The Development of Follicle Cells in the Ovarioles of *Carausius morosus* Br. (Orthoptera, Phasmidae)

The follicle cells surrounding the ovoid-shaped growing oocytes in the panoistic ovarioles of stick insects undergo several changes during oocyte growth[1–5]. The following description deals with the development of typical follicle cells of *Carausius morosus* Br., which ultimately secrete the egg-shell. The cells at the anterior pole of the oocyte behave differently, due to the development of the operculum, and are not considered here.

A few flat follicle cells are found scattered around the early growing oocytes (stage I; Figure 1). They multiply by mitotic divisions and form the one-layered follicle epithelium which is complete before stage II, though cell multiplication continues until stage V. Keeping pace with the growth of the oocyte, the epithelium grows only by cell multiplication up to stage IV, by both cell multiplication and increase in cell size up to stage V, and only by increase in cell size up to stage VIII. The cells elongate in a direction radial to the oocyte causing the epithelium to become thicker. At first they become cubical (stage II) owing to pressure through cell multiplication and then up to stage IV columnar as a result of nuclear growth due to endomitosis. These polyploid nuclei typically develop to a level of ploidy about eight times that of normal somatic cells (based on consideration of nuclear volumes). Cells in which endomitosis begins no longer show normal mitosis. The multiplying cells between the columnar cells lengthen also and may become as long as the latter. Their mitoses are often deformed, for the diameter of the dividing cells (up to 6 μ) is too small for the formation of a normal equatorial plate with a diameter of 9 μ. The equatorial plate and also the anaphase and telophase figures are then oblique with respect to the spindle figure. (The orientation of the mitotic spindles, variable in the thin epithelium, is normally radial between the columnar cells.)

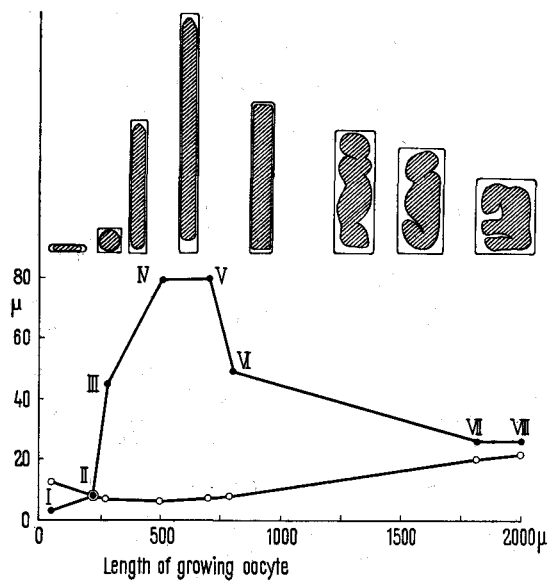

Fig. 1. Diagram showing the development and shape of follicle cells during oocyte growth. ●—● Length of the follicle cell = thickness of the follicle epithelium. o—o Diameter of the follicle cell. ▨ = Nucleus. (For description of individual stages I–VIII see text.)

The cells flatten after stage V. However, cell growth goes on until stage VIII, particularly after stage VI, and involves an increase of cytoplasm as the nuclear volume no longer increases. The cell shortening causes, through pressure, a coiling of the long nuclei (Figure 2a), which becomes evident just after stage VI. Later nuclear shape becomes gradually more variable, this is particularly obvious between stages VII and VIII (Figures 2b, c). These nuclei may appear to be in amitosis[6] when observed in sections (Figure 2d), and DAIBER[1], MARSHALL[2] and CAPPE DE BAILLON[3,4] have remarked that amitosis probably occurs in the follicle epithelium of various phasmid species. In squash preparations, however, these cells can clearly be seen never to contain more than one nucleus and thus amitosis does not occur in *C. morosus*. Only when the mature egg has left the follicle and the follicle epithelium has started to degenerate do the constricted nuclei give rise to smaller ones, which may best be considered as nuclear fragmentation[6] owing to degeneration.

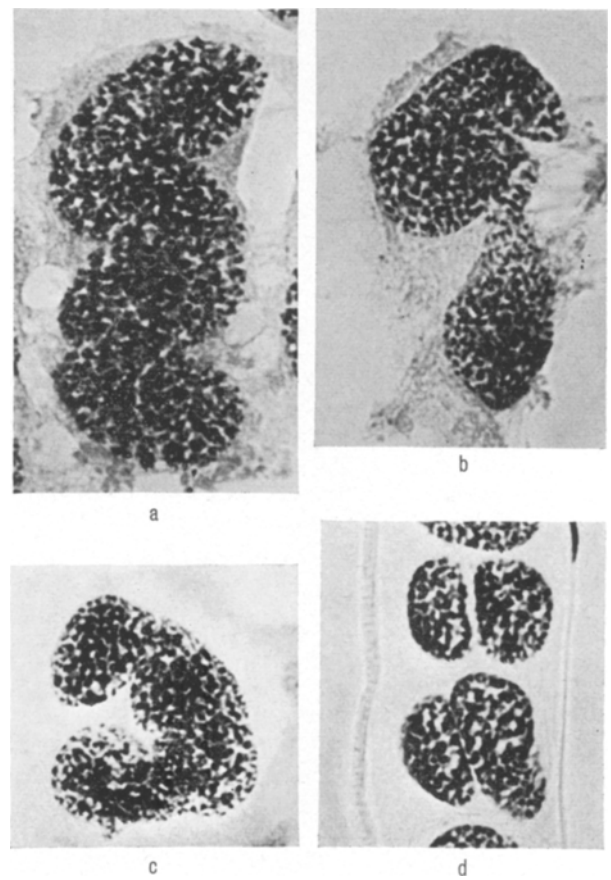

Fig. 2. Follicle cell nuclei as seen in squash preparations (a, b, c) and in section (d). × 710.

[1] M. DAIBER, Z. Naturw., Jena *39*, 177 (1905).
[2] W. S. MARSHALL, Arch. Zellforsch. *3*, 627 (1909).
[3] P. CAPPE DE BAILLON, C. r. Acad. Sci., Paris *196*, 809 (1933).
[4] P. CAPPE DE BAILLON, M. FAVRELLE, and G. DE VICHET, Bull. biol. Fr. Belg. *68*, 109 (1934).
[5] L. P. PIJNACKER, *The Cytology, Sex Determination and Parthenogenesis of* Carausius morosus *Br.* (Thesis, Groningen, 1964).
[6] O. BUCHER, Protoplasmatologia *6*, E/1 (1959).

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.

[1] J. S. COLTER, M. A. DAVIES, and J. B. CAMPBELL, Virology *24*, 578 (1964).
[2] C. SHALITIN, D. DANON, and E. KATCHALSKI, Arch. Biochem. Biophys. *99*, 494 (1962).
[3] S. S. COHEN, J. biol. Chem. *168*, 511 (1947).
[4] T. D. BROCK, Biochem. biophys. Res. Comm. *9*, 184 (1962).
[5] J. SCHINDLER, Folia microbiol. *9*, 269 (1964).
[6] B. L. BROWNSTEIN, Proc. nat. Acad. Sci. US *52*, 1045 (1964).
[7] J. SCHINDLER, Biochem. biophys. Res. Comm. *18*, 119 (1965).
[8] R. C. HUANG, J. BONNER, and K. MURRAY, J. mol. Biol. *8*, 54 (1964).
[9] G. C. BARR and J. A. V. BUTLER, Nature *199*, 1170 (1963).
[10] V. G. ALFREY, V. C. LITTAU, and A. E. MIRSKI, Proc. nat. Acad. Sci. US *49*, 414 (1963).
[11] L. S. HNILICA and D. BILLEN, Biochem. biophys. Acta *91*, 271 (1964).
[12] H. P. HAHN, Exper. *21*, 90 (1965).
[13] J. A. V. BUTLER and E. W. JOHNS, Biochem. J. *91*, 15 C (1964).
[14] S. NEČINOVÁ and M. BURGER, Folia microbiol. *10*, 23 (1965).
[15] A. GIERER and G. SCHRAMM, Nature *177*, 702 (1956).
[16] G. D. GUTHRIE and R. L. SINSHEIMER, Biochem. biophys. Acta *72*, 290 (1963).