Carcinogen-Induced Sarcoma in the Primitive Primate, *Tupaia glis** (32750)

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There have been few successful attempts to induce tumors in primates with chemical carcinogens. Sugiura *et al.* (1) obtained squamous cell carcinoma in rhesus monkeys with skin application of a high-boiling oil for 3–5 years. Krotkina (2) obtained a bone sarcoma in monkeys with methylcholanthrene after 3.5 years. In 1963 Levy (3) reported the induction of a fibrosarcoma in a marmoset, *Saguinus nigricollis,* 10 months after subcutaneous injection of methylcholanthrene. Recently O'Gara and Kelly (4) reported the induction of hepatomas in monkeys fed *N*-nitrosodiethylamine for 2 years. As far as we are aware, there have been no reports of tumor induction with chemical carcinogens in the primitive primates or prosimians, which include the Tupaioidea (tree shrews), Lemuiroidea (lemurs), Lorisoidea (loris and bush baby), and the Tarsiodea (tarsier). The Tupaioidae or tree shrews, widely distributed in Southeast Asia, are generally acknowledged as the most primitive representatives of the primate species and most closely resemble the ancestral primate stock (5–8). This is a brief report of the induction of sarcoma in the common tree shrew *Tupaia glis* with 3,4-benzpyrene.

Materials and Methods. The tree shrews were housed, 1 male–female pair to a cage, in galvanized wire-mesh cages 18 × 24 × 36 inches, similar to those described for marmoset monkeys by Levy and Artecona (9). Cages were provided with two wooden perches near the top to provide a means for arboreal activity, and with a wooden nesting box measuring 8 × 8 × 12 inches with a 4-inch diameter hole in the upper half of one side. The animals were fed once per day with approximately 100 gm of Calo dog food, 100 gm of Rx for Primates (Agway, Inc., Syracuse, N.Y.) and 1 handful of Purina Monkey Chow biscuits. The food was placed in disposable 8 oz paper Lily cups, which were held in place by a spring. This ration was supplemented with slices of apple and banana several times weekly. Water was supplied by a bottle hung on the side of the cage. The animals maintained excellent health on this diet, and only 1 animal in the colony of 20 was lost in 24 months, this death due to fighting when several males were housed together. Temperature of the room was maintained at 80°F with a relative humidity of 55%. The number of animals used in the experiments was necessarily small. Although these animals do not require as much space and care as the larger primates, these factors effectively limit the number of animals in any experiment.

Results and Discussion. Two adult animals, male and female 1–2 years of age, were inoculated subcutaneously with 10 mg of 3,4-benzpyrene in 0.2 ml of olive oil. One of these animals died in 24 hours, probably due to toxicity from the chemical, although the other showed no ill effects. The surviving animal (male) showed no abnormal changes, until at 6 months it developed a palpable nodule at the site of inoculation of the chemical. The tumor grew very rapidly and at 7 months measured 30 × 40 × 21 mm. The animal was sacrificed at this time as it appeared to be in a terminal state. At necropsy, the tumor was noted to be firm and vascular with very little necrosis. The lung surfaces had large numbers of raised, whitish areas, some measuring 2 or 3 mm in diameter. Several small nodules were noticed on the surface of the heart. All other organs appeared normal. Hematoxylin and eosin stained paraffin sections of the tumor were examined. The growth was classified as a sarcoma with involvement of muscle and nerve. A large number of metastatic tumor foci were present in the lungs and a smaller number in the heart muscle. The

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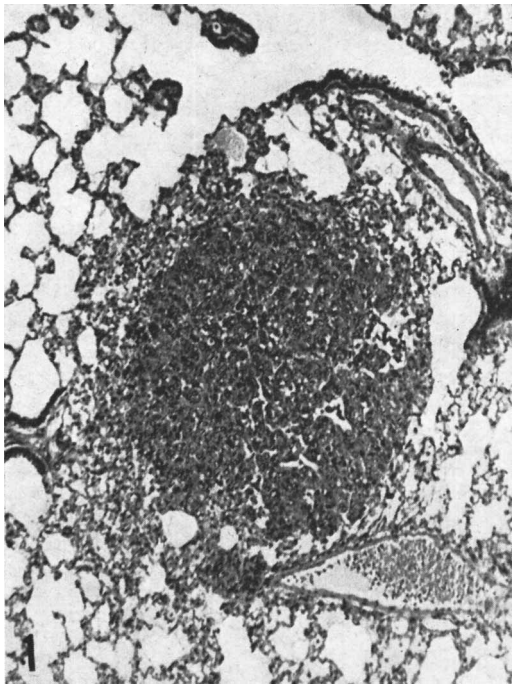

FIG. 1. Metastasis of carcinogen-induced sarcoma localized in the lung. Hematoxylin and eosin stain; 103 ×.

tumor showed no distinctive features. Some areas showed spindle formation while other areas were composed of round cells. Mitotic figures were frequently observed. Figure 1 illustrates a metastatic tumor focus in the lung at low magnification (103×). Figure 2 is a higher magnification (660×) of the central area of the metastatic focus, showing the morphology of the tumor cells.

Fragments of the tumor were transplanted into 2 adult animals, 1 of each sex, by trocar implantation. The female, which was approximately 3 weeks pregnant at the time of inoculation, developed a rapidly growing tumor which was removed at 5 weeks. It proved to be histologically identical to the original tumor. This tumor measured 20×50 mm and was necrotic centrally, but actively growing at the periphery. The male animal did not develop a tumor. Fragments from this successfully transplanted tumor were trocar-implanted into 4 additional animals which have remained negative. In a repetition of the original experiment, 3 animals, 2 males and 1 female 1–2 years of age, were inoculated subcutaneously with 10 mg of 3,4-benzpyrene in olive oil. Two of these animals developed tumors at 26 and 29 weeks. The remaining animal was negative at 8 months. Histological examination demonstrated the growths to be sarcomas similar in type to the original tumor. No metastases were observed, but the animals were sacrificed when the growths were relatively small.

The only previously reported observation of a tumor in the primitive primate, *Tupaia glis*, is by Elliot *et al.* (10). The tumor, a spontaneous mammary adenocarcinoma, was observed in a specimen trapped on Tioman Island, off Malaya. This was the only tumor observed by these investigators during a study of 400 animals trapped during a period of 4 years. We have observed no spontaneous tumors in our colony of 20 animals over a 2-year period.

Tissue cultures were established from trypsinized fragments of the transplanted tumor, with Eagle's basal medium containing 10% fetal bovine serum used as the growth medium. The cells possessed a fibroblastic type of

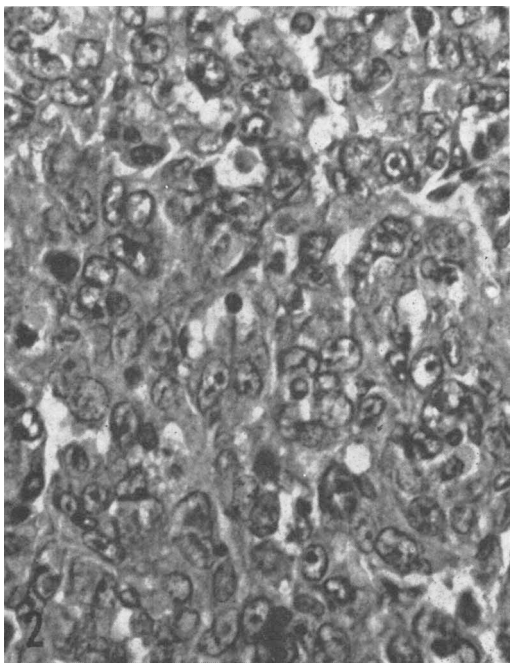

FIG. 2. Higher power view of lung metastasis illustrating tumor cell morphology. Hematoxylin and eosin stain; 660 ×.

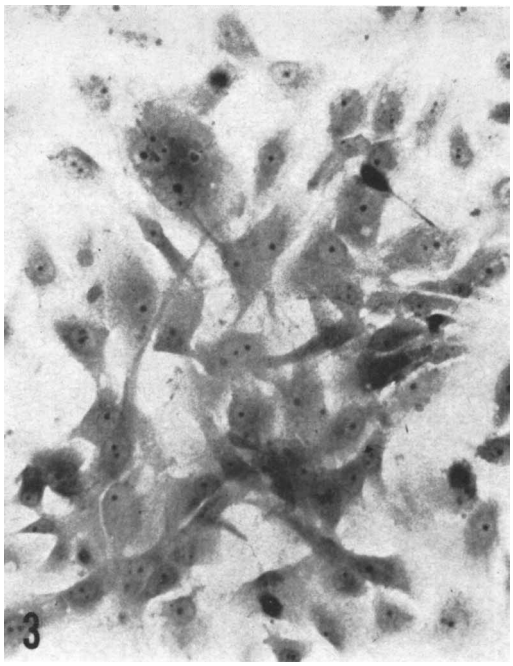

FIG. 3. Cell grown in tissue culture from trypsinized fragments of sarcoma. Note lack of contact inhibition, variation in size of nuclei and cells wth multple nuclei. Also note the long cellular processes. Hematoxylin and eosin stain; 262 ×.

morphology and were arranged in an irregular manner, demonstrating a defined lack of contact inhibition. The nuclei were highly variable in size and in the number of nucleoli they contained, and cells with multiple nuclei were frequently observed. Long cellular processes were present which stretched over other cells or connected with them. These features are illustrated in Fig. 3. Growth in culture appeared to be a balance between cell proliferation and cell degeneration. In some cultures, especially those recently transferred, cell degeneration was predominant. Small, dense, rounded cells were formed and released into the medium frequently in great abundance. In older cultures, cell proliferation was usually predominant. Large eosinophilic, cytoplasmic inclusion bodies were occasionally noted in young cultures up to 1 week of age; however, the inclusions were present in a high percentage of the cells in cultures 2 to 3 weeks old. In many cases these were multiple and pushed the nucleus to one side. The tissue cultures are presently being examined by electron microscopic methods to determine if a viral agent is present.

Summary. Sarcoma, metastatic in one instance, with a latent period of 6–7 months has been induced in the primitive primate, *Tupaia glis* by subcutaneous injection of 3,4-benzpyrene. Transplantation was successful in one instance with a pregnant animal. Growth in tissue culture was established from trypsinized fragments of the tumor. The fibroplastic cells demonstrated a lack of contact inhibition and contained cytoplasmic inclusion bodies.

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