVÁ and BURGER[14]).

This paper describes the effect of histone on the reproductive activity of phage f_2 and the interaction with infective f_2 RNA.

Material and methods. Bacteriophage f_2, *E. coli* K 13 and general techniques were described earlier (SCHINDLER[5]). Infective RNA was isolated by phenol extraction (GIERER and SCHRAMM[15]), omitting the final precipitation with ethanol. It contained 10^4 infective units/ml. The preparation of spheroplasts and their infection with infectious RNA was performed according to GUTHRIE and SINSHEIMER[16]. Histone was prepared from calf thymus by Dr. LIEBL. It was not fractionated. Its lysine:arginine ratio was 1:1.6 (analysis kindly performed by Dr. NOVOTNÝ). Protamine Hydrochloride Spofa in solution was used.

Results. Inhibition of reproduction of f_2 phage: Histone suppresses reproduction of f_2 phage in *E. coli* K 13. Following the addition of various concentrations of histone to the phage-bacteria system at the time of infection, a certain drop in the average yield of phage from infected cells could be observed (Table I). A similar effect can be observed after treatment with protamine. Histone, as a basic protein, can bind with the phage particle by electrostatic forces and thus inhibit infection of the host cell. Moreover, it can bind with RNA as well, inhibiting its

Table I. Inhibition of reproduction of f_2 phage by histone during 90 min incubation; inhibition by protamine during 5 min pulse or 90 min incubation

Histone μg/ml	Phage-yield pfu/inf.[a] cell	Relative titre	Protamine μg/ml	Phage-yield pfu/inf. cell 5 min exp.	90 min exp.	Relative titre 5 min exp.	90 min exp.
0	183	1.00	0	1220	1220	1.00	1.00
20	70	0.38	10	623	520	0.52	0.42
40	20	0.11	50	64	3	0.05	0.003
100	9	0.05	100	1.5	–	0.001	–
160	14	0.08	–	–	–	–	–

[a] 'pfu' means plaque forming unit.

Histone or protamine was added to respective concentrations of *E. coli* K 13 ($4.7 \cdot 10^7$ cells/ml) together with f_2 (0.2 pfu/cell). After 5 min infective centres were assayed ($4.6 \cdot 10^6$/ml), after 90 min chloroform was added and the phage was titrated. In one series of experiments with protamine, infected cells were centrifuged in the fifth minute and resuspended in warm broth. Average yield of phage from an infected cell was expressed as pfu/infective centre. Neither protamine or histone exerts any inhibitory effect on growing culture of *E. coli* K 13.

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Table II. The effect of histone on the reproduction of f_2 phage in spheroplasts by f_2 RNA infected

Histone μg/ml	Experiment I relative titre	Experiment II relative titre
0	1.00	1.00
5	0.71	0.87
10	1.04	–
50	–	0.95
100	0.84	–

0.2 ml of spheroplast stock was mixed with 0.2 ml of diluted infectious RNA at 37°C. After 5 min 0.4 ml of histone solution (double strength) was added. After 10 min infective centres were assayed in control tube ($1.9 \cdot 10^2$/ml) and respective mixtures were diluted in stabilized medium. 90 min after infection the mixture was freeze-thawed three times and subsequently titrated. Titres are relative values to controls.

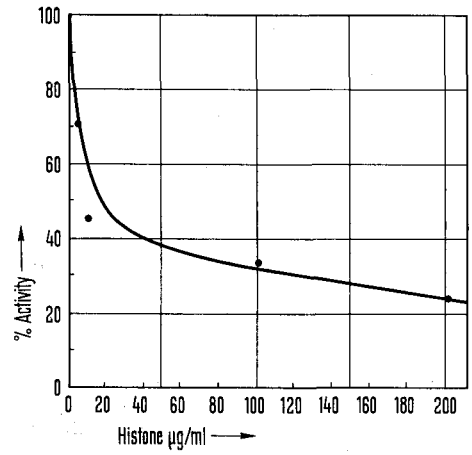

Fig. 1. Inactivation of f_2 phage by histone. $2 \cdot 10^8$ pfu/ml in 0.5% peptone and 0.25% NaCl solution were incubated 60 min at 37°C with histone in respective concentrations.

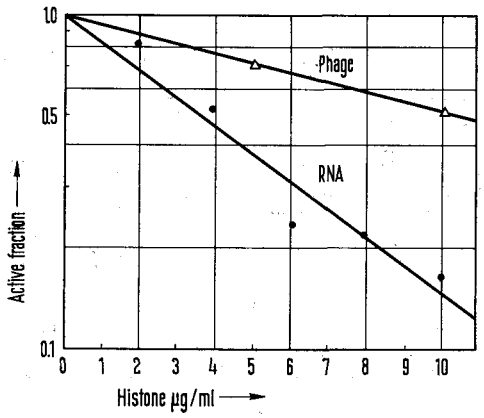

Fig. 2. Inactivation of infectious f_2 RNA by histone. 0.1 ml of 1:2.5 diluted infectious RNA stock solution was mixed with 0.1 ml histone to obtain the required concentrations. After 60 min at 37°C 0.2 ml of spheroplast stock was added, the mixture incubated 15 min at 37°C and the infective centres were titrated.

penetration into the cell. These two possibilities were therefore studied separately.

Inactivation of free f_2 phage: Histone inactivates f_2 phage in the range of 5 μg/ml to 200 μg/ml at 37°C in 60 min to a comparable degree as it inhibits the reproduction of this phage (Figure 1).

Inactivation of infectious f_2 RNA: Infectious f_2 RNA is more susceptible to histone than phage particles, as can be seen in Figure 2, where both phage activity and RNA activity are plotted against the concentration of histone. The slope of f_2 infectious RNA inactivation is steeper. The combined effect of histone could thus explain its inhibiting properties. Moreover, histone might interfere with the intracellular synthesis of phage. In this case it would be necessary to assume its penetration through the osmotic barrier into the spheroplast. Nevertheless, this could not be excluded when comparing its effect in this system to a similar one of ribonuclease and desoxyribonuclease respectively. Both these enzymes, according to FRASER and MAHLER[17], are able to penetrate into the spheroplasts of *E. coli* and to inhibit the early stages of phage T_3 development.

It was therefore necessary to investigate the influence of histone on f_2-reproduction following the infection of spheroplasts.

The effect of histone on phage reproduction in RNA-infected spheroplasts: By adding histone to the RNA-spheroplast mixture 5 min after infection, its effect on phage development can be studied. At the fifth minute, the infection of spheroplasts is almost completed, the titre of infective centres reaching 90–95% of the final value. As can be seen in Table II, histone exerts no significant inhibitory effect on the yield of phage particles in the range of 0–100 μg/ml.

Discussion and conclusions. As was demonstrated, histone inhibits the reproduction of phage f_2 in *E. coli* K 13 and the infection of spheroplasts by infectious RNA. Histone, as a basic protein, interacts with the phage capsid and with phage RNA.

The effect of histone depends on the concentration of univalent ions in the mixture, as shown in the detailed studies with polio-RNA (LUDWIG and SMULL[18]), where histone exerts a well-expressed enhancing effect in 0.05 *M* NaCl.

We conclude that the inhibitory effect of histone can be explained by its electrostatic binding to phage capsid alone in the primary phases of infection rather than by the interference with phage synthesis[19].

Zusammenfassung. Histon hemmt die Entwicklung der Phagen f_2 in *E. coli*-K 13-Zellen und in den Spheroplasten, die mit f_2 RNS infiziert wurden. Die inaktivierende Wirkung des Histons auf freie Phagenpartikel und infektiöse RNS wurde beschrieben.

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