Induced Tumors of the Parotid Gland*

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This experimental work was undertaken to study the effects of various carcinogens on salivary glands in the hope of producing primary epithelial neoplasms, and, among these, the so-called “mixed tumors” of the parotid and submaxillary glands. Indeed, this study has been stimulated by the meager results obtained in this field by a few authors and the uncertainty concerning the origin and pathogenesis of these epithelial tumors of varied patterns. Additional work will be described at a later date on the transplantability of the neoplasms obtained and the tumor development, under various conditions, which altered the physiologic activity of the glands prior to and following the introduction of various carcinogens.

Löwenstein (8), in 1910, injected scarlet red oil into the parotid glands of rabbits and produced inflammatory changes which he considered responsible for atypical epithelial proliferation of the small ducts. He claimed that the changes resembled human cylindromas.

Macchiarulo and Büngeler (9), in 1929, obtained “squamous epithelial cysts” containing large masses of keratin as a result of repeated injections of tar into the parotid glands of 70 rats. The initial alterations involved the nucleus and cytoplasm of the epithelial cells.

Abb (1), in 1932, injected scarlet red with and without Peru balsam, tar, or kieselguhr into nine rats and five rabbits. These treatments brought about the formation of cysts surrounded by squamous epithelium with keratinization and metaplastic changes.

Benecke and Schröder (2) injected benzpyrene in olive oil into the parotid glands of 53 rats and 55 mice but obtained positive results in only two rats. These were sarcomas. The oil cysts which developed were lined by papillary epithelial proliferations with massive keratin.

In their experiments Franseen, Aub, and Simpson (4) introduced methylcholanthrene and 1,2,5,6-dibenzoanthracene into the salivary glands of 30 mice, producing 17 tumors, 12 of which were epidermoid and 1 a fibrosarcoma. These neoplasms, which developed in about 165 days, displayed cyst formation. No mixed tumors were obtained.

In 1940 Rusch, Baumann, and Maison (13) surgically exposed the submaxillary gland of mice and introduced 1,2,5,6-dibenzoanthracene and 3,4-benzpyrene. In 2–3 months the resulting tumors measured about 2.5 cm. These authors described a metaplasia of the glandular epithelium to stratified squamous epithelium with occasional malignancy.

An investigation of the histogenesis of tumors of the salivary glands by Steiner (16) produced a comprehensive work on this subject. Into mice he inserted 1,2,5,6-dibenzoanthracene and also cholesterol. Rats were treated with benzpyrene and methylcholanthrene and guinea pigs with methylcholanthrene and cholesterol. The site of deposition of the chemical was the submaxillary gland. Methylcholanthrene was used in a similar manner in rabbits. All animals except the rabbits responded with tumor production—usually squamous-cell carcinoma. No adenomas were produced. The rabbits developed an early epithelial metaplasia which failed to become malignant.

EXPERIMENTAL
STUDIES WITH RABBITS

Because of the specificity of species response, the type of animal, as well as the carcinogen, was altered several times. Although little work had been done on rabbits, the size and accessibility of the rabbit parotid gland prompted its investigation.

Rabbits weighing 4–5 pounds were selected for implantation with the following chemicals: methylcholanthrene, 9,10-dimethyl-1,2-benzanthracene, 1,2-benzanthracene, and 1,2,5,6-dibenzoanthracene. The pellets for implantation were 5 mm. long, 19-gauge in cross section, and were composed of a 20 per cent solution of the carcinogen in beeswax. The pellets, usually 2 or 3, each weighing 1.2 mg., were introduced into the surgically exposed main duct and parotid gland proper from a small syringe. Twenty animals were treated with pellets in this way, but one death decreased the number

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to nineteen. In six animals, methylicholanthrene was the agent; in four, 9,10-dimethyl-1,2-benzanthracene, and in nine, 1,2-benzanthracene. The animals were killed at intervals of 3 months to 1½ years. The parotid glands, submaxillary glands, kidneys, liver, and spleen were removed for microscopic studies. Subserial sections were stained with hematoxylin and eosin.

Results.—No malignant tumor was observed in animals maintained for 1½ years. Two types of response were recognizable: one of inflammation, associated with extensive degenerative changes but without epithelial proliferation, the other of moderate inflammation with proliferation of ductal epithelium. Inflammatory edema destroyed the structural arrangement of the parotid gland and of the adjacent lymph nodes. Prominent findings were periductal round-cell infiltration and fatty and hyalin degeneration. The almost universal fibrosis in cases of long standing was grossly visible; it replaced portions of the gland and surrounding tissue and often showed areas of central liquefaction.

In other instances, the tissue in the vicinity of the pellets, which remained intact for many months, frequently revealed hyalinization. The hyalin appeared altered in its staining reaction by combination with the secretion of epithelial cells, the nuclei of which were scattered through this pink and bluish stained area. Eosinophiles and plasma cells were usually observed. There was proliferation of ductal epithelium together with the formation of a moderate number of new ducts which in some areas were expanded and lined by several layers of epithelial cells. This feature bears a definite resemblance with the pseudo-cartilage of the so-called “mixed tumors” of the salivary glands (Fig. 1). In a few sections, growth of adjacent lymphoid tissue into the glands was observed.

Studies with Mice

In view of the resistance of the rabbit to production of epithelial tumors of the salivary glands, further experiments were carried out with mice, the following strains being used: C57 black, C(b alb c), A, and AK. The carcinogens used were: 1,2,5,6-dibenzanthracene, methylicholanthrene, and 9,10-dimethyl-1,2-benzanthracene.

The experiment consisted of implanting 0.3 mg. of 20 per cent carcinogen in beeswax (four 20-gauge pellets 3 mm. long) into surgically exposed parotid and submaxillary glands.

Table 1 indicates the number of mice of the four strains treated with the various carcinogens.

Results.—Although the response to the carcinogens was characteristic to the strain, there was no relationship between the strain and the types of tumors produced by the chemical. Initial tumor production occurred simultaneously in C57, A, and C strains at about 14 weeks. However, in later attempts with about one-half the dose of carcinogen, the C type animals responded as early as 9 weeks.

The C57 black strain had a longer induction period, requiring 1—2 months longer for the relatively few tumors produced, and it was also considerably more sensitive to the toxic action of the agents.

The response of strain C was the most promising. The neoplasms presented an extraordinary variety of types of histologic patterns. Metastases to the lungs were observed in this group.

Although few strain A animals were used, they responded to all agents with early tumor production. Only one animal did not develop a malignant tumor by the third month.

TABLE 1

<table>
<thead>
<tr>
<th>Agent</th>
<th>C57</th>
<th>C</th>
<th>A</th>
<th>AK</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2,5,6-Dibenzanthracene</td>
<td>8</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Methylicholanthrene</td>
<td>9</td>
<td>61</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>9,10-Dimethyl-1,2-benzanthracene</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Strain AK, still under observation, responded poorly even at 6 months, only three malignant neoplasms having developed in the 25 animals examined.

Macroscopically, all tumors resembled each other. The few tumors that appeared connected with or that had perforated the skin were discarded, to avoid the study of possible epidermal tumors. The tumors varied in size from 1 to 2 cm. or more in diameter. Some of them appeared encapsulated, others did not. The tumors were solid or cystic. The latter occasionally contained mucoid material.

Microscopic Examination

Since the produced neoplasms did not differ fundamentally in their histologic pattern, regardless of the strain or carcinogen which had been employed, an individual analysis of the microscopic details was deemed unnecessary. The following description is generally applicable to all our cases.

Although the reaction to the carcinogen pellets started around the implant, subserial sections evidenced a multicentric growth that followed the initial response.

The inflammatory alterations were usually moderate, and the hyalinization of the tissue adjacent to the pellet was not so pronounced as in some rabbits. However, even in the cases of small tumors, the tendencies toward proliferation of ductal...
FIG. 1.—Rabbit parotid gland; 9,10-dimethyl-1,2-benzanthracene, 12-month duration. Proliferation of ductal epithelium and new duct formation in dense hyalinized cartilage-like tissue resembles so-called “mixed tumor” of salivary glands. × 120.

FIG. 2.—Mouse, strain C; 9,10-dimethyl-1,2-benzanthracene, 3-month duration; adenoma of parotid gland. × 200.

FIG. 3.—Mouse, strain C; methylcholanthrene, 9-week, duration; adenoacanthoma of parotid gland, 2 cm. in diameter. × 40.

FIG. 4.—Peripheral area of Figure 3. Large masses of whorled keratin bordered by cords of palisading polymorphous cells with hyperchromatic nuclei. × 250.
epithelium, transformation of its columnar cells into cuboidal or flattened epithelium, and formation of ductlike structures, lined by multi-layered epithelium, were noticeable. Participation of acinous cells in the response was uncommon.

This reaction, which was generally seen, was the initial stage of a great variety of morphologic features. In few cases (strain C with methylcholanthrene), we found adenomatous growths of low-grade malignancy (Fig. 2) composed of newly formed serous and few mucous acini with an admixture of tubules and solid aggregations of squamous epithelial cells. We traced the origin of these cells to the epithelium of the intercalated ducts which possesses a pluripotential plasticity. It has been established by Schaper and Cohen (14) and Skorpi (15), among others, that these columnar epithelial cells may differentiate into serous, mucous, and squamous epithelium. Our study of sections from 73 normal parotid glands, removed at random at the autopsy of adults, and from 134 primary epithelial parotid gland tumors corroborated not only this statement but also the findings of Hartz (6) and Meza-Chávez (16) that sebaceous glands may arise from the ductal epithelium of the salivary glands.

Most of the tumors in mice were adenoacanthomas which displayed papillary arranged epithelium with excessive keratinization and glandular structures. There was variation in the distribution of these features; in some instances, the pattern was that of a well differentiated acanthoma, while in others, however, less often, an adenoma prevailed. Some tumors of such a composition were frankly malignant, whereas others showed a low-grade malignancy, or none. A relatively small number of polymorphonuclear cells and lymphocytes was scattered throughout the epithelial and stromal tissue with some exudate in the lumina of the newly formed ductlike structures. Strands of proliferated cuboidal, columnar, and polyhedral epithelial cells of ductal origin with hyperchromatic nuclei encircled masses of whorled keratin into which cords of epithelium, with a dense fibrous-tissue center, extended. The keratin contained chromatin particles and pearl bodies (Figs. 3, 4). Ductlike structures surrounded by the epithelium described above were filled with sloughed cells and nuclear debris. Single cells also showed keratinization.

Since the transplantability of these tumors and the behavior of the transplants will be discussed in a separate study, it is sufficient to indicate that the adenoacanthoma took very well in the kidney and liver, maintaining its original structural features (Fig. 5).

There were also variants of this tumor which represented the features of a papillary cystadenoma and papillary cystadenocarcinoma. The papilae which contained either a very vascular loose or myxomatous connective tissue or hyalized material were lined by single or multiple layers of cuboidal epithelium and projected into blood-filled cavities (Fig. 6). In many instances, the base of the papillary carcinomatous projections merged with collections of poorly differentiated, malignant epithelial cells.

Another pattern of the tumor produced in a strain C mouse by 0.25 mg. methylcholanthrene in 4½ months displayed densely packed masses of vacuolated acinous cells with faint nuclei. Hyperchromatic nuclei, cells of fusiform, stellate, oval, and round shape were prominent between the vacuolated epithelial cells. We are inclined to regard them as myoepithelial cells within an adenocarcinoma with many mitotic figures (Fig. 7). It is of interest that there were metastases to the lungs.

A small percentage of the neoplasms which were produced were diagnosed as rapidly growing, undifferentiated carcinomata with an occasional admixture of glandular formations. In some instances, these tumors were composed of cells with hyperchromatic nuclei and scant cytoplasm; in others, the cells appeared vacuolated. No squamous differentiation was detectable. Some of these undifferentiated carcinomata grew well when transplanted into the kidney and liver.

Quite a few rapidly growing, large neoplasms of the parotid glands of mice of the AK and C strains were composed of variably sized cells with round to oval hyperchromatic nuclei and many mitotic figures. Numerous, rather sharply bordered, multinucleated giant cells were scattered throughout the tissue. An occasional cell was large, elongated, and contained a very large hyperchromatic, irregular or lobulated nucleus. The parotid glands were completely replaced by this type of tumor which was partly hemorrhagic and necrotic. Many tumor cells had drawn out cytoplasmic processes, thereby resembling malignant fibroblasts. However, a thorough examination of subserial sections clearly demonstrated a gradual transition from epithelial cells to polyhedral and giant cells. This led the authors to interpret the tumor as a giant-cell carcinoma (Fig. 8). Microscopically, the thyroids were not involved.

**DISCUSSION**

While the introduction of various carcinogens into the parotid glands of rabbits did not produce true neoplasms, 58 tumors were obtained by the same methods in 150 mice of different strains. The
FIG. 5.—Transplant of adenoacanthoma (Fig. 4) growing in the kidney of a mouse, strain C. × 125.

FIG. 6.—Mouse, strain C; 9,10-dimethyl-1,2-benzanthracene, 11-week duration; papillary cystadenoma of parotid. × 125.

FIG. 7.—Mouse, strain C; methylcholanthrene, 4½-month duration; undifferentiated carcinoma of parotid gland with proliferated myoepithelial cells. × 256.

FIG. 8.—Mouse, strain C; methylcholanthrene, 20-week duration; parotid gland, giant-cell carcinoma. × 250.
effort to incite the formation of the so-called "mixed tumor"—more adequately called polymorphic adenoma (Willis [18])—was unsuccessful, although small areas of pseudo-cartilage and adenomatous structures resembling this growth were found in some of our tumors.

The most commonly induced neoplasm of the parotid gland in the mouse was the adenoacanthoma, occurring in varied patterns. This tumor is seldom observed in its classic form, that is, displaying the characteristics of keratinization of epithelial cells associated with glandular structures in the parotid glands of human subjects. While the amount of keratin was great in many of the growths, there were others with little or no keratin. In the latter instance, the tumors had the features of a capillary cystadenoma and capillary cystadenocarcinoma.

We observed the participation of myoepithelial cells in the formation of an adenocarcinoma with metastases in the lung showing the morphologic features of the primary tumor. The myoepithelial cells stained brilliant red with hámalaun-erythrosin-safranin (trichrome stain of Masson), while the connective tissue appeared yellow. Adenoacanthomas of the salivary glands displaying many variations of structure recently received special attention in view of the work of McPeak and Arons (10) and McPeak and Warren (11) on adenoacanthomas in the esophagus and cardio-esophageal junction originating in the esophageal mucous glands, which are identical with the salivary glands.

The occurrence of rapidly growing parotid gland tumors in mice, showing an admixture of an adenoacanthoma with a sarcoma-like tissue containing numerous multinucleated giant cells, hemorrhages, and necrosis is of significance. The sarcoma-like tissue contained clearly bordered epithelial cells, occasional pearl bodies, and keratinized individual epithelial cells. Careful study of these tumors revealed a smooth transition of the cells of the solid acanthoma cords into elongated spindle-shaped cells and multinucleated giant cells. This finding induced us to conceive of these neoplasms as so-called giant cell carcinomata of the parotid glands. The thyroid was intact. The fact that these tumors have been produced by carcinogens does not diminish the importance of this finding. These growths were wholly of epithelial origin, even though collagenous fibers were demonstrable in the sarcoma-like part. Following the work of Foot and Day (3) the Tureen and Seelig (17), Hebbel (7) showed that the presence of collagenous fibers in the sarcomatous portions of an epithelial tumor of the thyroid does not interfere with the interpretation of this tumor as a giant-cell carcinoma. Hamperl (5) arrived at the same conclusion in his study of two "carcinosarcomas" of the breast but conceived of the spindle-shaped cells as myoepithelial cells, because their cytoplasm stained red with erythrosin. According to Willis (18), spindle cells and giant cells in a carcinoma result from the lack of differentiation. However, the morphologic structure of these giant-cell carcinomata differed considerably from the few poorly differentiated carcinomata which we produced. Spindle cells and giant cells were entirely absent from the latter.

We were not able to demonstrate that these induced tumors of the salivary glands of mice took origin from the acinous epithelium. However, we found evidence for the fact that they developed from the pluripotential epithelial cells of the intercalated ducts. This opinion agrees well with the so-called Heidenhain theory and with the study of Schaper and Cohen (14), who considered the cells of the intercalated ducts to be centers of proliferation. These cells may differentiate into squamous cells or, under neoplastic conditions, into mucous cells which are not found in normal parotid glands of adults.

SUMMARY

1. Rabbits seem to possess an unusual resistance to the production of induced salivary gland tumors.
2. Of the mice employed, strain C was the most responsive; strains A, C57 black, and AK, in that order, were less responsive.
3. Once the neoplasm had been induced, the strain of the mouse did not influence the type of tumor produced.
4. The adenoacanthoma with its many varied patterns was the most frequently produced tumor.
5. Related tumors were adenoma, papillary cyst adenoma, undifferentiated carcinoma, and giant-cell carcinoma. Metastases into the lungs occurred.
6. The site of origin of the initial metaplastic change and of the subsequent neoplasm was the pluripotential cuboidal epithelial cell of the intercalated ducts. These ducts may be regarded as proliferation centers.

REFERENCES


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