Induction of Sarcoma of the Liver in the Rat with Methylcholanthrene and Benzpyrene

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In studying the effects of the carcinogenic hydrocarbons these substances are most commonly applied to the skin or injected subcutaneously for obvious reasons. No doubt there are variations in the sensitivity of different animals, as well as of the different tissues of individual animals; as examples may be cited the extreme reactivity of mouse skin and of mouse and rat subcutis, which are in sharp contrast to the high resistance characteristic of rat skin. Nevertheless, as proof of the general effectiveness of these agents in the production of neoplasia, the literature abounds in examples of tumors induced in practically all tissues. But the ability of the carcinogenic hydrocarbons to produce new growths in the liver has been seriously questioned by a number of investigators, who found minimal or nonspecific reactions in this organ after exposure for considerable periods (5, 8, 9, 12). Shear, Stewart, and Seligman (9) state that this apparent resistance of the liver does not depend upon absorption or removal of the chemicals, for pellets that had lain in the mouse liver for as long as 16 months without inducing malignant disease were found intact, and surrounded by only a mild tissue reaction. Still, their protocols include a description of an adenocarcinoma that was found in the liver of a mouse of the C57 black strain, in which spontaneous hepatic neoplasms do not occur, 12 months after the insertion of a thread coated with dibenzanthracene; the authors favor an origin of this induced tumor in the biliary ducts or gall bladder because of its histology and location.

Oberling and the Guérins (7), on the other hand, recorded 2 examples of sarcoma of the rat liver in animals examined 16 and 25 months after an implant of benzpyrene crystals. Strong (10) succeeded in producing carcinoma of the liver in 19 of 1,367 NHO mice at an average age of 406.2 days, by injecting methylcholanthrene subcutaneously at 60 days of age; spontaneous hepatic tumors do not occur in this strain. Recently White and Eschenbrenner (11) reported the occurrence of multiple nodules, interpreted as hepatomas, in the cirrhotic livers of 2 of 6 rats that survived 14 months on a basal diet containing 60 mgm. of 1,2-benzanthracene per 100 mgm. of food. This parent hydrocarbon possesses only negligible carcinogenicity when injected subcutaneously or painted on the skin. That the liver is endowed with no general resistance to carcinogenic agents such as might be linked with its extensive detoxifying powers is clearly evident from the ease of production of parenchymal cell neoplasia in the rat with azo compounds administered orally or parenterally, although they are ineffective when applied directly to the liver (6); or of sarcoma in this animal with Cysticercus larvae (1). There is much of value in the observation of Dunning, Curtis, and Bullock (2) who, on the basis of a study of subcutaneous and hepatic tumors induced by the carcinogenic hydrocarbons and Cysticercus disease respectively in rats, concluded that "the potency for malignancy must be a universal cell characteristic and that the histogenesis of these tumors was determined by the fortuitous exposure to the irritant of the various types of cells."

The present note records the induction of 7 hepatic sarcomas in rats bearing implants of methylcholanthrene or benzpyrene in the liver. The experiments were performed between 1941 and 1943, and were not reported earlier because of the absence of the author in military service.

MATERIALS AND METHODS

Attempts were first made to inject the carcinogenic hydrocarbons dissolved in melted paraffin (m.p., 48° C.), as had been done with ease and excellent results subcutaneously (3, 4); the liver was exposed by a midline upper laparotomy. This method did not prove feasible, however, because of the technical difficulties produced by the small anteroposterior dimension of the organ, its mobility, and its friability. In almost all of several dozen attempts the material either solidified in the syringe before the injection could be started, or if sufficient speed had been at-
Fig. 1-4
tained to avoid this it was found subsequently to have entered the spaces between the hepatic lobes.

Insertion of a solid pellet of paraffin, 0.25 cc. in volume and containing 1 mgm. of methylcholanthrene or benzpyrene, in the exposed median or left lateral lobes proved a simple and adequate method of administration. Benzpyrene is readily soluble in paraffin in the 4 per cent concentration necessary to give the dose mentioned above, but with methylcholanthrene a small persistent sediment of insoluble material remained despite prolonged heating on a water bath at boiling temperature. By agitating these crystals before solidification of the paraffin, however, relative uniformity in dosage could be achieved. To make the pellets the melted material was drawn into a 5 cc. pipette of which the mouthpiece and tip had been cut off, leaving a cylindrical tube. Oil of sesame had been previously drawn into and drained from the pipette to coat it with oil and insure facility of expulsion of the solidified paraffin by gentle pressure at either end.

Quantities of 0.25 cc. were expelled, severed with a sharp blade, and the resultant flat discs fashioned into oval pellets by rolling them between gloved fingers. To insert a pellet in the liver a small incision was first made in the anterior aspect of the capsule, and the mass inserted into the yielding tissue with slight pressure of a small forceps. If the pellet was placed deeply enough it remained in situ without suture of the capsule.

A total of 51 rats were thus tested for the effect of carcinogenic compounds on the liver: 25 with methylcholanthrene and 26 with benzpyrene. As controls, 14 animals received these pellets subcutaneously in the right flank, and 15 other rats received pellets of paraffin alone in the liver.

In addition, cotton threads saturated with methylcholanthrene while this was in the molten state were sutured in the livers of 4 animals. The dose of carcinogen was calculated by weighing measured portions of the thread before and after impregnation. The prepared thread was pulled through the liver with an ordinary straight needle, and severed flush with the capsule at its points of entrance and exit. Approximately 1 cm., which contained 0.8 mgm. of the compound, was employed in each animal.

The rats, males and females of the August, Sherman, and Wistar stocks, varied in age from 91 to 161 days at the outset of the experiment, with the exception of one group of 10 older animals of 273 to 296 days. Their diet consisted of a mixture of Purina and Rockland chows, adequate water, and a generous portion of fresh carrots once weekly, and their nutritional status continued good, excluding those animals that developed the cachexia of late malignant neoplastic disease.

RESULTS

Three well defined stages of reaction occurred about the pellets containing methylcholanthrene or benzpyrene: acute inflammation, healing with fibrosis, and tumor formation.

1. Inflammatory reaction.—Pellets of animals that died during the first 3 weeks after implantation of a carcinogen were surrounded by an acute, nonspecific, inflammatory response (Fig. 1). The exudate consisted of fibrin, polymorphonuclear leukocytes, and lymphocytes and was accompanied by many newly formed small blood vessels. A considerable number of fibroblasts appeared early. The reaction was without doubt dependent upon a combined response to the insults of mechanical trauma and a foreign body, and did not differ from that produced by control pellets of paraffin alone. The hepatic cells beyond the immediate area of reaction remained unaltered, but a few, in proximity to the pellet, exhibited such signs of disintegration as shrunken nuclei, fatty cytoplasm, or frayed outlines.

2. Fibrosis of capsule of pellet.—With subsidence of the active inflammatory response after 2 or 3 weeks progressive fibrosis and hyalinization developed (Fig. 2). Pellets examined then, and at intervals up to 90 weeks as animals died of intercurrent disease, were surrounded by mature connective tissue fibers (unless neoplastic transformation had supervened). With increasing age of the pellet these fibers became progressively more acellular and appeared more dormant and better demarcated from the adjacent unaltered liver. Scattered lymphocytes often persisted in the sclerotic bundles, and the bile ducts ensnared in this fibrous tissue were often distorted, enlarged, or ab-

DESCRIPTION OF FIGURES 1 TO 4

Fig. 1.—Acute inflammatory reaction 10 days after insertion in liver of paraffin pellet containing 1 mgm. benzpyrene. Cavity to left represents pellet dissolved during preparation of histologic section. August male. Mag. X 100.

Fig. 2.—Fibrotic replacement of active inflammation 76 days after implant of 1 mgm. benzpyrene. Persisting distorted bile ducts and foci of lymphocytes can be observed. Sherman female. Mag. X 135.

Fig. 3.—Early sarcoma of liver in periphery of fibrotic tissue about pellet, 248 days after implant of 1 mgm. benzpyrene in paraffin. Hyperchromatic cells of bizarre shape, and mitotic figures, are present. Fibrotic acellular areas in upper left are a portion of reaction about pellet. Sherman female. Mag. X 100.

Fig. 4.—Spindle cell sarcoma of liver 485 days after implant of 1 mgm. methylcholanthrene in paraffin. Tumor contains remnants of distorted bile ducts. Fibrotic zone in upper right is a portion of inner, uninvolved zone of reaction about pellet. Sherman male. Mag. X 100.
normally grouped in small clusters. At times their individual cells, with their abnormally eosinophilic cytoplasm, were larger and more conspicuous than normal, but never gave any indication of neoplastic change. The morphology of this scarred reaction tissue about the pellets that contained the carcinogens differed in no detail from that surrounding the control pellets of paraffin.

A study of serial sections of the tissue around a considerable number of carcinogenic pellets, at various stages when no gross tumor or microscopic evidence of neoplasm was found in a single section, gave no clue to the changes that precede neoplastic transformation. A tumor, when present, exhibited the characteristic histologic criteria, and little could be learned of the important preliminary stage.

3. Tumor formation.— Sarcoma of the liver in the area immediately about the implanted carcinogen occurred in 7 of 27 rats that survived 248 days, the minimum period for initiation of the malignant process. Evidence of a tumor at this time was found only after the examination of serial sections (Fig. 3). The difference between this tissue and the benign reaction is striking. The relative cellularity and the presence of many hyperchromatic cells of bizarre shape, together with mitotic figures, among the bands of more delicate spindle elements, permitted a diagnosis of early sarcoma, especially as tumors in other animals consisted merely of larger accumulations of these altered cells. It is interesting to note that the earliest sarcoma in the series occurred at the periphery of the fibrous tissue about the pellet; at some distance, therefore, from the area of direct contact with the carcinogen, as others have observed, rather than at its point of strongest concentration.

Another tumor (Fig. 4) was a well developed but small spindle cell sarcoma, found in an animal dead 485 days after implantation of a methylcholanthrene pellet. This neoplasm, too, appeared to offer evidence of the initiation of the malignant alteration at the periphery of the reaction tissue, for it invaded hepatic parenchyma, engulfed a number of enlarged, distorted bile ducts, and encroached upon the hyalinized, acellular tissue immediately about the carcinogen pellet; but the persistence of a rim of benign fibrous tissue in the area closest to the carcinogen indicated that the neoplasm arose exterior to this site.

The remaining neoplasms arose in an animal dead 411 days after receiving benzpyrene, and in 4 others 389, 449, 510, and 630 days following the insertion of methylcholanthrene; all were larger growths, attaining a maximal size of 5 cm. in their largest dimension. Two of the methylcholanthrene tumors were in animals that had received the chemical in threads. In all cases the responsible agent was found embedded in the neoplasm, whose large size precluded discovery of the site of origin. Liver tissue at a distance, when not involved by the expanding tumor, remained unaltered both in the gross and microscopically. Widespread peritoneal implantation metastases from the pelvis to the under surface of the diaphragm were found in one animal of this group. The tumors proved to be spindle cell sarcomas with zones of a more polymorphous architecture distributed throughout at random. Neoplastic alteration of the hepatic parenchyma was not encountered. Inadequate specific irritation of its cells by the carcinogen might account for this; either because of their relative distance from the agent, separated as this was by the fibrous tissue about the pellets, or because a sarcoma destroyed the animal before a tumor could develop from the epithelium. On the other hand, insusceptibility of the epithelium to carcinogenic hydrocarbons may be an underlying factor.

With the exception of the opportunity of estimating the latent period for benzpyrene, in the 1 animal that died early in the course of sarcoma development, the time of onset of the tumors could not be determined. The other growths had obviously been present for a time when the animals died; in some cases slowly growing masses had been detected for some weeks prior to death, but palpation of a deeply situated internal neoplasm that does not grow rapidly cannot give even approximate information on its time of onset. Of the 15 rats receiving control implants of paraffin alone in the liver, 8 survived for periods of 505 to 553 days. The reaction about the pellets in these animals consisted of dense hyalinized connective tissue, with no evidence of neoplastic transformation.

Subcutaneous tumors produced by methylcholanthrene and benzpyrene pellets.—Pellets identical with those used in the liver experiment were implanted into 14 rats subcutaneously in the right flank, through a small skin incision. Tumors developed in 10 animals, and their relatively slow growth paralleled in most instances that of the hepatic neoplasms. The presence of a tumor was determined by the first palpable enlargement of the implanted pellets, and on this basis the minimal latent period was 119 days. No estimate can be given of the time of development of microscopic tumors. Tumor rats survived for 225 days to 528 days before the neoplasms had attained sufficient size, from 5 to 9 cm. in greatest dimension, to make it expedient to sacrifice the animals. All subcutaneous tumors were spindle cell or polymorphous cell sarcomas. The shorter latent period and greater yield of tumors in the subcutis as compared with the liver suggest speculation on the lesser sensitivity of the latter to the carcinogenic hydrocarbons.
however, the number of animals employed in this experiment was too small to warrant any extensive elaboration of such a hypothesis. Full consideration of differences in susceptibility of various tissues to the carcinogens has been given by Shear, Stewart, and Seligman (9).

SUMMARY
Sarcoma of the liver in the rat was produced in 7 of 27 animals that survived 248 days, the time required for the induction of the earliest tumor, after intraportal implantation of paraffin pellets containing 1 mgm. of methylcholanthrene or benzpyrene, or of cotton threads impregnated with 0.8 mgm. methylcholanthrene.

REFERENCES
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