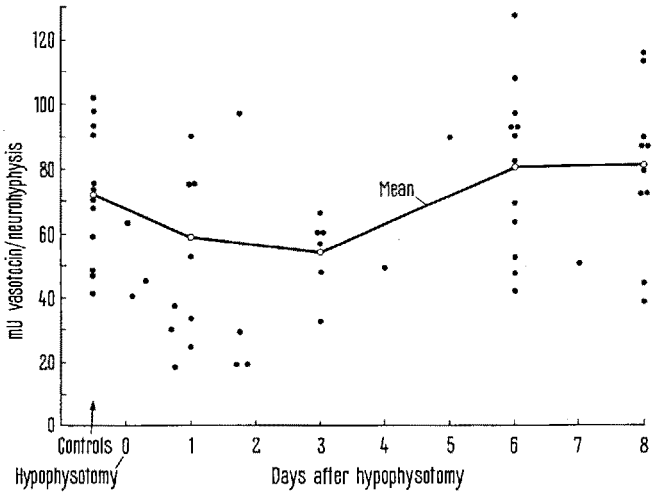

Fig. 7. Vasopressor activity of the distal part of the transected neurohypophysis in animals kept between 8° and 12 °C expressed in mU vasotocin/neurohypophysis plotted against days after hypophysotomy.

confirms observations by Fridberg et al.[13], and others[12]. The exact nature of these vesicles remains to be determined. Even if it is assumed that the primitive neurohypophysial ependymal and neuroglial cells can elaborate granules with hormone activity, it seems very unlikely, due to their small number, that the increase in vasopressor activity could be attributed to the activity of these glial cells.

On the basis of our light and electron microscopic findings, we conclude that the increased amount of PAF plus substance in the distal stump of the transected neurohypophysis is neurosecretory material. A local synthesis of this material is suggested by the increased vasopressor activity found in our bioassays.

Zusammenfassung. Nach Durchtrennung der proximalen Neurohypophyse wird im distalen Stumpf eine gegenüber Kontrolltieren erhöhte Menge paraldehyd-fuchsinpositiver Substanz gefunden, die im Elektronenmikroskop einwandfrei als Neurosekret identifiziert werden konnte. Die im Tierversuch ermittelte erhöhte, blutdrucksteigernde Aktivität des distalen Teils der Neurohypophyse spricht für eine lokale Synthese des Neurosekrets.

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[13] G. Fridberg, R. S. Nishioka, H. A. Bern and W. R. Fleming, J. exp. Zool. *162* (1966).

[14] Supported by NIH Grant No. NB 06641 and the Space Sciences Research Center of the University of Missouri.

DNA Synthesis in the Follicular Cells of *Carausius morosus* Br. (Phasmidae)

A study was carried out to determine the synthesis modality of desoxiribonucleic acid in the nuclei of the follicular cells of *Carausius morosus* using tritiated thymidine. The follicular cells of this insect vary considerably in shape and size during ovocyte growth[1]. At first, when the ovocytes are still very small, the follicular cells are flat and multiply actively by mitotic processes. During the later stages, which can be observed in the larger ovocytes, the nuclei of follicular cells increase in size considerably and arrange themselves perpendicularly on the surface of the ovocyte. The growth of the nuclei in follicular cells is the consequence of repeated endomitotic processes which take place at the same time as the ovocytic growth. Adult individuals of *C. morosus* were injected in the abdomen with tritiated thymidine (Amersham: specific activity 3000 mC/m*M*). Each subject received a dose of 5 μC. The ovaries were removed at intervals varying from 1 h to 4 days after injection and fixed in Carnoy. The slices, 5 μ thick, were coloured with Feulgen and then subjected to autoradiographic processing using liquid emulsion (Kodak NTB2).

Results. The study of the incorporation was carried out on ovocytes 800–1200 μ long. The sections used were mainly those cut perpendicularly to the length of the nuclei. Cylindrical nuclei look round in these sections and their diameters, in one and the same ovocyte, differ visibly. The follicular cells of the operculum, which are of a different shape and size from the other follicular cells, were not taken into consideration here.

The ovocytes examined were divided into 3 classes according to the different length and nuclear volumes of the follicular cells calculated for each class. These volumes are shown in the form of histograms (Figures 1–3). The dotted lines refer to the nuclei which are labelled. The arrows in the histograms indicate the nuclear volume corresponding to certain classes of ploidy. These volumes were calculated by assuming a linear relationship between volume and the level of ploidy and taking the calculated

[1] L. P. Pijnacker, Experientia *22*, 158 (1966).

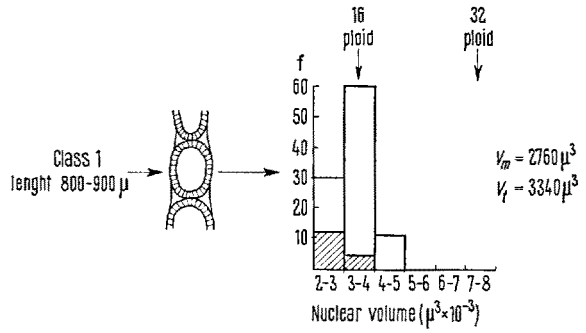

Fig. 1.

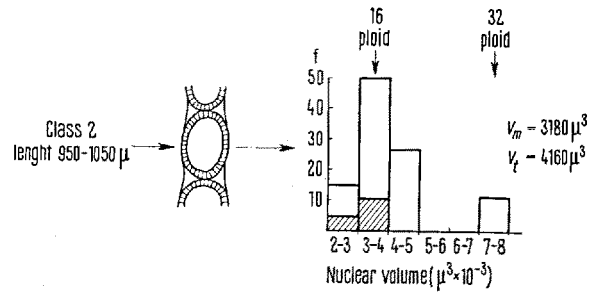

Fig. 2.

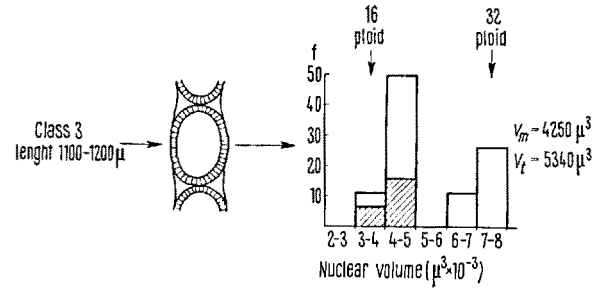

Fig. 3.

[///] labelled nuclei; [ ] unlabelled nuclei; v_m, average of the nuclear volumes of the labelled cells; v_t, general average of the nuclear volumes, f, frequency (%).

value '470 μ^3' for the diploid volume. The average of the nuclear volumes of the labelled cells and the general average of their class, which is always higher, are shown class by class in the histograms. It can be seen from the histograms that tritiated thymidine is preferentially incorporated into those follicular cells which, in each ovocyte, are of smaller volume than the others. This is so of all 3 classes of ovocytes examined.

Conclusions. Follicular cells have nuclei which increase their size gradually with the growth of the ovocyte. Differences in size exist between the nuclear volumes of the follicular cells of a singly ovocyte. The experiments with tritiated thymidine showed that in every class of follicular cells only the minus variants show incorporation. After incorporation there is a gradual increase in the size of the nuclei. Periods of rapid nuclear growth probably correspond to the empty spaces of the histograms of Figures 2 and 3.

Once growth has subsided a new wave of DNA synthesis starts in the nuclei as can be seen from the histograms of the follicular cells of the larger ovocytes.

Riassunto. Le cellule follicolari di un medesimo follicolo hanno nuclei che diversificano notevolmente fra loro per il volume. Ciò è ben osservabile nelle sezioni trasversali dove i nuclei, di forma cilindrica, appaiono circolari. La timidina tritiata viene incorporata preferenzialmente nei nuclei più piccoli. Per spiegare tale fenomeno è stata fatta l'ipotesi di una fase di accrescimento nucleare in assenza di sintesi di DNA.

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23 October 1967.

On the Mechanism of the Stimulating Action of Ribonucleic Acids on the Antibody Responses

The presence in lymphoid tissues and blood serum from immunized animals of a messenger RNA playing an important role in the antibody synthesis has been reported[1-5]. It has also been pointed out by several authors that there is an aspecific stimulating action of nucleic acids and nucleic acid-rich substances on antibody responses to different antigens[6-11]. In the following experiments we shall try to elucidate the mechanism of this aspecific adjuvant action and to show the possible differences between it and that of specific messenger RNA.

Rabbits weighing about 2 kg, fed on a standard diet and subdivided into several groups which were treated with different antigens, were used. Antigens were guinea-pig red blood cells (RBC), horse serum albumin and globulins (HSA, HSG) and bovine serum albumin (BSA) respectively. Nucleic acids used were: RNA from yeast (Schwartz) purified by precipiting and washing with 66% ethanol in the cold, and rabbit's liver RNA obtained with a phenol procedure (tissue was homogenized in ice with 7 vol. of acetate buffer pH 4.5 and then extracted and purified through several steps as described elsewhere[5]); the RNA recovery was about 500 mg/70 g fresh tissue.

RNA and antigens were injected in the following ways: (1) RNA and antigens were incubated for 30 min at 37 °C in separate tubes and then injected or separately or mixed immediately before injection. (2) RNA and antigen were mixed together, incubated for 30 min at 37 °C and then the mixture was injected. The whole cycle of immunization consisted of 4 i.v. injections made at 7 days intervals; circulating antibody titres were determined before each injection and 4 days after the last one, by the usual hemoagglutination technique for RBC and the zonal precipitation technique for soluble antigens.

RNA and soluble antigens were analysed both separately and after incubation together by paper and Tiselius electrophoresis (Perkin Elmer App. mod. 38 A)