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

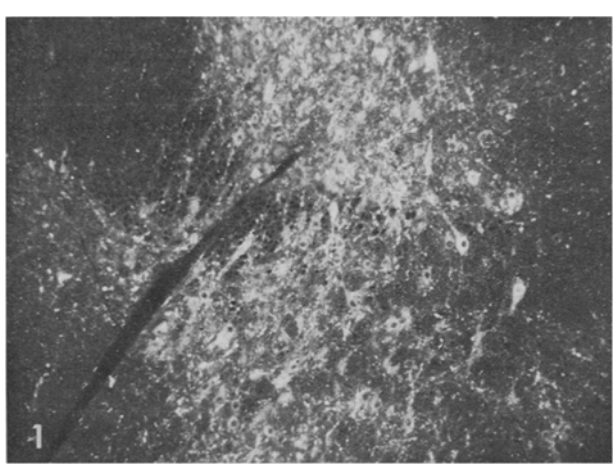

Fig. 1. The locus coeruleus and the subcoeruleus area of the African Green Monkey. A large number of nerve cell bodies with a specific green CA fluorescence of moderate intensity are observed. ×120.

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scattered in practically all parts of the cortex cerebri; they were also present in all cortical layers (Figure 3). These terminal plexa resemble the very fine cortical NA plexa found in the rat[2,11]. Also very fine densely packed CA nerve terminals were found in the neostriatum as has been observed in the rat[6] (Figure 4). Since high amounts of DA are found in the basal ganglia[12,13], these nerve terminals probably contain DA. The second type is fine to fairly large CA nerve terminals (varicosities range mainly from 1–1.5 μm) which mainly innervate the hypothalamus (Figure 5); dense meshworks of these terminals are also found in many areas of the preoptic region and subcortical parts of the limbic system. In view of the high amounts of NA found in these areas[14], these plexa are probably noradrenergic and are similar to those found in the same areas in the rat[1,2]. The third type consists of nerve terminals with a very strong fluorescence and varicosities, which show characteristic alterations in thickness from fine (around 1 μm) to thick (1.5–2 μm). The thick varicosities have the strongest fluorescence intensity. They are found scattered in many brain areas: e.g. the cortex cerebri, the thalamus and the globus pallidus. Such terminals have not been observed in the rat. However, they are present in various regions of the human brain[15]. They resemble the processes of the small intensely fluorescent cells in the ganglia which probably are part of a primitive nerve cell system[16]; these new types of terminals therefore may belong to phylogenetically older neuronal systems than the other monoamine terminal systems described.

The 5-HT nerve terminals were mainly observed in certain brain stem areas as found previously in the rat[1,2]. For example, a high density of 5-HT nerve terminals was observed in the nuc. suprachiasmaticus.

In sagittal sections through the lower brain stem also CA nerve bundles could be observed having a weak to mo-

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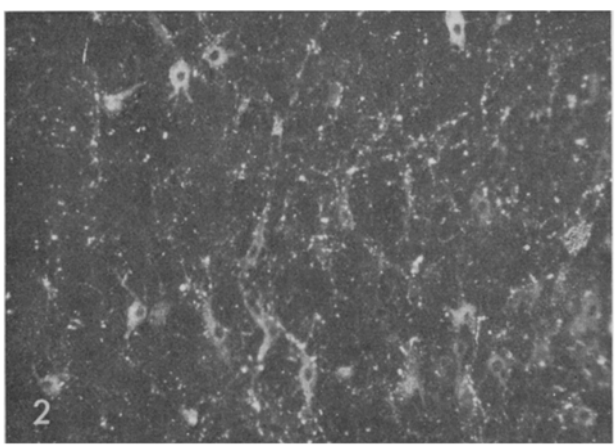

Fig. 2. The subcoeruleus area of the African Green Monkey. Nerve cell bodies with a specific green CA fluorescence of weak to moderate intensity are observed together with fine, varicose CA nerve terminals making close contacts with the CA cell bodies. ×300.

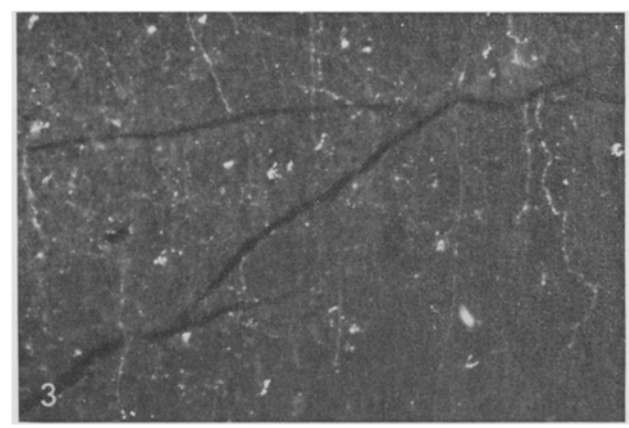

Fig. 3. The parietal cerebral cortex of the African Green Monkey. A plexus of very fine varicose nerve terminals with a specific green CA fluorescence is observed. ×260.

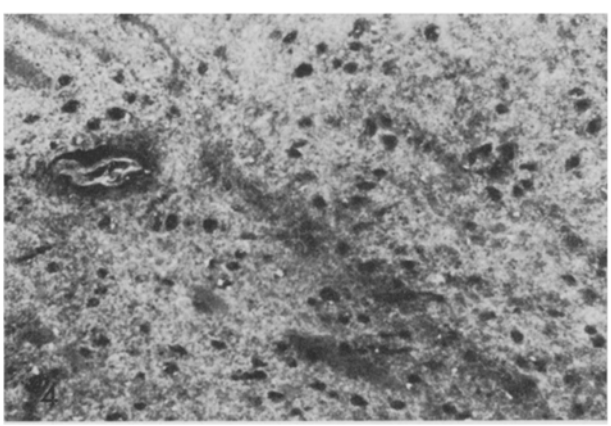

Fig. 4. The caudate nucleus of the African Green Monkey. A dense plexus of very fine dots with a strong specific CA fluorescence is observed probably representing closely packed CA nerve terminals. ×260.

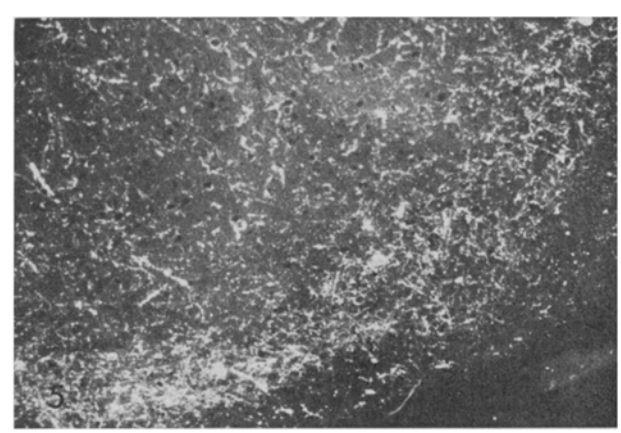

Fig. 5. The premammillary area of the African Green Monkey. Plexa of fine to fairly thick varicose nerve terminals with a strong specific CA fluorescence are observed especially in relation to the ventral border of the hypothalamus. ×100.

derate green fluorescence, the individual fibers having varicose-like enlargements. The fibers could be seen to traverse the reticular ventral nucleus in the medulla oblongata, the parvocellular reticular nucleus of the pons, the caudal and oral reticular nucleus of the pons, medial to nuc. mot. n. trigemini, and nuc. subcuneiformis of the mesencephalon. The course of these fibers is very similar to that of the ascending noradrenaline (NA) tracts in the rat[17,18]. SLADEK[19] has recently described this tract in kittens but interpreted it to represent CA nerve terminals due to the strong fluorescence in the varicose-like enlargements. It is known, however, that in young animals the fiber tracts have a relatively strong fluorescence intensity[20,21].

The present data indicate that in the monkey the principal architecture of the central monoamine neurons is similar to that in the rat and other mammals such as the rabbit and the cat. Thus, the CA and 5-HT neurons in the monkey are reticular lower brain stem neurons with inter alia long ascending monosynaptic connections with the tel-diencephalon[22].

Zusammenfassung. Verteilung und Morphologie von Katecholamin-(KA) und 5-Hydroxytryptamin-Neuronen im Affengehirn stimmt mit früheren Befunden über das Rattengehirn gut überein. Die Zahl der KA-Zellkörper in Area Subceorulea ist jedoch bedeutend grösser bei Affen, und eine neue Art terminaler KA-Nervenfasern von starker Fluoreszenzintensität und im Durchmesser variierender Varikosität wurde aufgefunden.

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[22] Acknowledgments. This work has been supported by a grant No. B72-14X-715-07 from the Swedish Medical Research Council, and by USPHS Grant NS 06801.

Alleviation of the Toxicity of Actinomycin D by Uridine and Thymidine on the Morphogenesis of Chick Embryos Cultivated in vitro

Teratogenic effects of Actinomycin D in various ambryonic systems have been reported by BRACHET et al.[1], BRACHET and DENIS[2], WALLACE and ELSDALE[3], FLICKINGER[4,5], and SAMESHIMA et al.[6] in amphibia and PIERRO[7,8], KLEIN and PIERRO[9], POHL[10], and GALLERA[11] in chick embryos and GROSS[12] and COUSINEAU[12] in sea urchin embryos.

The malformations in amphibians and chick embryos involved the nervous system, the eyes and cardiovascular system etc. In the present work actinomycin D has been shown to cause similar abnormalities in the chick embryos and can be reversed to a greater extent with subsequent treatment with thymidine or uridine.

Materials and methods. Fresh and fertilized eggs of white leghorn hens were obtained from a local farm and incubated at 37.5 ± 1 °C for required number of hours so as to obtain the proper stage for experiments. The experiments were performed at 2 different stages of development namely 1. primitive streak stage and 2. head process stage (stages No. 4 and No. 5, respectively, HAMBURGER and HAMILTON[13]). The glassware employed in the experiments was sterilized and the culturing solutions were autoclaved.

Stock solution of Actinomycin D (Merck, Sharp and Dohme) (500 µg/ml) was suitably diluted to give an effective concentration (0.05 µg/ml).

The embryos were explanted by the method of NEW[14] and treated with the above concentration of actinomycin D. Care was taken to add the antibiotic gently by the side of the blastoderm so that it is evenly exposed to the antibiotic. The preparation was kept at room temperature for 1 h for proper diffusion of the antibiotic before incubation. After 6 h of incubation with the antibiotic, the embryos were plunged 3 times separately into fresh PC saline to remove the antibiotic completely. The embryos were then divided into 2 experimental groups A and B in series 1. In group A the embryos were mounted in PC saline only. These embryos served as controls. In group B, the embryos were subsequently exposed to uridine (0.05 µg/ml). In series 2, the controls were run (A_1) as in the above series and the embryos in experimental group were subsequently treated with thymidine (0.05 µg/ml) (B_1) instead of uridine. 30 embryos were used in each set of controls and experimentals.

Identical sets of experiments were done using head process stage of embryos (stage V), first treated with actinomycin D and then subsequently treated either with uridine or thymidine at the same concentration (Series 3 and 4).

It is seen in the present work that actinomycin D causes microcephaly in 60% of the embryos treated at the primitive streak stage, inhibits the formation of somites and heart in 63% and 80% cases, respectively (Figure 1). Shortening of axis is also observed to the extent of 56%.

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