generationen, wie sie in der Literatur nach DÄNA beschrieben sind[3,4]. Bei den mit Thymusinjektionen vorbehandelten Ratten fanden sich keine Carcinome, ein nur geringes Ausmass an Degenerationen und als Zeichen einer abgelaufenen Parenchymschädigung atrophische Bezirke und Leberregenerate. Diese Befunde sind in der Tabelle zusammengestellt. Die Untersuchung zeigte, dass toxische Leberdegenerationen noch progredient sind, wenn die Vergiftungsperiode viele Monate oder 1 Jahr zurückliegt und nicht nur von der Dosis abhängen. Denn die Leberschädigungen sind bei den später getöteten Tieren schwerer und ausgedehnter als bei den früher getöteten.

Es ist eine gewisse Reaktionslosigkeit des Organismus dem angewandten Carcinogen gegenüber erkennbar, während die vorbehandelten Tiere gegenüber der toxischen Einwirkung stärkere Reaktivität und Regenerationsbereitschaft der Leber zeigten. Es führen nämlich die Degenerationen des Leberparenchyms zu Nekrobiosen, ohne dass eine zelluläre Reaktion darauf erkennbar wäre, während bei den vorbehandelten Tieren in solchen Fällen eher eine Ansammlung von Kupferschen Sternzellen und Regenerate von Zellbalken zu finden sind. Der Schutz, den die beschriebene Vorbehandlung hinsichtlich des Leberparenchymschadens und der Carcinomentstehung ausübt, ist möglicherweise einer immunologischen Sensibilisierung gegen neu auftretende Zellmutanten zuzuschreiben. Denn die hier beobachteten praecancerösen Parenchymveränderungen wurden in der Literatur wiederholt beschrieben als Folge von Mutationen im genetischen Apparat der Zelle, welche zu Regulationsstörungen wie Enzymdefekten nnd Spezifitätsänderungen führen[5–7].

Summary. Rats treated by i.p. injections of a homologous thymus are protected, probably by greater reactivity, against the toxic and carcinogenic influence of diethylnitrosamine on the liver, effects that increase with time in untreated controls, long after cessation of drug administration, as is shown by the greater extent of degeneration in animals killed at longer than at shorter intervals.

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The Effect of Membrane-Active Agents on the Mitotic Frequency in Normal and Carcinogen-Treated Epithelium

In previous studies, the effect of membrane-active substances on hamster cheek pouch carcinoma induced with 9,10-dimethyl-1,2-benzanthracene (DMBA) was investigated and it was shown that topical vitamin A palmitate resulted in increased tumour formation[1–4]. It was proposed that this co-carcinogenic effect might be related to the membrane-labilizing properties of this vitamin, resulting in activation and release of lysosomal enzymes, which may play an important role in the initial stages of cell division and tumorigenesis[5,6].

Subsequent studies, using the membrane-stabilizing agents cortisone acetate and chlorpromazine, known to suppress mitotic activity[7,8], demonstrated inhibition of DMBA carcinogenesis in the hamster cheek pouch[9,10]. This effect was interpreted as being related to the membrane-stabilizing properties of these agents.

In the present study, the effect of the above membrane-active substances on the mitotic frequency was investigated in normal and DMBA-treated hamster cheek pouch epithelium, in order to determine whether there is a relation between the effect of these substances on the mitotic frequency, and their promotion or inhibition of tumour formation.

Material and methods. Groups of 4 male Syrian golden hamsters, 55–65 g body wt., were treated during 12 weeks with the following substances or combinations: liquid paraffin; DMBA 0.5% in liquid paraffin; vitamin A palmitate 20% in liquid paraffin; cortisone acetate 0.05% in liquid paraffin; chlorpromazine 1.2% in liquid paraffin; DMBA 0.5% and vitamin A palmitate 20% in liquid paraffin; DMBA 0.5% and cortisone acetate 0.05% in liquid paraffin; DMBA 0.5% and chlorpromazine 1.2% in liquid paraffin.

The solutions and suspensions were administered 3 times per week to the right cheek pouch with a paint brush. At the end of the experimental period the animals were sacrificed. Groups of untreated animals served as controls. 4 h prior to sacrifice each animal received an i.p. injection of vinblastine (0.5 mg/100 g body weight, as a 0.1% solution in saline), which facilitates easier visualization of mitoses in the epithelium[11].

Histological sections of the treated cheek pouches were stained with hematoxylin and eosin, and were studied at a magnification of ×400. The epithelial layer was brought in the equator of the microscopic field, and the number of mitoses in this field was counted. This was repeated for 20 consecutive microscopic fields, excluding fields containing tumour tissue.

Results. The number of mitoses per microscopic field was determined in each animal, and the average values per microscopic field for each group (i.e. the average number of mitoses in 20 microscopic fields in each of 4 animals)

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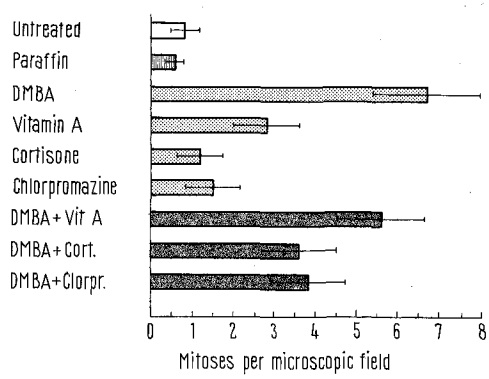
are presented in the Figure, together with the standard errors.

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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