An Antineoplastic Factor in Spleens of Mice Previously Inoculated with Methylcholanthrene. (20453)

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When carcinogenic compounds are applied to, inoculated into, or fed to rodents, the host may eventually develop a tumor either at the site of the exposure or, selectively, in such organ as the liver. The wide variety of such compounds has been well catalogued by Hartwell(1), but the mechanism of this response has not been clearly defined. Some compounds induce tumors more rapidly than others (*e.g.* methylcholanthrene vs. 1,2,5,6-dibenzanthracene), but all require relatively prolonged exposure to the host's tissues before a neoplasm appears.

It was observed in this laboratory(2) that when a carcinogenically-induced transmissible tumor involuted, the recovered host was refractory to reinoculation with the same tumor. This "immunity" was recorded by Jensen(3), Ehrlich(4), and more recently by Mazwell and Aptekan(5). In our study the "immune" phenomenon was associated with tissue cultures of growing splenic tissue. Using a similar tissue culture technic, involving two types of tissue per tube, the response of the mouse to methylcholanthrene inoculation was studied. Tumor and spleen tissues were grown simultaneously in the same tube, and observations were made on the effect of the spleen tissue on tumor tissue.

Methods. All of the C3H mice used in this study were purchased from the Jackson Memorial Laboratory, Bar Harbor, Maine. Groups of mice were inoculated subcutaneously with 0.5 mg of 20-methylcholanthrene* in cooking oil. Fibrosarcomas appeared at the point of inoculation by the 45th day and such tumors continued to appear in the rest during the 2 months following. The tumors and spleens from these mice were used for the study described below. Groups of 15 mice were each inoculated subcutaneously with 0.5 mg of 20-methylcholanthrene,* 0.5 mg of 3,4-benzpyrene,* 0.5 mg of 1,2,5,6-dibenzanthracene,* and with phenanthrene. All of these compounds were suspended in cooking oil. Cooking oil alone was also inoculated into a group of mice. At weekly intervals thereafter, 3-4 mice from each group were sacrificed and their spleens were studied for their destructive capacity on the methylcholanthrene-induced tumors referred to above. Each experiment was repeated at least 3 times, and the 2-week studies were repeated 7 times. The tumor and spleen tissues were individually minced with scissors and the fragments (ca. 1.0 mm in diameter) were washed 3 times in Hank's solution. Three or 4 pieces of tumor were explanted in plasma on one wall of a tube and to the opposite side about 3-4 times as many spleen particles were explanted. Each minced spleen was divided among 3-4 tubes. After the addition of one ml nutrient fluid,† the tubes were stoppered and rotated 6 times per hour at 35°C. Tubes with tumor alone, spleen alone, and tumor plus "normal" spleen were also simultaneously studied. In evaluating the significance of the various combinations of tissues, it was felt that in all instances the spleen tissues must show evidence of growth in order that the growth characteristics of the tumor tissue be significant. Furthermore, the tumor and spleen tissues alone must show good growth properties.

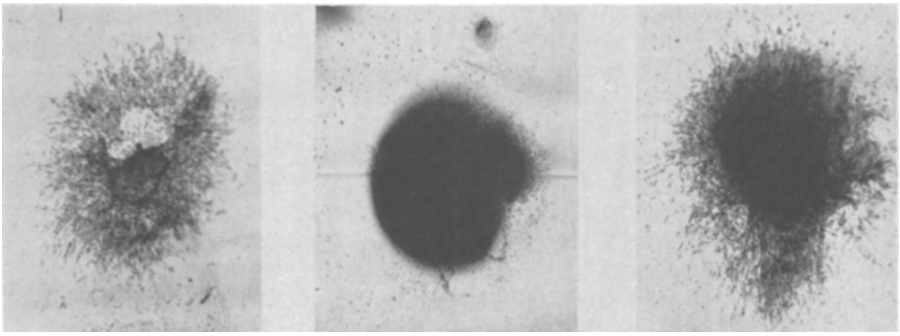
The magnified explants were photographed‡ through the wall of the tube thus obviating the need for disturbing the tissues. For simplicity, the spleen tissues are referred to as 1) homologous—from the mouse supplying the tumor, 2) methylcholanthrene—indicating the weeks from inoculation, 3) phenanthrene—indicating the weeks from inoculation, and 4) "normal"—from a mouse that had not been inoculated.

Results. Tumor explants grew profusely

* Eastman Kodak Co.

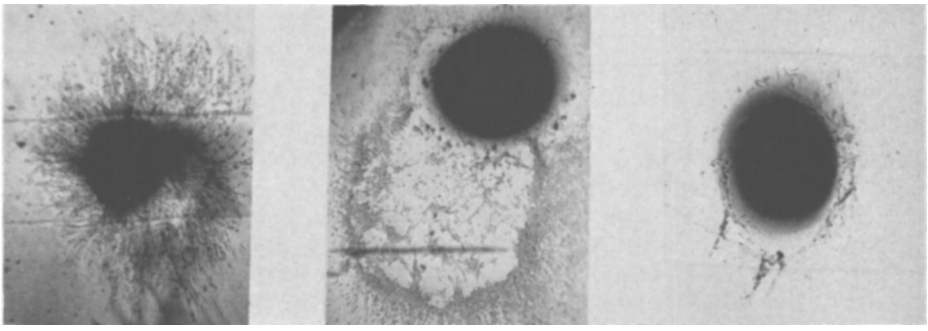
† Ox serum ultrafiltrate, Hank's solution, and chick embryo extract.

‡ We gratefully acknowledge the kind assistance of Mr. F. W. Schmidt, Dept. of Medical Photography, University of Texas Medical Branch.



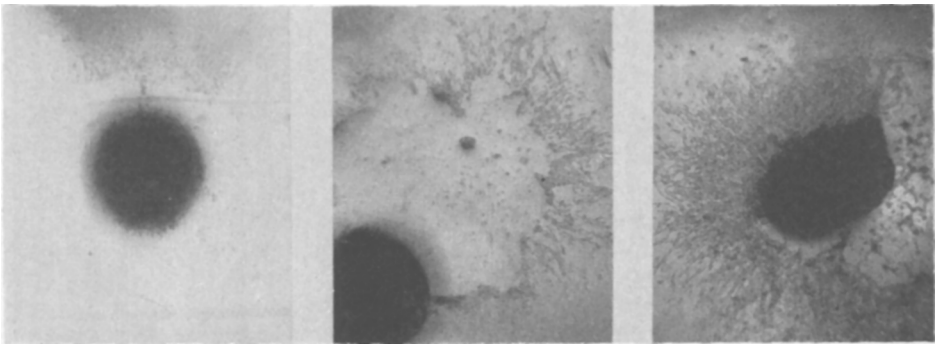
Tumor alone | With spleen of methylcholanthrene-inoculated mouse (1 wk previously) | With homologous spleen

FIG. 1. Tissue cultures of methylcholanthrene-induced mouse tumor—four days. ×38.



+ normal C_3H spleen | + C_3H phenanthrene spleen (2 wk) | + C_3H methylcholanthrene spleen (2 wk)

FIG. 2. Tissue cultures of methylcholanthrene-induced mouse tumor—two days. ×36.



+ meth. spleen (2 wk) | + meth. spleen (3 wk) | + meth. spleen (4 wk)

FIG. 3. Tissue cultures of methylcholanthrene-induced mouse tumor. ×30.

alone, and in the presence of homologous and "normal" spleen tissues (Fig. 1). Within 24 hours, fibroblasts grew out radially from the explant, with occasional areas of liquefaction within the growing "halo" of cells. Twelve tumors were grown with their homologous spleens and in no instance was any tumor suppressive effect detected.

Tumor explants grew poorly and most of them died when explanted in the same tube with spleen tissues from mice inoculated with methylcholanthrene one and 2 weeks previously (Fig. 1 and 2). This reaction was demonstrable in 6 of 8 experiments. The antineoplastic (AN) effect was less marked at 3 weeks after inoculation and thereafter the tumor growth resembled that which grew with homologous spleen (Fig. 3). The an-

tineoplastic effect appeared on the first day after explantation and appeared complete during the next 2 days. Under these conditions, most of the tumor explants failed to grow, or grew sparsely, were disintegrated and dead within the next 2 days. The mice supplying the spleens at 1-4 weeks after inoculation showed local evidence of the oily inoculum but no visible trace of neoplasm.

Tumor explants grew profusely in the same tube with phenanthrene spleens (Fig. 2), as well as with spleens from mice previously inoculated with benzpyrene and with 1,2,5,6-dibenzanthracene. Their growth pattern did not differ from those growing with "normal" and with homologous spleens. The growth of splenic tissue explants followed a pattern of cell migration within 24 hours of explantation. Within the next 2 days, fibrocytes and large lymphoid cells appeared at the periphery of the explant. Those spleen explants which exerted a tumor antagonistic effect were surrounded by the lymphoid cells, whereas the others exhibited a fibroblastic outgrowth.

Discussion. The A N phenomenon described above has not yet been associated with a soluble antibody; however, we shall refer to it as an "immune" reaction until evidence warrants a change. This transient phenomenon has manifested some degree of chemical specificity: phenanthrene and cooking oil injections alone did not induce the A N effect on methylcholanthrene-induced tumors, nor did 3,4-benzpyrene and 1,2,5,6-dibenzanthracene. The A N reaction was not uniform with all mice receiving methylcholanthrene. Absence of the A N reaction might be attributable to 1) an inhomogeneity among the animals used, 2) multiple influences of methylcholanthrene, 3) a delayed A N response, or 4) an A N period which disappears before one week. Exploratory tests for the latter proposal have indicated that the A N factor does not appear in the spleens of inoculated animals in less than 6 days.

The 3-week period of A N response might coincide with the latent period of carcinogenesis in the host. Since carcinogenic agents vary in this property, some of the slower acting agents should be studied with the technic described above, to see if the A N period is prolonged.

The mechanism of the A N reaction may simulate the "immunological paralysis" described by Felton and Ottinger(6). When they inoculated massive doses of pneumococcus polysaccharide into mice, the immunogenic mechanism failed. Strong's statement(7) that "all carcinogens are mutagens" may be pertinent to this A N process: if a somatic mutation induces a new antigenic specificity in tissues, then the antigenic burden thus imposed through methylcholanthrene might eventually cause the protective mechanism of the host to fail. This may explain the observed response of the host to methylcholanthrene, in which the host's spleen tissues manifested chronologically an initial destructive effect then a tolerant effect on tumor tissue to which it was exposed.

Summary. C3H mice were inoculated subcutaneously with 0.5 mg of 20-methylcholanthrene. At weekly intervals thereafter, spleens were examined from groups of mice for growth effect on methylcholanthrene-induced tumors. By growing spleen and tumor explants in the same tube, a tumor-destructive effect could be demonstrated in spleens for as long as 3 weeks after inoculation. Thereafter, this effect could not be detected. The tumor-destructive effect could not be demonstrated with "normal" spleens, nor with the spleens from the mice supplying the tumor tissue. This reaction could not be induced with phenanthrene, 3,4-benzpyrene, 1,2,5,6-dibenzanthracene, and the cooking oil in which these compounds were dissolved.

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