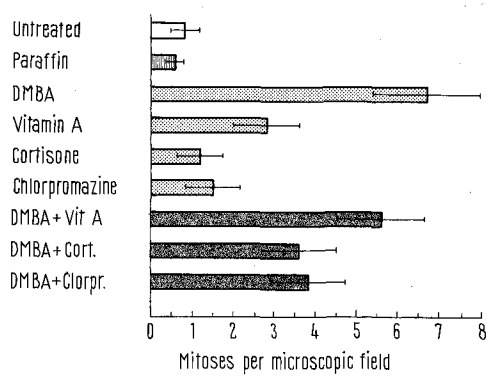
are presented in the Figure, together with the standard errors.

Discussion. After application of DMBA the number of mitoses in the epithelium was 11 times higher than after application of paraffin alone. Vitamin A palmitate also caused an increase in the number of mitoses, to 4.5 times the control level. After cortisone acetate and chlorpromazine the number of mitoses was 2–2.5 times higher than after paraffin only. It is possible that this small increase is in the range of biological variations, and is of no practical importance, since others have shown that both cortisone and chlorpromazine inhibit mitotic activity[7,8].

Treatment with a combination of DMBA and vitamin A palmitate resulted in a mitotic frequency approximately 85% of that found after DMBA alone. It is obvious that vitamin A did not increase the number of mitoses, when applied to epithelium treated with DMBA. Thus the cocarcinogenic effect of vitamin A palmitate during DMBA-carcinogenesis[1–4] does not appear to be due to the increase in mitotic frequency induced by each of these 2 substances separately, but to the increased permeability of cellular and subcellular membranes induced by vitamin A, which probably facilitated easier and more effective penetration of DMBA into the cells.



Mitotic frequency in hamster cheek pouch epithelium after local application of various compounds and combinations during 12 weeks. Averages of 20 fields in 4 animals per group ± S.E.

After treatment with a combination of DMBA and cortisone acetate or chlorpromazine, the number of mitoses was approximately 50% of that found after DMBA alone. Thus it might be possible that the inhibition of carcinogenesis recorded in previous studies[8,9] is due to the depression of the mitotic activity by these 2 compounds. However, although the decrease in the number of mitoses was similar after cortisone acetate and chlorpromazine, the inhibition of tumour formation was much more striking after chlorpromazine. Thus no direct correlation was found between the effect of these 2 substances on the mitotic activity, and their effect on tumorigenesis, and the inhibition of DMBA-carcinogenesis by these compounds is more likely to be due to stabilization of membranes, with decreased permeability and consequent less effective penetration of the carcinogen into the target cell[12].

Zusammenfassung. Die Mitosehäufigkeit im Epithel der Backentasche des Goldhamsters nach 12 Wochen dauernder Behandlung mit Dimethylbenzanthracen (DMBA) Vitamin A-palmitat, Cortison-Acetat, Chlorpromazin sowie Kombinationen dieser Stoffe wurden untersucht und die beobachteten Wirkungen werden mit der Modifikation der carcinogenen Wirkung von DMBA durch die letzteren drei Substanzen verglichen.

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Polyteny and Salivary Gland Secretion in the Melon Fly, *Dacus cucurbitae*

Studies on the functional significance of puffs in the polytene chromosomes of Diptera have shown that they are sites active in RNA synthesis, and thus can be considered as sites of localized gene activity[1]. Appearance of puffs could herald the beginning of new synthetic activities in the cytoplasm, since biochemical studies on puff-RNA have shown that its base composition is similar to DNA, and thus could be considered as specific messenger RNA[2–4].

During the course of the study of the puffing patterns in the salivary gland chromosomes of the melon fly, *Dacus cucurbitae*, 3 peaks of puffing activity were noticed: one in the early third instar larvae (120 h after oviposition), another in the mid-third instar larvae (168 h) and the third at the end of the larval life, just before pupation (240 h)[5]. In order to investigate the relationship of these peaks in puffing activity to the cytoplasmic events, functional characteristics of the glands were studied with histochemical methods over the period from the beginning of larval life to pupation.

Breeding and synchronization of larval development was performed as described in an earlier paper[6]. For the determination of carbohydrate and protein-containing material, histochemical tests as stated in PEARSE[7], with appropriate controls, were performed on salivary glands fixed in San Felice's fixative. Autoradiography, determination of DNA content, and nuclear volumes were performed as previously described[6,8].

Observations and discussion. The salivary gland secretion in *Dacus* is PAS-positive, resistant to diastase treatment and is not completely removed by chloroform-methanol. This indicates the absence of glycogen and glyco-

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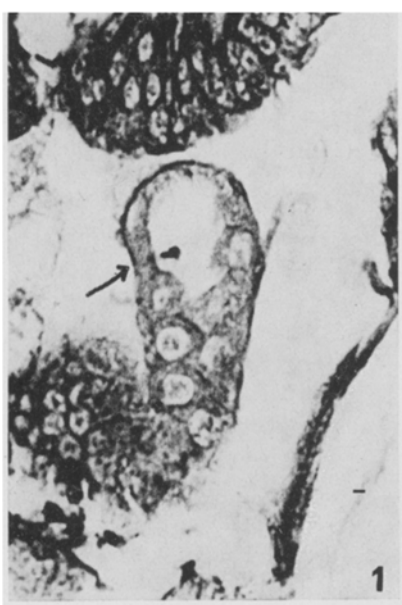
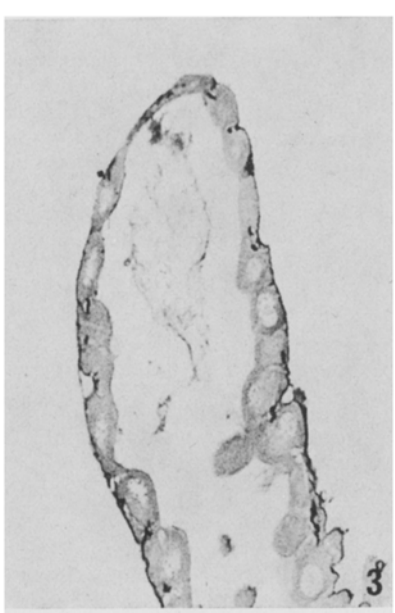
lipids in the secretion. The PAS reaction was blocked by acetylation, but was revived after alkali treatment, indicating the presence of 1:2 glycol groups. The secretion did not stain with alcian blue nor did it react on a dialysed-iron treatment, indicating the absence of acid-mucopolysaccharides. The secretion is bromophenol blue-positive but negative to Millon's reaction to tyrosine, which indicates the presence of proteins but the absence of tyrosine in the secretion. It may be concluded that the salivary gland secretion in *Dacus cucurbitae* is a glyco- or mucoprotein.

The secretory epithelium and the glandular lumen are PAS-negative from the beginning of larval life up to 168 h (Figure 1 and 2). The cytoplasm in the earlier stages (up to 168 h) is faintly PAS-positive (Figure 1 and 2). Furthermore, the cytoplasm is bromophenol blue-positive in the earlier stages. At about 168 h, the larvae stop feeding and leave the food-medium. Simultaneously, there is an increase in the PAS-positive material in the cytoplasm, and secretory material, with characteristics similar to the material previously present in the cytoplasm, starts appearing in the glandular lumen (Figure 3 and 4).

Concurrent with the appearance of the secretion in the glandular lumen, a large number of puffs are noticed in the salivary gland chromosomes. The 45 changes in puffing pattern recorded at this time are far more than those that occur in any of the other stages in which puffing activity patterns were followed[5]. The appearance of a large number of puffs and a shift in cytoplasmic activities, like the synthesis of mucoproteins, may not be just casually coinciding events. It is, therefore, tempting to consider some of these puffs, at least, as being required for the synthesis and transport of the mucoprotein secretion in *Dacus*.

The salivary gland secretion in *Dacus* starts after DNA synthesis in the chromosomes has been completed (Figure 5)[6]. This indicates that the cells synthesize and secrete the mucoprotein only after they have reached their





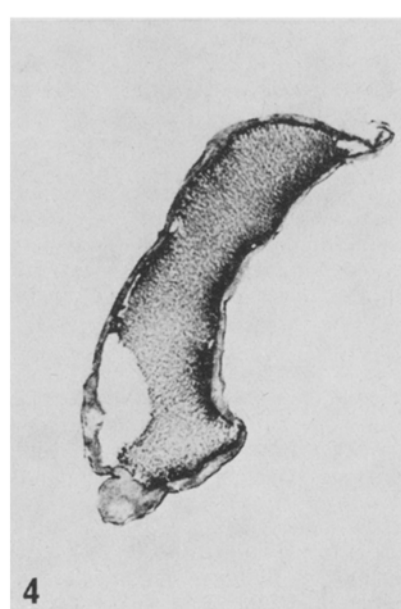


Fig. 1–4. PAS-Hematoxylin stained sections.
Fig. 1. 96 h old salivary gland (indicated by arrow) showing the absence of secretory material in the lumen. ×550.
Fig. 2. 144 h old salivary gland showing PAS-positive cytoplasm and negative lumen. ×550.
Fig. 3. 168 h old salivary gland showing secretory material beginning to appear in the glandular lumen. ×130.
Fig. 4. 240 h old salivary gland showing the glandular lumen being completely filled with PAS-positive material. ×130.

maximum ploidy levels. At this stage the salivary gland nuclei incorporate ^{3}H-thymidine only in about 2% of the cells and there is no further increment in the DNA content of the glands (Figure 5). BERENDES[9] reported a similar case in *Drosophila hydei*. Recently, POELS[10] and POELS et al.[11] have shown that the synthesis and secretion of the mucopolysaccharide substance in *Drosophila hydei* are closely related with the presence of ecdysterone in the hemolymph. They have further shown that the hormone not only inhibits the synthesis of the secretory product, but also stimulates the secretion of the already synthesized material. We are currently investigating which of the two processes (cessation of DNA syntheis or stimulation of secretory activity) is under the influence of ecdysone, in *Dacus*.

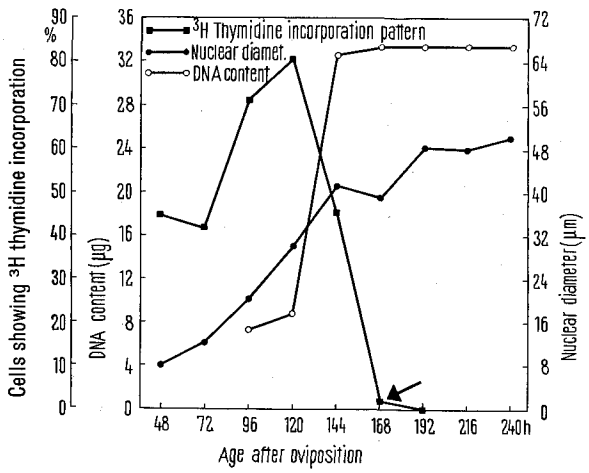


Fig. 5. Graph showing the changes in DNA content (100 pairs of glands), ^{3}H-thymidine incorporation pattern and nuclear diameter during larval development of *Dacus cucurbitae*. Arrow indicates the time at which secretory material starts appearing in the glandular lumen.

Zusammenfassung. Die Speicheldrüsenabsonderung der Melonenfliege, *Dacus cucurbitae*, besteht aus Muco- oder Glycoproteinen. Ein Maximum der Puffing-Aktivität der Chromosomen fällt mit dem Auftreten der Sekretsubstanz im Lumen zeitlich zusammen. Die Sekretion der Drüse beginnt erst, nachdem die Chromosomen ein Maximum des Polytäniegrades erreicht haben.

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Effect of Phenobarbital on Growth of a Metastasising, Allogeneic Sarcoma in the Rat

Barbiturates cause reversible depression in vitro and in vivo of a wide range of biological functions in various mammalian tissues, including those concerned with immunological reactions, principally by interfering with oxidative and Embden-Meyerhof glycolysis and obstructing the generation of energy rich phosphate[1,7]. Such metabolic obstruction by sodium phenobarbital has been shown to depress the formation of ribose, NADPH and ATP in lymphocytes, and consequently inhibits generation of oxy- and deoxy-nucleoside triphosphates necessary for DNA synthesis[2–4,7]. These metabolic effects of phenobarbital appear to explain its immunosuppressive action in vitro and in vivo, which has been reported to occur with dosages prescribed in conventional clinical practice[5] and also post-operatively in patients after general anaesthesia and surgery[6]. Thus concentrations of phenobarbital of 1.5 µg/ml, which is equivalent to those produced in human tissues by therapeutic dose of the drug, reduced incorporation of ^{14}C-thymidine into DNA of lymphocytes cultured in the presence of phytohaemagglutinin by nearly 90%, and 1.5 mg phenobarbital/kg body weight prevented the delayed-hypersensitivity reaction to dinitrochlorobenzene in rabbits[5].

Barbiturates are widly used as sedatives and for induction of anaesthesia in patients with malignant disease, in which cell-mediated immunity may influence rates of tumour growth and dissemination. Therefore, it was decided to study whether sedative, but non-toxic doses of phenobarbital administered to rats daily would reduce their partial immunity to an allogeneic solid sarcoma (Y-P388) which has been found to metastasis rapidly and regularly to lymph nodes and lungs[8]. This tumour is clonogenic in vivo in that single sarcoma cells injected i.v., form macroscopic tumour colonies in high yield in the lungs, which are readily counted as pleural nodules after 6–7 days growth. Spontaneous dissemination of tumour cells to the lungs causes similar colonies to form where cells arrest and clone.

Female Caworth Farm strain rats from a specific pathogen free derived colony, 30 days old and weighing approximately 90 g were used for tumour transplantation. Techniques for weighing the primary tumour (Pr) following inoculation of Y-P388 cells into leg muscle and the principal metastases produced in regional groups of lymph nodes (ipsilateral crural CN, lower abdomino-pelvic PN and upper abdominal UAN) to which the tumour spreads, have

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