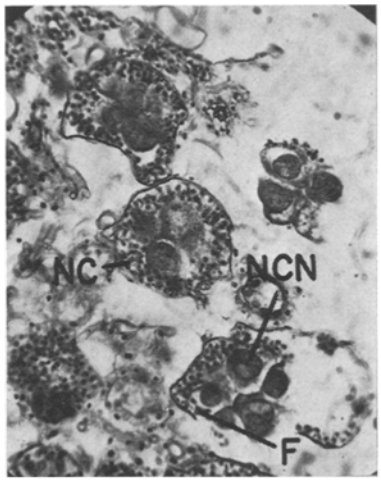

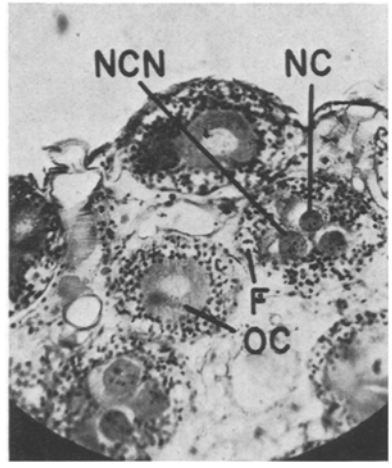

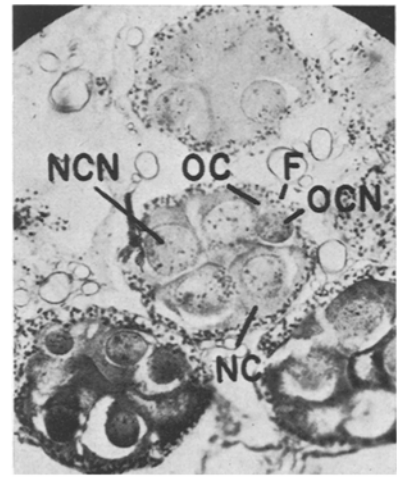

Fig. 4–6. Sections of ovaries of 48-, 72- and 96-h-old *Sarcophaga ruficornis* treated with thiourea.

become smaller in size and finally after 96 h they get squamous.

After 24 h of treatment with 1.5% thiourea, a slight general decrease in the size of ovaries and their components has been recorded. The follicle cells become more columnar and their nuclei get somewhat oval-shaped, showing only 1 or 2 irregularly placed nucleoli. The nurse cells and the developing oocyte do not undergo any marked histological change. After 48 h the follicle cells appear to have lost their cellular arrangement. Their intervening walls disappear, giving rise to a syncytial condition. The nuclei get pycnotic and appear as darkly stained round masses. The developing oocyte and nurse cells show a further decrease in size. The nurse cell nuclei also show some loss of chromatin material (Figure 4). After 72 h the most conspicuous change recorded was the total absence of yolk granules from the ooplasm. The nurse cells and the developing oocyte show a further general decrease in size (Figure 5). The ovaries of *Sarcophaga*, after being treated with thiourea for 96 h, show well marked histological changes. The size of the ovary and ovarioles was greatly reduced as compared to those treated for 72 h. The oocyte now occupies only less than half the follicular space. The nurse cells also get considerably smaller, with great reduction of the chromatin material in their nuclei. No trace of yolk granules was visible in the ooplasm (Figure 6).

Discussion. It has been shown by BORKOVEC[9] that, depending upon dosage and concentration of the chemosterilant used, insects either do not oviposit or the eggs remain infertile. In *Sarcophaga ruficornis* the application of thiourea impairs the development of the ovary, resulting in the formation of inviable eggs.

The primary target of thiourea in the ovary of *Sarcophaga* is the follicle. The transformation of the columnar follicle cells to a syncytial condition with pycnotic nuclei is very significant of their morphological and functional degeneration. It is well known that the follicular epithelium plays an important role in the formation of yolk granules in the ooplasm[10]. The histological damage caused to the follicle cells by thiourea in *Sarcophaga* must, therefore, be acting adversely on the physiological activity of the oocytes. The total disappearance of the chromatin material from the nuclei of the nurse cells in the thiourea treated *Sarcophaga* further indicates the disruption of yet another source of egg nutrition. Such nurse cells, deficient in their nutrient material, obviously do not attain their full morphological and physiological development and thereby impair the normal development of the oocyte as well. The consequent result is that the oocyte remains under-developed in size and cytological organization.

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Nuclear Pores in the Spermatozoon of the Rat

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Summary. The paper describes a hexagonal array of nuclear pores in a non-redundant region of the nuclear envelope underlying the basal surface of the rat spermatozoon head. It is concluded that intranuclear material protruding through these pores is the cause of the characteristic rows of circular 'bumps' found in surface replicas of this region.

WOOLLEY[2] described a region of the postacrosomal surface of the rat spermatozoon whose appearance in surface replicas (as more or less regular rows of circular protruberances about 90 nm in diameter) distinguished it from the postnuclear sheath, from which it was separated by the 'posterior ring'. In this region, which was termed the 'basal surface', there was no cytoplasmic element between the nuclear envelope and the plasma-

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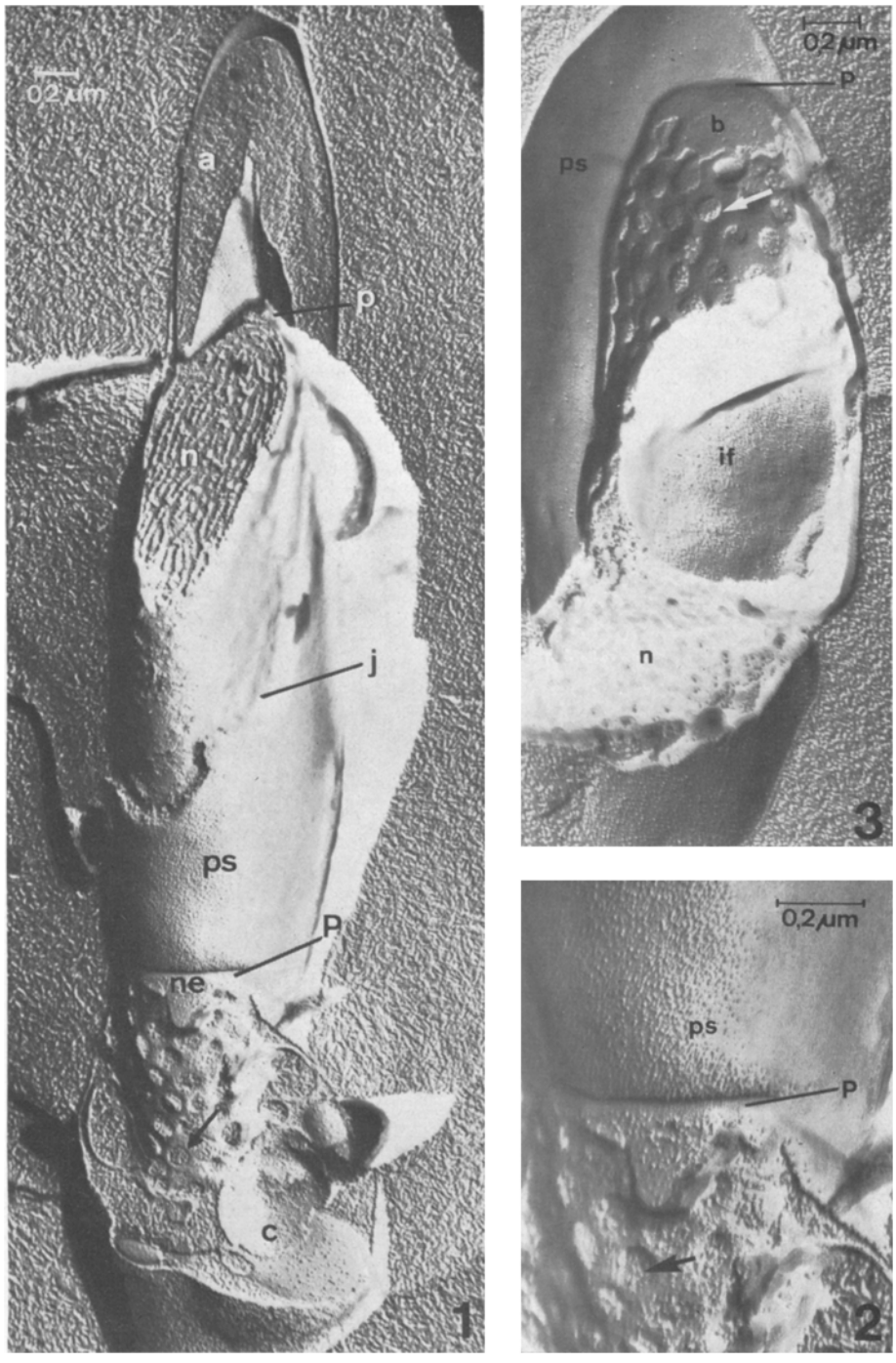

Fig. 1. A view of the head of a rat spermatozoon clearly showing the posterior ring (P) and the nuclear envelope (ne) underlying the basal surface with its nuclear pores (↙) surrounded by the cytoplasmic droplet (c). The acrosome-postnuclear sheath junction (j) is also visible, and the head has been fractured in an oblique transverse direction showing the arrangement of the acrosome (a) and perforatorium (p) around the condensed chromatin of the nucleus (n).

Fig. 2. A higher magnification view of the posterior ring (P) in Figure 1 showing the fine patterning characteristic of this structure when cross-fractured. The plasmalemma has been split along its middle plane over the postnuclear sheath (ps), but completely removed caudal to the posterior ring.

Fig. 3. A view of the posterior region of the sperm head showing the basal surface of the plasmalemma (b) and, beneath it, the nuclear envelope with its pores (↙) in hexagonal array lying between the postnuclear sheath (ps) (delimited by the posterior ring (P)) and the basal plate or implantation fossa (if) where there are no nuclear pores. The nucleus has been cross-fractured to reveal condensed chromatin (n) within this region.

lemma[3]. Evidence presented here, obtained by the freeze-etching method of specimen preparation for transmission electron microscopy, clearly shows that the circular features represent nuclear pores.

Spermatozoa were obtained from the uteri of oestrous females after normal mating and immediately suspended in a cryoprotective mixture of 25% glycerol in 0.9% saline without prior chemical fixation. That the sperm were still motile was checked by observing a sample of the suspension with phase contrast optical microscopy. The suspension was then centrifuged at 1,000 *g* for 5 min, the sperm resuspended in fresh cryoprotectant and centrifuged again. After removal of the supernatant, aliquots of the paste-like sperm concentrate were transferred to small gold discs which were then plunged into liquid Freon 22. Replicas were prepared according to the procedure outlined by THOMPSON[4] using a Balzers 360M freeze-etching plant, and were viewed in an AEI EM6B transmission electron microscope.

This technique revealed circular pores arranged in a regular hexagonal pattern (the usual consequence of the packing together of circles of equal size) in the envelope surrounding the condensed chromatin of the sperm nucleus (see Figure 1). The latter was easily recognised by its characteristic lamellar appearance, analogous to that seen in bull and rabbit spermatozoa[5–7]. Such nuclear pores were confined to that region of the nuclear envelope which directly underlies the basal surface. This is shown in Figure 3, which also shows clearly the so-called 'implantation fossa' or basal plate, this region of the nuclear envelope being devoid of such pores. Evidence that this is truly the basal surface and not a region of membranous scrolls is provided by Figure 2 which shows the fine patterning usually associated with the posterior ring of the spermatozoon in freeze-etch electronmicrographs[5,7].

Nuclear pores have been shown to be present in the region caudal to the posterior ring of spermatozoa of humans[8,9], rabbits[6], rodents[10–12] and water buffalo[7] – but situated in what is usually described as 'redundant nuclear envelope'. This term is generally taken to apply to those parts of the nuclear membrane in excess of that required to enclose the sperm nucleus in its condensed form (e.g. as in the membranous scrolls of the neck region of the spermatozoon). However, in rodent spermatozoa at least, condensed chromatin is present within that part of the nucleus enclosed by the 'redundant nuclear envelope' (see Figure 3, also, for example, Figure 11 of STACKPOLE and DEVORKIN[11]) and therefore the term 'redundant' would not seem applicable.

Therefore it seems that pores appear in the nuclear envelope of the rat spermatozoon in a non-redundant region caudal to the posterior ring. From freeze-etching studies it has become clear that it is the protrusion of intranuclear material through such pores which causes the characteristic topography of the basal surface in surface replicas of air-dried smears of spermatozoa. The great advantage of the freeze-etching method of replica preparation is that the process is a physical one, eliminating the danger of chemical fixation artifacts; although a number of other workers do actually fix their material (e.g. in glutaraldehyde) before freezing. Also, the procedure does not involve a 'drying-down' step, and so removes the possibility of any shrinkage of the chromatin, resulting from such a process, being the direct cause of the origin of the circular features (as has been suggested[2]).

The function of these nuclear pores in the basal region of the spermatozoon head is at present open to speculation, but it may well be the facilitation of the transport of informational and other molecules between the nucleoplasm and the cytoplasm of the sperm cell, not only during the various stages of spermiogenesis, but also in immature spermatozoa within the epididymis and also possibly in mature forms. It is of interest that the basal surface is the only part of the sperm head associated with the cytoplasmic droplet.

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Activité mitotique lors de la période prénéoplasique précédant la cancérisation du foie par la diethylnitrosamine

Study of the Mitotic Activity During the Preneoplastic Period in the Liver of Rats Treated by Diethylnitrosamine

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Summary. The chronic administration of diethylnitrosamine in drinking water induces liver tumors in the rat. The preneoplastic period may be divided in two phases differing mainly in the loss of circadian control of mitotic activity in hepatocytes.

L'administration chronique de nitrosamines per os provoque chez le rat un nombre élevé de cancers hépatiques. L'apparition de tumeurs est précédée par une période prénéoplasique pendant laquelle l'homéostasie du tissu hépatique est probablement modifiée. Nous connaissons peu de choses sur la dynamique cellulaire du foie au cours de cette période. Le cancérigène produit des nécroses qui sont suivies d'une activité mitotique. D'autre part, il apparaît dans le foie des cellules présentant des déficits enzymatiques en glucose-6-phosphatase et en adénosine triphosphatase[1,2]. On ignore si ces cellules restent soumises aux mécanismes qui normalement contrôlent l'homéostasie du tissu.

De tels mécanismes peuvent être mis en évidence non seulement en étudiant la capacité du foie de répondre à une hépatectomie par une activité mitotique mais aussi