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*.

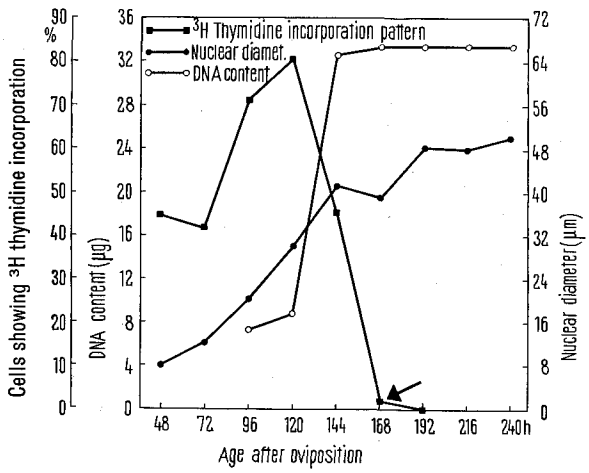

Fig. 5. Graph showing the changes in DNA content (100 pairs of glands), ^{3}H-thymidine incorporation pattern and nuclear diameter during larval development of *Dacus cucurbitae*. Arrow indicates the time at which secretory material starts appearing in the glandular lumen.

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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Table I. Effects of wholebody irradiation (WBI)[a] and daily injections of phenobarbital (1.5 mg kg^{-1} i.m.)[b] on growth of primary tumour (Pr) and its metastases in lymph nodes and lungs in rats following inoculation of right leg with 10^4 Y-P388 cells

Treatment	ΔW (g)	W_2 (g)	Tumour weights ($g \times 10^2$) Pr	CN	PN	UAN	Organ weights ($g \times 10^2$) Spleen	Thymus	N_L
Nil (killed day 7)	+24 ± 1	135 ± 2	85 ± 11	1 ±0.5	3 ±1.0	2 ±0.3	65	33	0.0
WBI (killed day 7)	+17 ± 3	122 ± 5	126 ± 16	4 ±0.5	8 ±1	3 ±0.7	25	20	8 ± 1 (6,5,3,13,9,13)[c]
Water (killed day 10)	+42 ± 4	128 ± 2	115 ± 11	4 ±0.7	12 ±2	3 ±0.5	58	47	0.3 ± 0.2 (1,1,0,0,0,0)
Phenobarbital (killed day 10)	+41 ± 2	130 ± 3	110 ± 7	5 ±0.5	13 ±2	3 ±0.7	60	39	0.7 ± 0.3 (1.2.1.0.0.0)

[a] 570 rads (^{60}Co γ-rads) 24 h preceding tumour inoculations. [b] Commenced 1 day preceding tumour inoculations and terminated 7 days after inoculations. [c] Number of lung colonies counted in individual rats (in brackets). Tumour growth measured 7 days after WBI and 8 days after commencing phenobarbital treatment, respectively. Pr, CN, PN, UAN represent primary tumour and crural, pelvic and upper abdominal lymph nodes, respectively; ΔW and W_2, gain in body weight after inoculation and final body weight; N_L, mean number of tumour colonies for lungs. Each group consists of 8 rats.

been described in detail previously, as well as counting and incidence of lung metastases[8]. Tumour colonies produced in lungs by i.v. inoculation of $5 \times 10^2 - 10^5$ tumour cells were counted 7 days after inoculation, a) after rats were injected with 1.5 mg/kg phenobarbital daily for 8 days, the first injection being given 1 day prior to inoculation with tumour and b) after rats were exposed to whole body irradiation (WBI) from a ^{60}Co source 24 h preceding inoculation to suppress immunity. Effects of phenobarbital and WBI on spontaneous spread of the tumour to lymph nodes and lungs were measured after inoculating the leg muscle with 10^4 cells and killing the animals 7 days (WBI groups) and 10 days (phenobarbital treated groups) later, respectively. Treatment with phenobarbital had no significant effect on rate of growth of primary tumour in muscle, nor on growth of metastases in lymph nodes and lungs (Table I); nor did it significantly affect rates of gain in body weight of rats or the weights of spleen and thymus. Prior exposure of rats to sublethal whole body X-irradiation (570 rads ^{60}Co γ-rays) to suppress immunity caused significant increases in growth of Pr and metastases (including lung metastases) as reported previously[8].

When the tumour was assayed intravenously, to measure survival and growth of individual cells arrested in the lungs, treatment with phenobarbital was found to cause significant increases in cell survival (Table II). Approximately double the number of colonies were present 7 days following inoculation 10^3 and 10^4 tumour cells. Too many colonies to count had formed after 10^5 cells were incoulated, in both phenobarbital treated and control groups, and caused mean increases in lung weight of nearly 50%. Immunosuppression by WBI given 24 h before inoculation had a more marked effect than treatment with the barbiturate in increasing the yield of lung colonies when a low number (5×10^2) of tumour cells were injected intravenously.

The i.v. technique of assaying this antigenic tumour provides a more sensitive means of measuring immunity to its growth in lungs than spontaneous dissemination to the lungs from a primary solid tumour, since growth of the latter in situ stimulates immunity to further growth of the tumour; it causes immunosurveillance to develop rapidly and effectively destroy a considerable proportion of tumour cells deposited as single or small groups of cells in the various organs, and particularly in lymph nodes. Thus in the immunologically intact animal, growth of Pr stimulates immunity. This allows relatively few cells to leave the lymph nodes, enter thoracic duct lymph and thus be car-

Table II. Effect of phenobarbital[a] given daily to female rats inoculated intravenously with 10^3–10^5 Y-P388 tumour cells, on growth of lung tumour colonies scored 7 days post incoculation

N	Treatment	ΔW	W_2	Mean organ weights ($g \times 10^2$) Spleen	Thymus	Liver	Lung	N_L
10^3	Water	+23 ± 2	113	47	35	463	89 ± 4	5.5 ± 2.0
10^3	Phenobarbital	+34 ± 1	113	54	41	464	91 ± 1	11.5 ± 2.9
10^4	Water	+28 ± 3	118	53	43	489	93 ± 3	64 ± 15
10^4	Phenobarbital	+30 ± 3	116	66	47	469	93 ± 4	128 ± 37
10^5	Water	+30 ± 1	116	60	42	485	145 ± 20	>500
10^5	Phenobarbital	+37 ± 2	118	65	40	500	135 ± 14	>500
5×10^2	Nil	+38 ± 1	129	62	38	—	92 ± 3	5.5 ± 1.5
5×10^2	WBI[b]	+24 ± 3	110	18	15	—	90 ± 1	25 ± 3

[a] phenobarbital (1.5 mg kg^{-1}) injected i.m. daily for 8 days, rats receiving the first dose 1 day preceding inoculation; controls received equal volumes of destilled water daily. [b] 570 rads (^{60}Co γ-rays) whole body irradiation given 24 h preceding inoculation with tumour cells. Results compared with incidence of lung colonies in untreated rats inoculated with 5×10^2 tumour cells and in immunosuppressed rats exposed to wholebody irradiation (WBI). ΔW (g), mean increase in body weight 7 days post inoculation; W_2 (g) final body weight; N, number of tumour cells inoculated; N_L, mean number of lung tumour colonies per rat.

ried via the blood to the lungs. Cells which remain viable, and are arrested, face further exposure to well developed immunosurveillance, which reduces colony formation further.

It is concluded that phenobarbital, at therapeutic levels of dosage used in humans, caused modest increases in growth of single tumour cells in rats and could conceivably reduce the effectiveness of immunosurveillance in patients with antigenic tumours. However, should some spontaneous tumours be antigenic and induce a significant autoimmune response in the host of origin, early stimulation of such immunity by growth of the primary tumour is likely to occur. In this situation the modest immunosuppressive effects attributed to barbiturates and presumably due to their general cytotoxic and cytostatic actions are unlikely to be of significance, since a potent immunosuppressant (sublethal whole body irradiation) is also relatively ineffective in reducing immunity to a transplanted tumour once tumour growth causes immunity to become established and presumably the same conditions would apply to autochthonous growth in humans if immunity similarly depends on cell mediated reactions.

Résumé. Chez des rats traités au phénobarbital (1.5 mg/kg par jour) on a trouvé une faible augmentation du nombre de colonies produites dans les poumons des rujets inoculés, par voie intraveineuse avec les cellules Y-P388 (un sarcome métastasisant, allogénique). Cet effet semble être dû aux propriétés immunosuppressives de la drogue[5]. Celles-ci cependant paraissent faibles, puisque le phénobarbital n'a pas stimulé l'accroissement d'une transplantation primaire de Y-P388 dans le muscle ni ses métastases aux nodules lymphoïdes et aux poumons.

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Mapping of Central Monoamine Neurons in the Monkey

The distribution of central monoamine terminals[1,2] and cell bodies[3] have been described in detail in the rat. However, no comparable histochemical fluorescence studies have been reported in the monkey. Thus, it has only been reported in the monkey that a lesion placed in the ventromedial tegmental area involving the medial aspect of the substantia nigra produces a marked decrease in the dopamine (DA) content of the ipsilateral corpus striatum[4,5], whereas a nigro-neostriatal DA pathway has been described in the rat[6]. The present paper shows that the distribution of the monoamine neurons in the monkey resembles in many aspects the distribution of these neurons in rats.

Adult monkeys Macaca-Irus (cynomolgus) and the African Green Monkey (*Cercopeithecus sabaeus*) ranging from 2.5 to 4 kg body wt. were used. The monkeys were anesthetized with barbiturates given i.v. in order to perform as much as possible of the brain dissection in vivo. By means of serial transverse sections, the brains were divided into slices about 4 mm thick. Various anatomical areas in the transverse sections were then isolated and divided into small 4 mm cubes. These were rapidly frozen in liquid propane and processed for histochemical fluorescence analysis of monoamines[7–9].

The distribution of the catecholamine (CA) and 5-hydroxytryptamine (5-HT) cell bodies in the lower brain stem of the monkey was similar to that in the rat. The CA cell bodies were localized to the lateral reticular formation of the medulla oblongata and the pons, within the locus coeruleus (Figure 1), the subcoeruleus area (Figures 1 and 2), the substantia nigra, the ventromedial part of the cranial mesencephalon (particularly the nuc. paranigralis, nuc. pigmentosus parabrachialis) and the nuc. arcuatus. It should be noted that the number of CA cell bodies in the subcoeruleus areas of the monkey constituted a larger part of the CA cell population than in the rat. The 5-HT cell bodies were distributed in the raphe nuclei of the lower brain as described previously in the rat. No 5-HT cell bodies, however, were found in the nuc. ruber which by JONES[10] has been claimed to contain 5-HT cell bodies in the rat.

Three different kinds of CA nerve terminals were observed. One type consisted of very fine nerve terminals (varicosities range mainly from 0.3–0.7 μm) which were

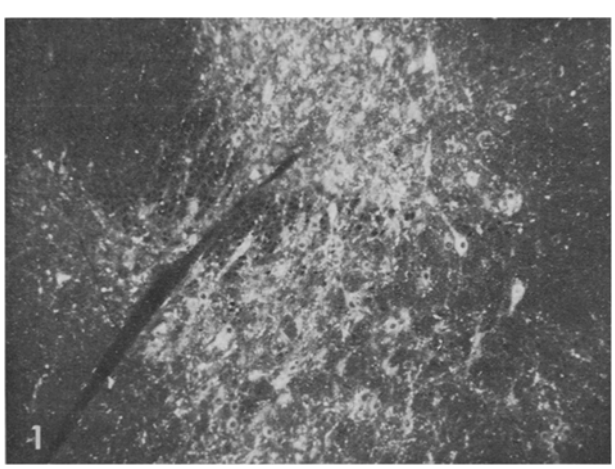

Fig. 1. The locus coeruleus and the subcoeruleus area of the African Green Monkey. A large number of nerve cell bodies with a specific green CA fluorescence of moderate intensity are observed. ×120.

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