Production of Reticulum Cell Sarcoma and Fibrosarcoma by Methylcholanthrene Adsorbed on Activated Carbon

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Many neoplasms have been produced in experimental animals by introducing by various methods carcinogenic chemicals into different tissues or organs. Relatively few of the tumors induced by these experiments have arisen in the reticuloendothelial system. In 1938 Furth and Furth (1) reported the experimental production of monocytic leukemia in mice by the intrasplenic injection of 1,2-benzpyrene suspended in lard. In 9 per cent of the mice they found a massive infiltration of the spleen, liver and cervical lymph nodes by large cells resembling histiocytes with some of the characteristics of Hodgkin’s disease. In 1939 Midor and Morton (5) painted methylcholanthrene in benzene on the skin of 60 dilute brown mice and produced generalized lymphomatosis, localized mediastinal lymphoma, extramedullary myelopoiesis or reticuloendotheliosis in 48 animals. Only 1 mouse had reticuloendotheliosis with tumor cells in the spleen, liver and lymph nodes. In 1940 Roussy, Guérin and Guérin (8) reported the production of reticulum cell sarcoma in the spleen of 2 white rats by implantation of 3,4-benzpyrene within that organ.

The experiment reported in this paper was designed to ascertain if an active carcinogenic agent, such as methylcholanthrene, would tend to produce reticulum cell sarcoma if it were localized in the reticuloendothelial system. Since the cells of the reticuloendothelial system are active in phagocytizing particulate matter, it seemed possible that a carcinogenic agent could be prepared which would be selectively taken up by these cells, and could be readily detected in their cytoplasm. With this in mind methylcholanthrene was adsorbed on activated carbon and injected intraperitoneally into mice.

MATERIALS AND METHODS

Sixty milligrams of methylcholanthrene were dissolved in 100 cc. of boiling absolute ethyl alcohol to form a pale yellow solution with a definite blue fluorescence. To this boiling solution 1.2 gm. of activated vegetable carbon were added and the mixture was stirred for 5 minutes. The mixture was allowed to cool. The carbon particles settled rapidly, leaving a clear, colorless supernatant fluid with no fluorescence. The entire mixture was filtered and the carbon was washed with boiling absolute ethyl alcohol. The filtrate and the washings were dried in a weighed evaporating dish. Since no color was observed in the evaporating dish and there was no increase in its weight it was assumed that the methylcholanthrene had been completely adsorbed on the carbon. The resulting methylcholanthrene-carbon complex was suspended in 60 cc. of sterile physiological saline solution so that each cc. contained 1 mgm. of methylcholanthrene and 20 mgm. of carbon.

On January 8, 1944, 30 male white mice, 3 weeks old, were injected intraperitoneally with 1 cc. of the methylcholanthrene-carbon-saline suspension. The mice were from the stock strain used by the Department of Bacteriology at the University of Colorado School of Medicine for the past 8 years. During this time only one spontaneous tumor was noted at autopsy of an estimated thousand mice. The tumor was not examined microscopically. Most of these animals were sacrificed when 9 to 12 months of age. Sixty mice of the same strain were used as controls in this experiment. The controls were kept under identical conditions as to diet and care in the same room, but did not receive the injections given to the test animals.

OBSERVATIONS

At the end of 21 days 2 of the injected animals were sacrificed and examined. On gross inspection, small black masses of carbon were found to be
lightly adherent to the parietal peritoneum, and the mesenteric lymphatic vessels and lymph nodes were distinctly darkened. There was a black film between the liver and spleen and between the liver and diaphragm. The mediastinal lymph nodes were also blackened with carbon. Microscopic examination of paraffin sections, stained by hematoxylin and eosin, showed carbon particles free in the sinuses of the lymph nodes, and within the cytoplasm of the reticuloendothelial cells just beneath the capsule and adjacent to and in the sinuses. The liver contained no carbon in the parenchyma but carbon followed the distribution of the capsule and the fibrous connective tissue septa. The spleen contained no carbon.

At the end of 90 days 3 more animals were sacrificed. The carbon masses were definitely adherent to the peritoneum by what appeared to be a fibrotic reaction. The mesenteric lymph nodes were somewhat enlarged and blackened. The liver was covered with a film of carbon and was adherent to the diaphragm and spleen. Microscopic examination of the black masses adherent to the peritoneum revealed a central nucleus of carbon surrounded by young cellular connective tissue with little inflammatory reaction. Evidence of neoplastic change was not seen. The lymph nodes of all 3 animals contained free carbon in the subcapsular and radial medullary sinuses, and the reticuloendothelial cells in and adjacent to these areas showed hyperplasia and contained carbon in their cytoplasm.

After 132 days, mouse 10 (Table I) had an easily palpable, firm, irregular mass in the left upper quadrant of the abdomen. Two weeks later this animal was sacrificed. Autopsy showed a glistening, firm mass, measuring $15 \times 15 \times 10$ mm., lying between the liver and spleen and adherent to these organs and to the retroperitoneal tissue and intestines (Fig. 1, A). A second tumor of similar size and appearance was attached to the superior surface of the liver and extended through the diaphragm (Fig. 1, B) into the right thoracic cavity where it displaced but did not invade the lung. These tumors were located in areas in which an abundance of carbon had been found in the animals examined at 21 and 90 days. Gross sections of these tumors revealed a central radiating accumulation of carbon pigment surrounded by pale gray, rather firm tissue (Fig. 1). Sections from the area where the tumor invaded the diaphragm (Fig. 2) showed carbon between the liver and diaphragm with a rather abrupt transition from normal diaphragm to tumor, which did not invade the liver. Microscopically the tumor showed a central area of carbon embedded in hyalinized fibrous tissue with an outer zone of anaplastic, spindle cells of various sizes with a few mitotic figures (Fig. 3). This tumor was similar to the methylicholanthrene fibrosarcomas produced in other rodents (4). Five other mice exhibited intra-abdominal tumors of this type in from 139 to 150 days after injection (Table I).

**Table I: Results Observed in 30 Mice Injected with Carbon-Methylicholanthrene, and in 60 Controls, over a Period of 180 Days**

<table>
<thead>
<tr>
<th>Number of mice</th>
<th>Mouse No.</th>
<th>Tumor noted, days*</th>
<th>Autopsy, days*</th>
<th>Fibrosarcoma</th>
<th>Reticulum cell sarcoma</th>
<th>No. tumor noted</th>
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<td>60 Controls, not injected</td>
<td></td>
<td>190</td>
<td>60</td>
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</tbody>
</table>

* Days after injection.

The first reaction to the presence of carbon-carrying methylicholanthrene was the production of numerous normal-appearing fibroblasts as seen in the animals that were sacrificed early. In those animals that developed fibrosarcoma, it would ap-

**DESCRIPTION OF FIGURES 1 to 4**

Fig. 1.—Photograph of 2 fibrosarcomas in mouse 10, 146 days after injection of methylicholanthrene adsorbed on carbon, showing central carbon within the tumors. A.—Tumor (T) between spleen (S) and liver (L) and intestine (I). B.—Tumor attached to superior surface of liver and extending through the diaphragm (D). Mag. $\times 1.25$.

Fig. 2.—Photomicrograph of section from lesion depicted in Fig. 1, B showing fibrosarcoma (T) invading diaphragm (D) but not liver (L). Carbon masses are visible in the tumor and between the liver and diaphragm. Mag. $\times 30$.

Fig. 3.—Photomicrograph of the fibrosarcoma of Fig. 2 showing carbon masses among the tumor cells. Mag. $\times 400$.

Fig. 4.—Photomicrograph of reticulum cell sarcoma in mesenteric lymph node of mouse 12, 170 days after injection of methylicholanthrene adsorbed on carbon. Mag. $\times 450$.
pecar that the continuous action of the carcinogenic agent was responsible for the malignant change. Whether the tumors were the result of the action of methylcholanthrene on the normal-appearing fibroblasts first produced in response to the foreign agent, or whether the fibroblasts were potentially malignant from the beginning could not be definitely decided. However, only 6 of the 30 mice developed fibrosarcomas in the 180 days of this experiment. At any event, fibrosis apparently preceded the formation of fibrosarcomas.

On the 165th day a firm mass was felt in the abdomen of mouse 12 (Table 1). This animal was sacrificed on the 170th day. Autopsy revealed a smooth, firm, oval tumor, measuring 15 × 10 × 10 mm., at the root of the mesentery in the usual position of the mesenteric lymph nodes. It had a rather firm gray, homogeneous cut surface with a central mass of carbon extending towards the periphery on one side. Microscopic examination showed a lymph node with no follicles and the architecture completely altered. The central area contained large carbon granules surrounded by a few fibroblasts and collagen fibers. Among them were many poorly defined plomorphic cells with distinctly outlined hyperchromatic nuclei. Some of these cells were quite large, had homogenous, pale, acidophilic cytoplasm, and contained large round, oval, sometimes slightly indented, nuclei with 1 to 3 large nucleoli. There were a moderate number of mitotic figures. A few normal-appearing lymphocytes were in this area. Peripherally, the large type of cells became more numerous forming almost a syncytial arrangement (Fig. 4). Some were giant cells with 1 to 3 large nuclei. Carbon pigment was in the cytoplasm of some of the tumor cells. Foot's modification of Bielschowsky's stain showed an abundance of reticulum fibers throughout the tumor, surrounding single or small groups of cells. Van Gieson's stain and Mallory's connective tissue stain showed collagen only in the central area of fibrosis.

The cells making up this tumor were very similar to, but larger than, the hyperplastic, carbon-containing reticuloendothelial cells seen adjacent to the sinusoids in the lymph nodes of mice examined earlier. This tumor was thought to be a reticulum cell sarcoma derived from the reticuloendothelial cells of the lymph node.

Call to military service necessitated the termination of this experiment on the 180th day. All remaining test animals and the 60 control animals were then sacrificed. Autopsy revealed no evidence of neoplasm in any of them.

DISCUSSION

Since the descriptions of malignant reticulum cell tumors of lymph nodes by Goornaghdy (3), Oberling (6), and Roulet (7), a divergence of opinion has existed as to the exact nature of these neoplasms and there is considerable confusion in their nomenclature. The most popular term in this country is "reticulum cell sarcoma." This has been carelessly broadened to include almost any lymphoid tumor with proliferation of reticular tissue. In 1941 Warren and Picena (9) called attention to the confusion in nomenclature associated with the term "reticulum cell sarcoma" and adopted the restricted criteria of Oberling (6) for diagnosing this tumor. They regarded the reticulum cell sarcoma as the most undifferentiated form of the lymphoid tumors and subscribed to the concept that it originates in the cellular reticulum of the primitive mesenchymal syncytium. In 1942, Gall and Mallory (2) reviewed the subject of malignant lymphomas and divided them into seven categories, two of which were grouped together under the heading of "reticulum cell sarcoma." They stated that various authorities regard the type of cell of this tumor "(1) as an immature cell of the lymphocytic series, (2) as a pluripotential cell of variously assumed potentialities of development including the formation of lymphocytes, and of reticulum and collagen, and (3) as a relatively well differentiated cell of the monocyte or clasmatoocyte series." Their own observations led them to believe that the reticulum cell sarcoma must be divided into two types: "(1) tumors composed of relatively well differentiated wandering cells with phagocytic propensities resembling monocytes or clasmatoocytes, and (2) tumors made up of highly undifferentiated, presumably pluripotential cells" which they chose to call "stem cells."

The tumor that appeared in mouse 12 of our series, apparently fulfilled the criteria of the "undifferentiated or syncytial reticulum cell sarcoma" of Warren and Picena and the "stem cell lymphoma type of reticulum cell sarcoma" of Gall and Mallory.

This experiment was specifically designed to ascertain if methylcholanthrene, when carried within the cells of the reticuloendothelial system, would induce reticulum cell sarcoma. It did so in 1 out of 30 animals. It also produced fibrosarcomas in the peritoneal cavities of 6 other animals of this series. This experiment also demonstrated the case with which methylcholanthrene can be combined with carbon and incorporated within the cells of the reticuloendothelial system by phagocytic action. The carcinogenic agent was readily tagged by this proce-
duration and made visible in the gross and microscopic examinations. Thus it was possible to observe the development of the tumors in relation to the carbon particles. In the animals sacrificed early, the carbon-methylcholanthrene complex was found in the medullary and cortical lymphatic sinuses and in the reticular cells of these sinuses but not in the cells of the germinal centers of the lymph follicles. The reticular cells near the carbon and those containing carbon were the cells showing hyperplasia, and presumably the reticulum cell sarcoma that developed in mouse 12 arose from these cells. The carbon was equally useful in locating the carcinogen in the fibrosarcomas.

It is possible that a larger number of tumors of both types may have developed had this experiment been continued over a longer period. It is conceivable that more tumors might be produced if the quantity of methylcholanthrene were increased to the maximum amount that could be adsorbed by the carbon. Further experiments have been planned whereby higher concentrations of methylcholanthrene will be used with the carbon, the complex will be given both intraperitoneally and intravenously, and the animals will be observed for longer periods.

SUMMARY

This experiment was designed to ascertain if a known carcinogenic agent would tend to produce reticulum cell sarcoma if it were localized in the cells of the reticuloendothelial system.

Methylcholanthrene was adsorbed on activated carbon to tag the carcinogenic agent visibly and permit it to be phagocytized by cells of the reticuloendothelial system. Thirty white mice were injected intraperitoneally with the carbon-methylcholanthrene complex.

During the 180 days of the experiment, 5 mice were sacrificed within the first 90 days in order to observe the initial reactions. Of those remaining, 6 animals developed typical methylcholanthrene fibrosarcomas within the peritoneal cavity in 132 to 150 days, 1 developed a reticulum cell sarcoma of a mesenteric lymph node in 165 days, and 18 were found free of neoplasms. Omitting the 5 mice sacrificed early, 28 per cent of the injected animals developed tumors. Sixty control mice developed no tumors.

REFERENCES

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