Experimental Tumors in Lymph Nodes and in Endocrine and Salivary Glands*

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In recent years, numerous neoplasms of the endocrine glands in the human have been described which have produced abnormal amounts of the natural secretions of these glands. This is true of certain tumors of the thyroid, parathyroid, pituitary, testis, ovary, adrenal, and pancreas. A need has long been felt for experimental material of similar nature in small animals to permit further study of the characteristics of these infrequently occurring tumors, and to have a source of material for the study of abnormal endocrine states. During the last 4 years, we have, therefore, attempted to induce the development of tumors in some of these highly differentiated endocrine glands as well as in the salivary glands and lymph nodes of rats and mice.

Methylcholanthrene was first chosen as the agent because it had been shown by Shear (23), Iball (13), and Twort and Twort (26) to be one of the most active carcinogens. To avoid the necrosis and severe reaction caused by this agent in its pure form, less active materials in the form of 5 per cent 1,2,5,6-dibenzoanthracene and 10 per cent methylcholanthrene in cholesterol were also used. These did not produce tumors before the animals died of intercurrent diseases. Radon seeds, crystals of thorotrast, of theclin, and of arsenic trioxide have also been tried, all without success in the production of tumors.

The C3H strain of mice has previously been shown by Andervont (1) to be very susceptible to the development of tumors by carcinogenic chemicals. The ancestors of the animals of this strain used in these experiments were obtained from him. A disadvantage of the C3H strain for the present purpose is the high incidence of spontaneous mammary carcinomas in the females. Males were therefore largely used in these experiments. Occasionally, spontaneous mammary tumors were removed surgically to give the carcinogenic agent a longer period to act. The ancestors of our strain A mice were obtained from the Roscoe B. Jackson Memorial Laboratories at Bar Harbor, Maine. Mice of this strain were found also to be very susceptible to carcinogenic chemicals.

TECHNIC

Mixtures of methylcholanthrene or of 1,2,5,6-dibenzoanthracene with cholesterol were made by heating the mixed crystals to the melting point several times and agitating the container while the substance was in a molten state. In earlier series pellets were formed by the technic described by Shear (23) by drawing the molten substance into glass tubes having a small bore, and expelling the material after cooling. The long rods obtained could be cut into any desired length. The pellet was then inserted into a cannula for introduction into the tissues. In later series the pure methylcholanthrene was melted and permitted to harden. Small pellets were then chipped off and, when used, were introduced into the tissues directly without the use of a cannula.

Implantation was performed in the earliest series by the use of either a steel or glass cannula provided with an obturator. A small pellet was introduced into the end of the cannula, a minute stab wound made in the capsule of the exposed gland, and the cannula introduced to the approximate center of the gland. The pellet was then expelled by advancing the obturator. In later series a pellet about 0.5 mm. in diameter was insinuated with a sharp pointed instrument into the substance of the gland through a small stab wound in the capsule under the magnification provided by a Beebe loupe. The latter method was used for the introduction of crystals of arsenic trioxide, theclin, and thorotrast. The fact that the pellets had been placed accurately in the substance of the lymph nodes and glands was proved subsequently by histological sections.

Thyroid.—A small pellet of 5 per cent 1,2,5,6-dibenzoanthracene in cholesterol was implanted into the right lobe of the thyroid gland in 10 mice of the C3H strain. All died of intercurrent disease without the development of tumors. Because of the vascularity and the very thin structure of this gland it was most
difficult to be certain that the agent was placed within its substance and, therefore, the observation was not repeated.

We have been unable to find a report in the literature of the production of a tumor of the thyroid gland at the site of a carcinogenic agent. Dobrovol'skaia-Zavad'skaia and Adamova (9) reported a strain of mice in which there seemed to be a susceptibility to thyroid neoplasia after subcutaneous injection of 1,2,5,6-dibenzanthracene in olive oil or lard at some distance from the thyroid gland.

**Thymus.**—Pellets of 5 per cent 1,2,5,6-dibenzanthracene in cholesterol were introduced into the thymus glands of 12 mice of the C3H strain. All died of intercurrent disease without the development of tumors. A pellet of pure methylcholanthrene was introduced into the thymus of 4 rats only a few days old, and a pellet of 5 per cent 1,2,5,6-dibenzanthracene in cholesterol into the thymus of 8 additional rats of the same age. None developed tumors, although 1 animal lived almost 3 years after introduction of the agent.

Lewis has reported the development of a lymphosarcoma of the thymus in a mouse of strain C3H which had received a small amount of dibenzanthracene in the pleural cavity 115 days previously.

**Pituitary.**—A pellet of pure methylcholanthrene was introduced between the lobes of the pituitary gland in 6 rats after exposure of the gland by the technic previously described (12). None developed tumors although the last animal did not die until 584 days after implantation. In a second series of 4 rats a pellet of 10 per cent methylcholanthrene in cholesterol was placed between the pituitary lobes. One animal developed widespread tumors with the histological characteristics of a lymphosarcoma 8 months after implantation, but there was no tumor at the site of implantation of the chemical. No tumor developed in the other 3 rats, although the last animal lived 17 months after treatment.

Radon seeds, two at 1.2 mc. and two at 0.2 mc., were placed between the lobes of the pituitary gland in 4 rats, 2 of which were only 18 days old. They all died within 5 months without tumors.

No experimental pituitary tumors have been described at the site of introduction of a carcinogenic agent. Oberling, Guérin, and Guérin (19) reported 3 adenos of the pituitary in the rat, one composed of chromophobe cells, another of spongiosocytes, and a third of castration cells, which appeared after introduction of a pellet of 3,4-benzpyrene under the pia mater in contact with or within the brain tissue at a site apart from the pituitary. Another of their rats which had received a drop of 0.1 per cent solution of 3,4-benzpyrene in oil in the brain developed an epithelial tumor which invaded the anterior lobe of the pituitary. However, in a later report, these investigators suggest that these tumors may have been of independent spontaneous origin as it was found that hypophysial tumors were more common in normal rats than they had previously supposed.

Weil (27), after injections of styryl 430 into the brains of rats, reported that "neoplastic transformation took place in the pars nervosa and glandular portion" of the pituitary gland. Seligman, Shear, and Alexander (22) have succeeded in producing brain tumors in mice with methylcholanthrene, but have reported no pituitary tumors.

**Testis.**—A pellet of 5 per cent 1,2,5,6-dibenzanthracene in cholesterol was introduced into the right testis of 10 mice of the C3H strain, and a pellet of 50 per cent dibenzanthracene in cholesterol was similarly used in 5 mice of strain A. No tumors developed, the longest survivor having lived 6 months.

Few experimental tumors of the testis have been reported. Teratomas of the testes have been produced in fowls by injections of zinc chloride by Michalowsky (17), Bagg (2), and Falin (11); and malignant seminomas of the testes have been produced by regeneration after partial castration in fowls by Champy and Lavedan (6).

**Pancreas.**—A pellet of 5 per cent 1,2,5,6-dibenzanthracene in cholesterol was placed in the pancreatic tissue in 10 mice of the C3H strain and a crystal of pure methylcholanthrene in 7 mice of strain A. No tumors resulted before the death of the last animal 6½ months after introduction of the chemical. No experimental carcinomas of the pancreas have been found reported in the literature.

**Adrenals.**—The adrenal gland adapted itself very well to introduction of a small pellet within its substance in spite of its small size. The left adrenal was used and was exposed through a left flank incision. A minute hole could be made in the capsule of the gland and a pellet introduced with minimal contamination. The animals and substances used are summarized in Table I. It is interesting to note that without exception the resulting tumors were sarcomas. The glands were examined at various stages after introduction of a crystal of carcinogenic agent, and it was observed that although the pellet was placed at the center of the gland, the small tract of introduction admitted fibroblasts for the repair process, presumably from the pericapsular connective tissue. The pellet then became surrounded by a wall of fibrous tissue from which the fibrosarcomas developed, and the parenchyma of the adrenal was destroyed by invasion by the tumor.

The only experimental adrenal tumor we have found reported is that of Lacassagne (15). A tumor of the testis was produced in a rabbit 6 months after the introduction of 1,2,5,6-dibenzanthracene in lard. After careful study he suggested that the tumor might be derived from remnants of the adrenal cortex which may occur in the rabbit's testis.

**Lymph nodes.**—The inguinal lymph node of both rats and mice was found suitable for introduction of various substances by the same technic as was used for the adrenals. This could easily be done through
a skin incision about 5 mm. long and the pellet introduced with the aid of a Beebe magnifying loupe. The animals and substances used are tabulated in Table II.

By examining the nodes in the animals dying of intercurrent diseases at various intervals after the introduction of the carcinogenic pellets, it was observed that the same sequence of histological changes occurred here as in the adrenal. A small focus of necrosis appeared about the pellet in the center of the node and gradually became replaced by fibroblasts entering along the tract of introduction. When tumors developed they were usually fibrosarcomas. In two instances the tumors appeared to be hemangio-endotheliamas. No tumors of the lymphocytic series appeared. The observations on the lymph nodes are tabulated in Table II.

An attempt was made to enhance the action of the methylcholanthrene by producing hyperplasia of the node from a sterile inflammation in the hamstring muscles by the injection of 0.1 cc turpentine in gum arabic. No tumors resulted although the longest survivor lived 21 months.

Beck (3) also found that the action of the carcinogenic chemical is not enhanced by inflammation.

Lewis (16) has reported a lymphosarcoma arising from a lymph node at the site of injection of dibenzanthracene in olive oil in the right axilla of a BA strain mouse, after 237 days, and another tumor apparently composed of lymphoblasts arising from a mediastinal lymph node. Morton and Miller (18) and also Brues and Marble (5) have reported that under appropriate conditions the application of carcinogens to the skin of mice may give rise to lymphoblastoma or lymphatic leukemia in certain strains of mice. A lymphosarcoma occurred in our series of animals following the introduction of 10 per cent methylcholanthrene in cholesterol in the pituitary region.

Salivary gland.—Pure methylcholanthrene in the form of a pellet was introduced into the right submaxillary salivary gland of 4 rats. One epidermoid carcinoma resulted after 196 days. Two of the animals died with abscesses in the salivary glands in the third month after the introduction of the carcinogen. Subsequent experiments have been carried out on mice because of the shorter period usually required for the development of tumors with carcinogenic agents in these animals. A total of 30 mice of strain A has been used. When tumors developed, the animals in many instances were allowed to live until death re-

### Table I: Experiments on the Adrenal Gland

<table>
<thead>
<tr>
<th>Animals</th>
<th>Number</th>
<th>Substance</th>
<th>Tumors</th>
<th>Day of first observation of palpable tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H mice</td>
<td>5</td>
<td>100% methylcholanthrene</td>
<td>3 fibrosarcomas</td>
<td>160, 166, 178</td>
</tr>
<tr>
<td>A mice</td>
<td>9</td>
<td>100% methylcholanthrene</td>
<td>2 fibrosarcomas</td>
<td>263, 280</td>
</tr>
<tr>
<td>A mice</td>
<td>17</td>
<td>100% methylcholanthrene + glycerine</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>A mice</td>
<td>5</td>
<td>Thelin crystals</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>A mice</td>
<td>10</td>
<td>50% theelin in cholesterol</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>A mice</td>
<td>10</td>
<td>Thorotrast crystals</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Rats</td>
<td>8</td>
<td>100% methylcholanthrene</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

### Table II: Experiments on Lymph Nodes

<table>
<thead>
<tr>
<th>Animals</th>
<th>No. used</th>
<th>Substance</th>
<th>No. of tumors</th>
<th>Day of first observation of palpable tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H mice</td>
<td>9</td>
<td>100% methylcholanthrene</td>
<td>5 fibrosarcomas</td>
<td>204, 220, 205, 196, 205</td>
</tr>
<tr>
<td>C mice</td>
<td>16</td>
<td>100% methylcholanthrene</td>
<td>8 fibrosarcomas</td>
<td>185 to 328</td>
</tr>
<tr>
<td>A mice</td>
<td>8</td>
<td>100% methylcholanthrene</td>
<td>2 hemangio-endotheliamas</td>
<td>283, 308</td>
</tr>
<tr>
<td>A mice</td>
<td>10</td>
<td>AsO₃ crystals</td>
<td>5 fibrosarcomas</td>
<td>128, 196, 153, 153, 196</td>
</tr>
<tr>
<td>A mice</td>
<td>5</td>
<td>50% theelin in cholesterol</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>A mice</td>
<td>11</td>
<td>Pure theelin crystal</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Rats</td>
<td>8</td>
<td>0.1 cc. turpentine in gum arabic in</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>hamstring muscles</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100% methylcholanthrene in lymph node</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
to the skin at their first appearance, so that it may safely be assumed that they did not arise from the skin.

At the Third International Cancer Congress in 1939, Steiner (24) reported the induction of epidermoid cysts, squamous cell carcinomas, and carcinoaromas in the submaxillary gland of rats and mice by the use of 3,4 benzpyrene and methylcholanthrene. Benecke and Schröder (4) have reported the production of polymorphous spindle cell or round cell sarcomas in rats, and sarcomas with 1 squamous cell carcinoma in mice, from the injection of benzpyrene into the parotid glands. Rusch, Baumann, and Maison (21) have also reported squamous cell carcinomas in mice and spindle cell sarcomas in rats following injections of 3,4 benzpyrene and 1,2,5,6 dibenzanthracene dissolved in corn oil into the submaxillary salivary glands.

Because a specific virus has been described as occurring in the submaxillary salivary gland in a large percentage of guinea pigs (7) and also less commonly in rats (25) and white stock mice (14), Dr. Olive Gates has examined a number of normal salivary glands from our stock of mice and rats without finding any intranuclear inclusions which are reported invariably to be found when the virus is present. Injection of a cell-free and bacteria-free filtrate of a submaxillary gland tumor from a mouse produced no intranuclear inclusions in other normal mice of the same strain. This investigation was carried out to determine whether the presence of a salivary gland virus might have enhanced tumor formation in this location.

**DISCUSSION**

In all the glands used, with the exception of the submaxillary salivary gland, the parenchymal cells have been either nonsusceptible to the production of tumors, or the connective tissue taking part in the repair process has proved more susceptible to the production of tumors than the more differentiated parenchymal cells. When a tumor of connective tissue origin developed, the death of the animal invariably occurred before a tumor of the parenchymal cells appeared.

We have, as yet, found no solution to this problem in mice or rats, as the glands are so small that even a minute break in the capsule permits fibroblasts of capsular or extracapsular origin to penetrate to the center of the gland along the small channel of insertion and thus to come in contact with the carcinogenic agent. It is possible that the use of somewhat larger animals would eliminate the difficulty occasioned by the premature development of fibrosarcomas, as fibroblasts from the capsule would probably be less likely to penetrate to the center of a larger organ. However, one must be prepared to wait a much longer period of time for the development of tumors from carcinogenic chemicals in the larger animals as judged by the reported periods required for the development of tumors from the skin and subcutaneous tissues in these animals.

The mechanism of the production of malignant tumors by chemical compounds has previously been described by Wolbach (28), and the more recent literature has been reviewed by Cook and Kennaway (8). There is general agreement that foci of necrosis appear which produce a characteristic repair reaction, leukocytic infiltration, and dilatation of capillaries. The reactions we have observed in the tissues are in keeping with these observations and, although the chemical was placed in the parenchyma of the gland, the repair reaction was excited almost exclusively in the connective tissue.

**SUMMARY**

Attempts have been made during the past 4 years to produce tumors of the more highly differentiated tissues as represented by the pancreas, lymph nodes, and the thymus, thyroid, adrenal, pituitary, and salivary glands with 1,2,5,6 dibenzanthracene, methylcholanthrene, radon seeds, crystals of thorotrace, of theelin, and of arsenic trioxide in rats and mice. Five fibrosarcomas developed in the adrenal region and 18 fibrosarcomas appeared at the site of implantation of methylcholanthrene in lymph nodes, but all were thought to have originated from the pericapsular connective tissue. Two hemangioendotheliomas developed from methylcholanthrene implanted in lymph nodes. In only the submaxillary salivary glands were tumors of parenchymatous origin produced. In this location, of 17 tumors produced, 12 epidermoid carcinomas and 5 fibrosarcoma were microscopically verified.

The authors are indebted to Dr. Shields Warren and Dr. Olive Gates for assistance in interpreting the microscopic sections of the tumors.

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