Neoplasia in the Parakeet

IV. Transplantable Methylcholanthrene-induced Rhabdomyosarcoma

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In the epidermis of the parakeet, unlike that of the chicken, 20-methylcholanthrene readily induces squamous-cell carcinoma (36). On the other hand, sarcomas easily produced in chickens by the subcutaneous or intramuscular injection of methylcholanthrene (17) are difficult to elicit in the parakeet. After a number of trials with solutions of 20-methylcholanthrene (MC) in benzol or sesame oil, positive results were obtained with crystalline MC mixed with diatomaceous earth.

MATERIALS AND METHODS

The parakeets (Melopsittacus undulatus) used in these experiments were genetically heterogeneous; their age at the beginning of the various experiments was about 3 months. Sex distribution was equally divided between male and female. The 20-methylcholanthrene (MC) was injected subcutaneously and intramuscularly in the breast as a 3 per cent solution of benzol or sesame oil. In one experiment crystalline MC was mixed with 3 parts of diatomaceous earth. Quantities of the mixture containing 0.5 mg. MC were introduced into the muscle and subcutaneous tissue of the breast by trocar and stylet.

Homotransplants of the induced sarcoma and of subsequent passages were transferred as a tissue mince in a small volume of 0.9 per cent NaCl with needle and syringe. The implants were injected into the subcutaneous tissue and muscle of the left breast. Pieces of tumor approximately 1 cu. mm. were placed on the chorioallantoic membrane of chick embryos by the usual technic for opening and sealing the egg (40). Inoculation of chicks was carried out in the manner described for the parakeets.

RESULTS

Injection of 3 per cent MC in benzol. — Ten birds were given injections intramuscularly (I.M.) of 0.15 ml. of 3 per cent MC in benzol. Six birds died within 24 hours showing toxic symptoms due to the benzol. The remaining four birds died within 3 months; none showed neoplastic changes at the site of injection or elsewhere.

Injection of 3 per cent MC in sesame oil. — Each of 53 birds received a subcutaneous-intramuscular injection of 0.15 ml. of the solution. Twelve of these birds survived over 42 weeks after injection, and eight were sacrificed at 75 weeks; in none was there evidence of neoplasia at the site of injection.

Injection of crystalline MC. — With a trocar and stylet 0.5 mg. of crystalline MC was placed beneath the skin of the breast in each of sixteen parakeets. Six died within 7 months, and ten were sacrificed 30 months after inoculation; in none was there evidence of any carcinogenic effect.

Injection of crystalline MC and diatomaceous earth. — With a small trocar and stylet, a mixture of 0.5 mg. crystalline MC and 1 mg. of diatomaceous earth (kieselguhr) was inoculated into eighteen parakeets. The injection site was the subcutaneous tissue and superficial muscle bundles of the breast. Ten birds died within 40 weeks of inoculation. In each there was a foreign body inflammatory reaction at the site of inoculation. The reaction was most pronounced about the doubly refractile fragments of the calcareous foraminifera shells; these were often surrounded by multinucleate giant cells. Histiocytes and fibroblasts comprised the bulk of the cell population. Of the surviving birds, one that died after 366 days bore a 2 × 3-cm. tumor at the site of inoculation; another bird died 5 days later and was found to have a 6-mm. tumor. On the following day, 371 days after inoculation, a bird with a 2.5 × 3-cm. tumor at the injection site was sacrificed. The remaining
six birds were killed 459 days after inoculation; none showed evidence of neoplasia. Of eighteen birds inoculated with only kieselguhr 6 months previously, two died showing necrosis and a foreign body inflammatory reaction at the injection site; in neither of these birds nor in any of the survivors was there evidence of cancerogenesis.

Gross and microscopic character of the induced tumors.—The tumor mass in each of the three birds was moderately firm; the overlying skin was intact. On section the cut surface was variegated pink to dark red and meaty in appearance; in the two large tumors there were several yellow-white foci of necrosis (Fig. 1). Only in the first bird were metastases found; these appeared as a single 2 × 3-mm. gray nodule in the myocardium, multiple similar nodules in the liver, and as replacement of the right testis by a 3 × 4-mm. tumor. The metastasis in the right femur was not recognized grossly.

Histologically the appearance of three primary tumors was similar. At the site of inoculation of the carcinogen in the subcutaneous tissue and between the most superficial muscle bundles was a pleomorphic collection of cells composed of multinucleate giant cells, histiocytes, and fibroblasts. Within the giant cells bits of included foraminifera may be identified. The tumor itself had a pleomorphic pattern which, however, was clearly differentiated from that of the inflammatory reaction. The bulk of the tumor was composed of large fusiform or strap-shaped cells with a rather pale amphophilic cytoplasm and bluntly oval vesicular nuclei containing one to three prominent nucleoli; mitoses were frequent (Figs. 2 and 3).

The fusiform and strap cells probably are neoplastic derivatives of the dedifferentiated muscle cells produced by the original injury and inflammation that followed inoculation of kieselguhr and MC. In his study of mammalian muscle regeneration Godman (12) writes: "Most individual fusiform cells applied to the inner aspects of the tube wall represent sarcoplastic elements formed from ‘dissociative degeneration’ of muscle segments." This author also remarks that where the sarco blasts are in apposition with the connective tissue of the endomysium it is difficult or impossible to distinguish them from activated fibroblasts. In the absence of cross-striations and occasionally even of myofibrils, it is equally difficult to determine whether all the cell components of the tumor are sarco blasts or whether some are fibroblasts.

Between these cells, in the periphery of the tumor, were narrow ribbons of darkly eosinophilic striated muscle containing large numbers of nearly spherical nuclei arranged in single or double rows in the axis of the fiber (Fig. 2). These were identical in appearance with the early stage of regenerating mammalian (6, 10, 12) and avian (38) muscle fibers. These elements probably represent the regeneration of muscle fibers that have persisted following destruction of most others by the invading tumor. Similar changes have been observed at the periphery of metastases to striated muscle in man (11, 18). Scattered throughout the tumor were very large multinucleate cells that closely resemble the club-shaped stage of the regenerating muscle fiber (Fig. 3). These cells, however, were far removed from any remnants of the normal host muscle, and the nuclei were larger and more pleomorphic than those found in normal regenerating muscle.

It is most significant that the large multinucleate cells were also found in metastases to the liver (Fig. 4), bone marrow (Fig. 5), and testis. The congnous, often-faceted nuclei, and the abundant granular and fibrillar cytoplasm are all characteristic of a regenerating muscle fiber, in this instance a neoplastic one. The cells closely resemble the giant muscle cells found in tissue culture of chick striated muscle by Hogue and de Renyi (20). In them, as in the tumor cells, cross-striations were absent, but strong contractions were elicited by stimulation with a microneedle. It is of interest that in the tumor cells, as in those of regenerating muscle, mitoses were absent; amitosis is generally regarded as the mode of nuclear division. In other details the histological appearance and cellular composition of the metastases was the same as that of the primary tumors (Figs. 4 and 5).

Homotransplantation of the tumors.—Transplantation of the two large primary tumors was attempted, but only the tumor that had already metastasized grew. The minced tumor was inoculated beneath the skin of the breast of eighteen parakeets; it grew in eleven. Since the fourth passage the tumor has grown in nearly all inoculated birds. The latter are young adults of either sex; occasionally a 3–4-year-old bird is used, but no significant difference in the growth rate has been observed. The tumor has been growing as a transplant for 24 months and is now in the 42nd passage. The transplants grow rapidly, usually attain a diameter of 2–3 cm within a month, are frequently hemorrhagic, and are the site of massive necrosis (Fig. 6). Metastases are common in the liver (Fig. 8), less frequent in the lung. The histological pattern of the transplants has remained similar to that of the primary tumor; occasional myoblastic syncytia are still seen (Fig. 7), although they are less common. The dominant element is the fusiform, frequently strap-shaped cell.
identified as a neoplastic myoblast. The cytological detail as well as the histological pattern is quite different from that of spontaneous and of transplantable fibrosarcomas of the parakeet.

Transplantation to chick chorioallantoic membrane.—Pieces of tumor from the first, seventh, tenth, and 21st passages have been grown on the chorioallantoic membrane (CAM) of the chick embryo. Inoculation was usually carried out on the 8th day of incubation; the eggs were opened and examined for tumor growth 11 days later. A 2–5-mm. yellow white tumor nodule was found on about one third of the CAM inoculated (Fig. 9). The tumor grew in the mesenchyme of the CAM, the overlying chorial epithelium often proliferated, producing concentric layers of keratinized cells that resembled epithelial "pearls." The allantoic membrane adjacent to the tumor was often the seat of papillary hyperplasia (Fig. 10). However, both these changes in the epithelium of the CAM are probably nonspecific (25) and not related to growth of the tumor. Histologically the neoplastic cells are less densely packed in the CAM, but cytological characteristics such as the presence of syncytiat myoblasts are those of the primary tumor (Fig. 11).

Heterologous transplantation.—It is believed that immunological tolerance to heterotransplants can be accomplished by inoculating tumor tissue into embryos or onto the CAM (21, 39). For this reason and because the tumor can be grown on the CAM of the chick, an attempt was made to transplant the parakeet tumor to young chicks. In a series of three experiments the CAM was inoculated with tumor on the 3d, 8th, and 14th day of incubation, respectively. A total of 32 chicks hatched; within 24 hours these were given inoculations subcutaneously with tumor from the same passage that had supplied the original inoculum. Occasionally a 1–2-mm. fibrous nodule developed at the site of inoculation; no progressive tumor growth was observed. In another experiment sixteen newly hatched chicks received intraperitoneal injections of tumor brei; 14 days later minced tumor from the same passage was inoculated subcutaneously; no growth was obtained.

DISCUSSION

Malignant tumors of striated muscle are uncommon neoplasms in all classes of vertebrates. Among the invertebrates, where beautifully differentiated striated muscle is found in a number of phyla and where it often occupies a position taken by smooth muscle in vertebrates, e.g., the intestinal wall of insects, no muscle tumor has been reported.

Large series of rhabdomyosarcomas in man have been recently reviewed (31, 41); the tumor has been found in the dog and other domestic animals (43), as well as in the mouse (29) and rat (22), in reptiles and fishes (37). Approximately twelve cases have been reported in birds, all chickens (1, 3, 9, 13, 14, 22, 24, 30). Peyron (34) has described the occurrence of large areas of striated muscle in Rous sarcomas; these he regarded as true tumor elements and not regenerating host muscle at the periphery of the tumor. In a series of 625 spontaneous tumors in parakeets, we have seen one example of a rhabdomyosarcoma. This tumor measured 1.5 × 1 cm. and arose in the lateral aspect of the neck in an adult female. Histologically, it resembled mammalian rhabdomyosarcoma; the tumor failed to grow when transplanted subcutaneously in eighteen parakeets. The rarity of these muscle tumors in the parakeet can be compared with the frequency of fibrosarcoma, for in this series 92 of the latter were found in the subcutaneous tissue and fasciae.

In view of the large proportion of body tissue made up of skeletal muscle, it is surprising that these neoplasms are so rare. Some explanation of this may be sought in the highly differentiated character of muscle cells and the infrequent occurrence of extensive regeneration following most forms of injury. With the notable exception of in situ necrosis (6), muscle is usually replaced by a connective tissue scar. The introduction of a large amount of diatomaceous earth in control birds was associated with inflammation and an extensive in situ necrosis of muscle that was followed by regeneration. The development of rhabdomyosarcoma when both kieselguhr and MC were introduced, and the absence of neoplastic growth when MC alone was employed, may depend upon the presence of regenerating muscle in the former instance. This would also lend support to the view that in some human cases the history of trauma is more than coincidental (7).

Rhabdomyosarcoma has been produced with the use of chemical carcinogens in mice (19, 26) and rats (4, 5, 15, 16, 42). In birds the intramuscular injection of carcinogens has been followed by the development of fibrosarcoma in pigeons (8, 28), ducks (8), guinea fowls (8), and chickens (2, 27, 32, 35), but no instances of rhabdomyosarcoma are reported. However, in a recent review Peacock (33) writes: "The type of sarcoma that can easily be induced in fowls is a fleshy, whorled, spindle cell tumor, probably derived from voluntary muscle though never showing the transverse striation characteristic of mammalian rhabdomyosarcoma." Other than a remark that the staining reac-
tions of the cells are those of muscle he offers no support for this generalization.

The cytological identification of the parakeet tumor as a rhabdomyosarcoma is based largely on the presence of syncytial masses and strap cells, both often having prominent myofibrils. Distinct cross striations were not seen. Regenerating muscle is associated with proliferating endomysial fibroblasts that may be indistinguishable from sarco blasts; it is therefore possible that some of the elements of the tumor are neoplastic fibroblasts. Such cells were not disclosed by use of Mallory's trichrome, Van Giesson, and reticulum stains, but these stains, particularly the first two, will fail to identify undifferentiated fibroblasts that are not producing collagen. It may be said that the parakeet tumor has all the characteristics frequently seen in human tumors identified as rhabdomyosarcomas (41), but the possibility that it is a mixture of striated muscle and fibroblasts—a malignant mesenchymoma—has not been ruled out.

SUMMARY

A rhabdomyosarcoma was induced in three of eighteen parakeets, Melopsittacus undulatus, at the site of an intramuscular injection of 20-methylcholanthrene mixed with diatomaceous earth. The primary tumors were 6 mm. to 3 cm. in diameter; histologically they were composed of spindle and strap cells and of multinucleate syncytia; myofibrils were prominent, but cross-striations were not clearly defined. In one of the birds metastases were found in the liver, femur, heart, and testis. The tumor grew readily on transplantation, and in the elapsed 24 months it has been carried through 42 passages by subcutaneous inoculation in para keets. The transplants reach a diameter of 2-3 cm. within a month, frequently undergo massive necrosis, but have retained the histological characteristics of the primary tumor. Metastases are common in the liver, less frequent in the lung. The tumor grows readily on the chorioallantoic membrane (CAM) of fowl embryos, in which the characteristic strap cells and syncytia are still in evidence. Following inoculation of the CAM some chicks were permitted to hatch and were then immediately inoculated with the rhabdomyosarcoma. In another series chicks were given an intraperitoneal injection of tumor brei followed 2 weeks later by the subcutaneous inoculation of the tumor. In none of these heterologous transplants was there evidence of growth.

REFERENCES


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Fig. 1.—Cut surface of tumor that developed at site of intramuscular injection of 0.5 mg. methylcholanthrene mixed with 1 mg. of diatomaceous earth. Interval of 366 days from injection to death of bird. "X × Nat. size." Bird No. 1.

Fig. 2.—Periphery of tumor. Remnants of damaged muscle fibers appear as darker bands with axially arranged rows of nuclei. Nucleated erythrocytes appear as rectangles within capillaries. Between these structures are pale-staining cells, often with clusters of nuclei, identified as neoplastic sarco blasts. H. & E. stain. X 310. Bird No. 1.

Fig. 3.—Section from deeper region of tumor; no residual host muscle is present. Two large neoplastic syncytia resembling regenerating muscle are conspicuous. Fusiform sarco blasts and strap cells, many of the latter showing two or three congruous nuclei characteristic of young sarcoblast ribbons, make up the bulk of the tumor. Several cells are in mitosis; whether they are sarco blasts or endomysial fibroblasts cannot be determined with certainty, but they are provisionally identified as sarco blasts. H. & E. stain. X 420. Bird No. 1.

Fig. 4.—Metastasis in liver showing syncytia and sarco blasts similar to those of the primary tumor. H. & E. stain. X 140. Bird No. 1.

Fig. 5.—Metastasis in bone marrow; the strap-shaped syncytia are prominent. H. & E. stain. X 110. Bird No. 1.
Fig. 6.—Appearance of first-passage transplant 40 days after inoculation. Left, feathers removed, tumor covered by intact skin. Right, cut surface of same tumor showing characteristic large central area of necrosis. Bird No. 2.

Fig. 7.—Histological appearance of transplant in twelfth passage. A single large syncytium is surrounded by neoplastic sacroblasts. H. & E. stain. ×160. Bird No. 3.

Fig. 8.—Metastases in the liver from transplant growing in 31st passage. ×2.5. Bird No. 4.

Fig. 9.—Tumor growing on each of three chorioallantoic membranes. CAM inoculated on 8th day of incubation, harvested 10 days later. Transplants of tumor in 10th parakeet passage.

Fig. 10.—Section of transplant on CAM. H. & E. stain. ×15.

Fig. 11.—Higher magnification of previous figure to show strap-shaped syncytia and sacroblast ribbons. H. & E. stain. ×140.
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