HISTOGENESIS OF SEBACEOUS GLAND CARCINOMAS PRODUCED IN RATS BY 2-ACETYLAMINOFLUORENE*, ‡

By STANLEY C. SKORYNA,§ M.D., RODERICK C. ROSS, M.D., and L. A. RUDIS, M.D.

(From the Departments of Experimental Surgery and Pathology, McGill University, Montreal, Canada)

Plates 1 to 5

(Received for publication, March 23, 1951)

Tumors produced by 2-acetylaminofluorene occur with differing frequencies in various organs. The squamous-cell carcinomas of the auditory canal region, reported in the original experiment of Wilson, De Eds, and Cox (23), appear to be of particular interest. There has been considerable discussion in the literature as to their site of origin and mode of development. In our experiment this type of tumor was the most frequent one. This stimulated our interest in further investigation of the factors involved in their pathogenesis.

Wilson, De Eds, and Cox (23) introduced 2-acetylaminofluorene (hereafter referred to as 2-AAF) as an experimental carcinogen in 1941. Their report included descriptions of tumors produced in the bladder, kidney, liver, lungs, and head, although the emphasis was placed on bladder tumors. In the experimental studies by Bielschowsky (2, 6), Harris (12), Cantarow, Paschkis, and Stasney (7), and Engel and Copeland (11), tumors developed in additional sites, including the gastrointestinal tract and reproductive organs. The majority of tumors have been epithelial. In a few instances sarcomas and leucemias were found. The most effective route of administration appears to be oral, although remote tumors were also produced on parenteral application. When 2-AAF is added to a standard diet, tumors occur in 70 to 90 per cent of the animals receiving it. 2-AAF appears to have almost exclusively remote carcinogenic action, no tumors occurring at the site of application, although Heiman and Meisel (13) reported local production of tumors by intraesophageal instillation of 2-AAF by means of a curved needle. The compound has been found to be particularly useful administered in combination with cocarcinogens (3), and in the investigation of changes preceding the development of malignancy (20).

Detailed studies of the metabolism and excretion of 2-AAF have been carried out by Westfall (22), Morris and Westfall (17), and Bielschowsky (4). Westfall, using a

* This work was supported by a grant-in-aid from the National Cancer Institute of Canada.

‡ Presented at the annual meeting of the American Association for Cancer Research, Cleveland, April, 1951.

§ Research Fellow in Experimental Surgery, National Cancer Institute of Canada.

method of quantitative estimation of 2-aminofluorene depending on diazotization of the amino group, found the compound in a conjugated form in tissue extracts of many organs and body fluids. Bielschowsky (4) isolated a metabolite, 2-acetyl-7-hydroxyfluorene, from the urine of rats fed 2-AAF. The metabolite had no carcinogenic properties.

Variations in the frequency and site of the tumors have been reported when different strains of rats were used (10). Mice were generally found to be less susceptible to this carcinogen (1).

The incidence of "head" tumors has been variously reported, ranging from 16 per cent in the experiment of Harris (12) (17 out of 104) to 78 per cent in a piebald strain by Bielschowsky (5) (30 out of 38). They were reported to occur exclusively in the external auditory canal region and not to be adherent to the skin of the head in the early stages of development. Cox, Wilson, and De Eds (9) identified them as squamous-cell carcinomas arising from a structure adjacent to the external auditory canal, probably a sebaceous gland. Bielschowsky (2) classified them as external acoustic duct tumors. Harris (12) considered them to be of low grade malignancy because of the sharp circumscription from the surrounding structures by a fibrous capsule. The invariable presence of necrotic material in the midst of the tumor has been associated with secondary infection.

Wilson, De Eds, and Cox (24) considered the possibility of the carcinogenic effect being local, the 2-AAF in the food coming into contact with the ear during feeding. Powdered 2-AAF was blown into rats' ears for 13 months at weekly intervals without gross evidence of ear tumors in animals autopsied up to 24 months. The 2-AAF powder was found to dissolve the cerumen with resulting continuous exposure of the external duct to the carcinogen. This work seems to exclude the possibility of local action of 2-AAF in the production of these tumors. The relatively early occurrence of tumors of the head, as compared to other sites, Bielschowsky (2) explained by the fact that the external location of the tumors leads to early recognition of them.

Methods

2-acetylaminofluorene was administered to a colony of hooded rats derived from a local strain from the McGill University Clinic, Royal Victoria Hospital. They had been highly inbred by continuous brother and sister mating since 1933. The carcinogen was added to a standard Purina meal diet. The concentration in the food was 0.05 per cent for the first 7 weeks, and 0.04 per cent for the following 28 weeks of administration. The rats were 7 to 8 weeks old at the beginning of the experiment with the exception of 20 older male rats. The sex distribution was equal.

EXPERIMENTAL OBSERVATIONS

Out of 150 rats, 124 survived a 6 month period of administration of the compound. The deaths were due to liver damage from the carcinogen, and in a few cases to extensive pulmonary infection. Tumors occurred in 80 per cent

of the surviving rats. The sites included head, liver, breast, lungs, bladder, kidney, and other locations. Five female rats were still living at the end of the 20th month, when the experiment was terminated.

"Head" tumors were observed in 71 out of 124 animals (57 per cent), in some cases developing coincidently with liver and breast tumors. The sex distribution was 43 females to 29 males. This sex difference should probably be discounted in view of the higher mortality among male rats in the early months of administration, from hepatotoxic effects and cirrhosis. The first "head" tumors were observed at the end of the 6th month of feeding with 2-AAF. The majority of tumors occurred between the 9th and 12th months, although they continued to appear as late as the 17th month of the experiment. Usually the animals were left until they died from tumors or were killed in the terminal stage of extreme cachexia, when death appeared imminent. The average length of survival between the time the tumor was first noticed and death of the animal was 5 to 10 weeks.

Gross Pathology

In the early stages subcutaneous lumps were observed to develop anterior to the external auditory canal. At first the skin was freely movable over the tumors, but as they enlarged, ulceration of the skin usually occurred (Fig. 1), with discharge of greasy, greyish-yellow material. In some cases there was a similar discharge from the auditory meatus, apparently due to perforation of the tumor into the auditory canal. Frequently, especially in the advanced stages, infection led to putrefaction of the discharge with the formation of subcutaneous abscesses. In one case an internal perforation behind the neck musculature was observed, with the formation of a paracervical fluctuant abscess. In several cases massive hemorrhage from the tumor led to death of the animal. This was found to be due to erosion of the large stapedial vessel, located close to the tumor.

In all 71 animals which developed "head" carcinomas, the location of the tumor was identical. This was particularly well observed in 12 animals in which the tumors were bilateral. They were located antero-inferior to the auditory canal, and adherent to its medial end. They were in contact with the skull, and in some cases erosion of it was macroscopically evident.

The tumors were roughly spherical and their diameter ranged from 0.5 to 5 cm. On reflection of the skin small tumors appeared as firm grey-white nodules with a lobulated, somewhat brain-like surface. On section they were seen to consist of grumous grey-white material, similar to that seen in sebaceous cysts, enclosed by firm, grey tissue a few millimeters in thickness.

Anatomy of the Auditory Compound Sebaceous Gland

In view of the fact that these tumors always developed in the same location, dissections of the auditory region were performed on rats fed a normal diet,

without the carcinogen. A compound sebaceous gland was found to be present at the medial end of the external auditory canal. Similar location of the tumors and this gland led us to believe that they might have originated from it. Serial histological sections revealed that the main excretory duct of the gland opened into the external auditory canal close to the tympanic membrane. The opening could be visualized macroscopically.

On subsequent review of the literature it was found that the structure and development of this gland had been described in detail in two simultaneously published papers by Zawisch-Ossenitz (25) and Zymbal (26) in 1933. Spitz, Maguigan, and Dobriner (21) reported tumors developing from this gland following the administration of benzidine.

The auditory compound sebaceous gland, as described by Zawisch-Ossenitz (25) and Zymbal (26), is a specialized gland observed only in rodents and insectivores. Zawisch-Ossenitz distinguished thirteen lobules, Zymbal three. The gland measures approximately 3 to 5 mm. in adult rats. In our experience the number of lobules varied but usually three or four major lobules were evident. Each lobule is composed of clusters of sebaceous glands ending in a minor excretory duct. The collecting ducts of the lobules form a single excretory duct ending in a groove on the antero-inferior wall of the external auditory canal. The entrance of the main duct is 2 to 4 mm. from the tympanic membrane in the gap between the bony ear and the first cartilage of the auditory canal. Virtually no sebaceous glands occur distal to this compound gland, but proximal to it numerous simple sebaceous glands are found lining the osseous portion of the external auditory canal and extending to the tympanic membrane (Fig. 2).

Histopathological Characteristics

The tumors characteristically consist of lobules of various sizes. This is particularly evident in early lesions in which the neoplastic change may be confined to one or more lobules of the gland (Fig. 3). At this stage of development the tumor lobules are frequently cystic and filled with keratin or sebaceous material. Papillary processes, often with well vascularized connective tissue cores, project into these cysts (Figs. 4, 5). Fusiform melanophores occur infrequently in the connective tissue stroma.

The tumor cells are oval or polyhedral in shape. Their cytoplasm varies from homogeneous basophilic to foamy eosinophilic, the latter identical in appearance with that of the cells of sebaceous gland acini (Fig. 6). The latter areas exhibit the same sudanophilic character as do the cells of sebaceous glands. The majority of tumors consist predominantly of sebaceous cells (Figs. 5 to 7). In other tumors the production of keratin is the dominant feature (Figs. 8, 9). In a smaller number both of these characters are prominent (Fig. 10).

The nuclei are round or oval with clear-cut nuclear membranes. There is usually a single clearly defined nucleolus, often appearing to be in contact with the nuclear membrane. The chromatin is finely stippled. In cells undergoing keratinization, pyknosis and caryorrhexis are prominent. This is also true of the more superficial cells in sebaceous areas (Fig. 7). As the cells mature and are exfoliated the shadowy remnant of their preexisting structure frequently

remains, producing the appearance that has been termed "mummification" (15). This picture is seen to best advantage in sebaceous areas of the tumor (Figs. 5, 7). In the "mummified" areas the outline of cells and nuclei and the foamy character of the cytoplasm persist. In keratinizing areas of the tumor "mummified" cells are also seen (Fig. 8). Here again the cellular and nuclear shadows persist, but the cytoplasm is not foamy. In some keratinizing tumors parakeratosis is marked, and visible nuclei are present well out into the keratinized layer (Fig. 9).

The tumors are frequently infected, and such areas of the tumor are heavily infiltrated with polymorphonuclear leucocytes (Fig. 11). The marked tendency of these tumors to become infected is probably directly related to the invariable presence of otitis media.

Mitotic figures are rare in the better differentiated tumors, but they are present in moderate numbers in more anaplastic growths. The latter are more cellular and show marked variation in nuclear size and staining (Fig. 11).

The smaller tumors show little tendency to infiltrate and growth is chiefly centripetal (Fig. 4). However, as they develop, peripheral infiltrative growth becomes evident (Fig. 12). Invasion of bone, striated muscle, and brain, as well as permeation of lymphatics, was noted (Fig. 13).

Pulmonary metastases were present in two cases. These consisted of multiple small round nodules of infected squamous carcinoma. Although in some instances the tumor bulged into bronchial lumina, there was no evidence of origin from bronchial epithelium (Fig. 14). The sebaceous character is not apparent in these metastatic lesions, and this is also true of the more anaplastic, infiltrative tumors generally.

Preexisting Lesions

In a few rats which developed ear tumors, labyrinthitis had been present for some preceding time, as evidenced by the characteristic tilting of the head to one side and disequilibrium symptoms. King (14) and Nelson and Gowen (18) reported that labyrinthitis complicates bacterial infection of the middle ear in a small percentage of cases, usually less than 4 per cent. Dissections of the middle ear cavity revealed that the incidence of middle ear disease in our strain was high, up to 80 per cent of the total number of animals examined being affected. In all tumor-bearing rats in which the middle ear was examined, chronic purulent otitis media was present. This led us to believe that the ear tumors might be associated somehow with latent middle ear infection.

The presence of otitis media did not seem to affect significantly the health or life span of the animals. No perforation of the tympanic membrane was found. However, in a few instances, marked bulging of the tympanic membrane into the external auditory canal was observed. This bulging resulted from the accumulation of purulent and necrotic material in the middle ear cavity. In

further studies in older rats which had not received 2-AAF in the diet, stasis of sebum and dilatation of the lobules of the gland were observed (Fig. 15). It seems probable that these are related to the chronic otitis media with bulging of the tympanic membrane or accumulation of exudate in the auditory canal, interfering with drainage from the gland (Fig. 15).

In rats which had been fed 2-AAF, including some which did not develop actual tumors, similar stasis of secretion and dilatation of one or more lobules of the gland were noted (Fig. 16). Zawisch-Ossenitz (25), referring to photographs of this lesion, called it "cystic degeneration." The development of this lesion does not appear to be related to the administration of 2-AAF. However, no tumors were found to originate from normal gland tissue, but only in such cystic lobules of the gland (Figs. 3, 4).

DISCUSSION

The sebaceous gland carcinomas undoubtedly arose as a result of administration of the carcinogen, since no spontaneous tumors in this location were observed in rats fed a normal diet either in our colony or in the original strain of several thousand rats. However, the administration of 2-AAF does not appear to be sufficient to produce tumors in a normal sebaceous gland. The tumors invariably were observed to begin in the epithelium of cystic lobules, whose existence seems to be a prerequisite for tumor formation. On the other hand, the cystic degenerative lesions, occurring independently of the carcinogen administration, do not *per se* lead to tumor formation. It seems probable that the presence of cystic degeneration of one or more lobules of the sebaceous gland had a localizing effect on the carcinogenic action of 2-AAF.

It is doubtful that these findings could be interpreted in the sense of the subthreshold existence of tumors, the malignancy being evoked by a non-carcinogenic process (19, 16), in this case the chronic obstructive-cystic lesions in the sebaceous glands. It seems more likely that the initiation of malignancy in the cystic lobules of the gland is a result of the action of the carcinogen on the previously injured tissue.

Cystic dilatation of lobules of the compound sebaceous gland might be compared to sebaceous cyst formation in man. The fact that the cystic lesions in rats do not *per se* lead to tumors corresponds to the low incidence of carcinomas arising in sebaceous cysts. Caylor (8) found an incidence of 3.4 per cent of malignancy in simple sebaceous cysts.

SUMMARY AND CONCLUSIONS

1. Tumors of the external auditory canal region were produced by oral administration of 2-acetylaminofluorene in 71 rats out of 124 which survived a 6 month period of administration of the compound. In 12 animals the tumors were bilateral. Tumors in other locations, such as the liver, breast, etc., were less frequent.

2. Tumors were found to originate in the compound sebaceous gland located at the medial end of the external auditory canal.

3. Histologically the tumors were sebaceous or keratinizing squamous-cell carcinomas.

4. The tumors were found to arise in cystic lobules of the gland, produced by stasis of secretion.

5. Cystic lesions of the sebaceous glands appear to be a frequent condition in older rats. The outward bulging of the tympanic membrane with interference in drainage from the gland seems to be a promoting factor in the stasis of sebum.

6. The localizing effect of the preexisting cystic lesions in the sebaceous glands on the carcinogenic action of 2-acetylaminofluorene is discussed.

The authors wish to thank Dr. D. R. Webster and Dean G. Lyman Duff for their interest and helpful criticism.

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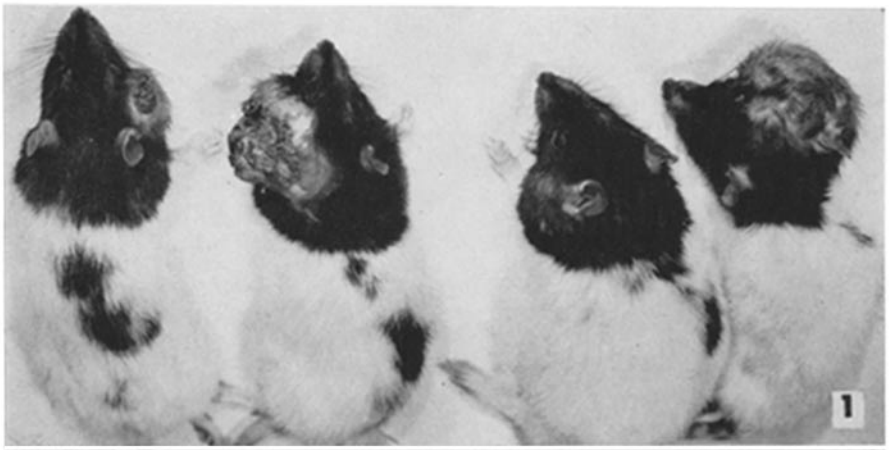
EXPLANATION OF PLATES

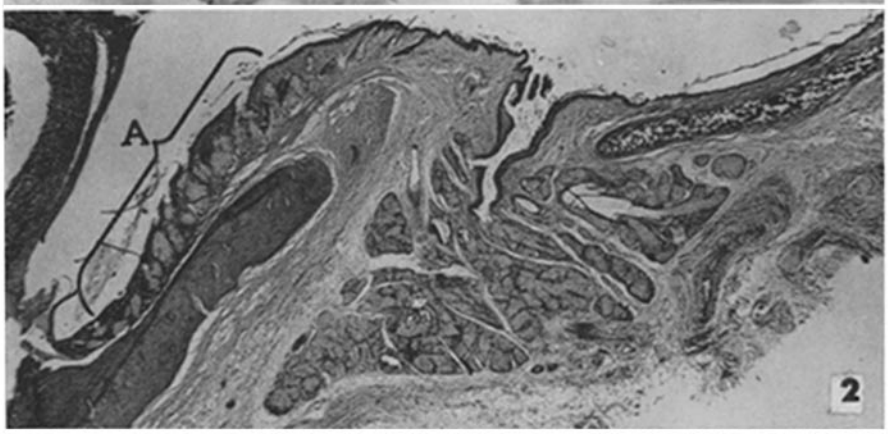
PLATE 1

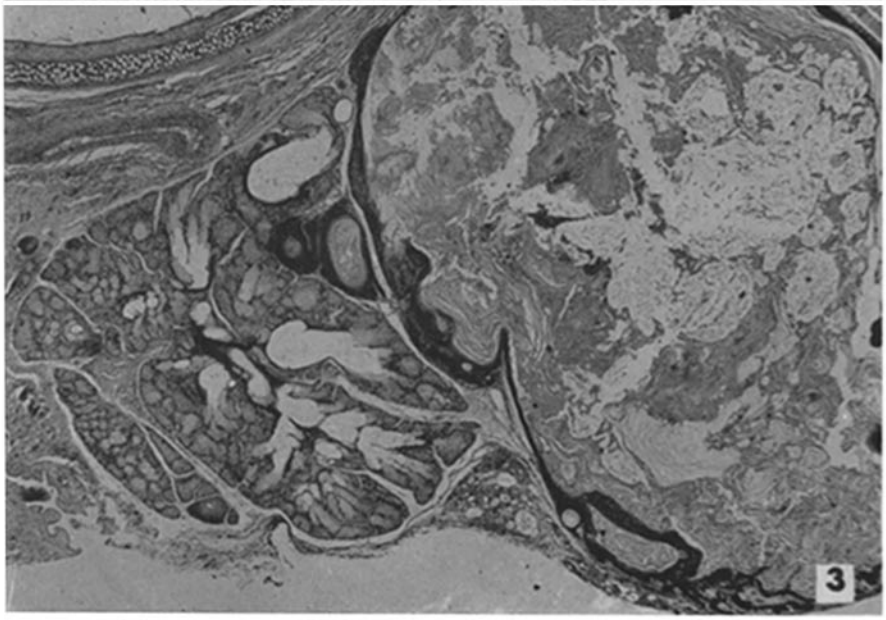
FIG. 1. Group of rats with tumors of the external auditory canal region in different stages of development. Three tumors are ulcerated.

FIG. 2. Normal compound sebaceous gland showing duct entering auditory canal between bony ear and first cartilage plate. There are numerous simple sebaceous glands (A) proximal to the compound gland but few distal to it. Purulent exudate is evident on the extreme left, internal to the tympanic membrane. Hematoxylin and eosin. × 26.

FIG. 3. Carcinoma developing in cystic lobule of compound sebaceous gland. The cyst is filled with keratin and sebaceous material. A small portion of the auditory canal can be seen at the upper left. Hematoxylin and eosin. × 26.







(Skoryna *et al.*: Histogenesis of sebaceous gland carcinomas)

PLATE 2

FIG. 4. Partial involvement of compound sebaceous gland by tumor. At the lower left a papillary process of tumor projects into a cystic lobule. In the upper field a portion of the gland shows dilatation of several ducts. Hematoxylin and eosin. × 26.

FIG. 5. Sebaceous area of tumor showing papillary process with vascularized connective tissue stalk. The exfoliating superficial cells show the shadowy outline of previous structure. Hematoxylin and eosin. × 100.

FIG. 6. Sebaceous tumor in upper field and adjoining sebaceous gland. A ductule of the latter shows dilatation and stasis of sebum. Hematoxylin and eosin. × 100.

FIG. 7. Sebaceous carcinoma showing maturation of the cells and "mummification." Hematoxylin and eosin. × 100.

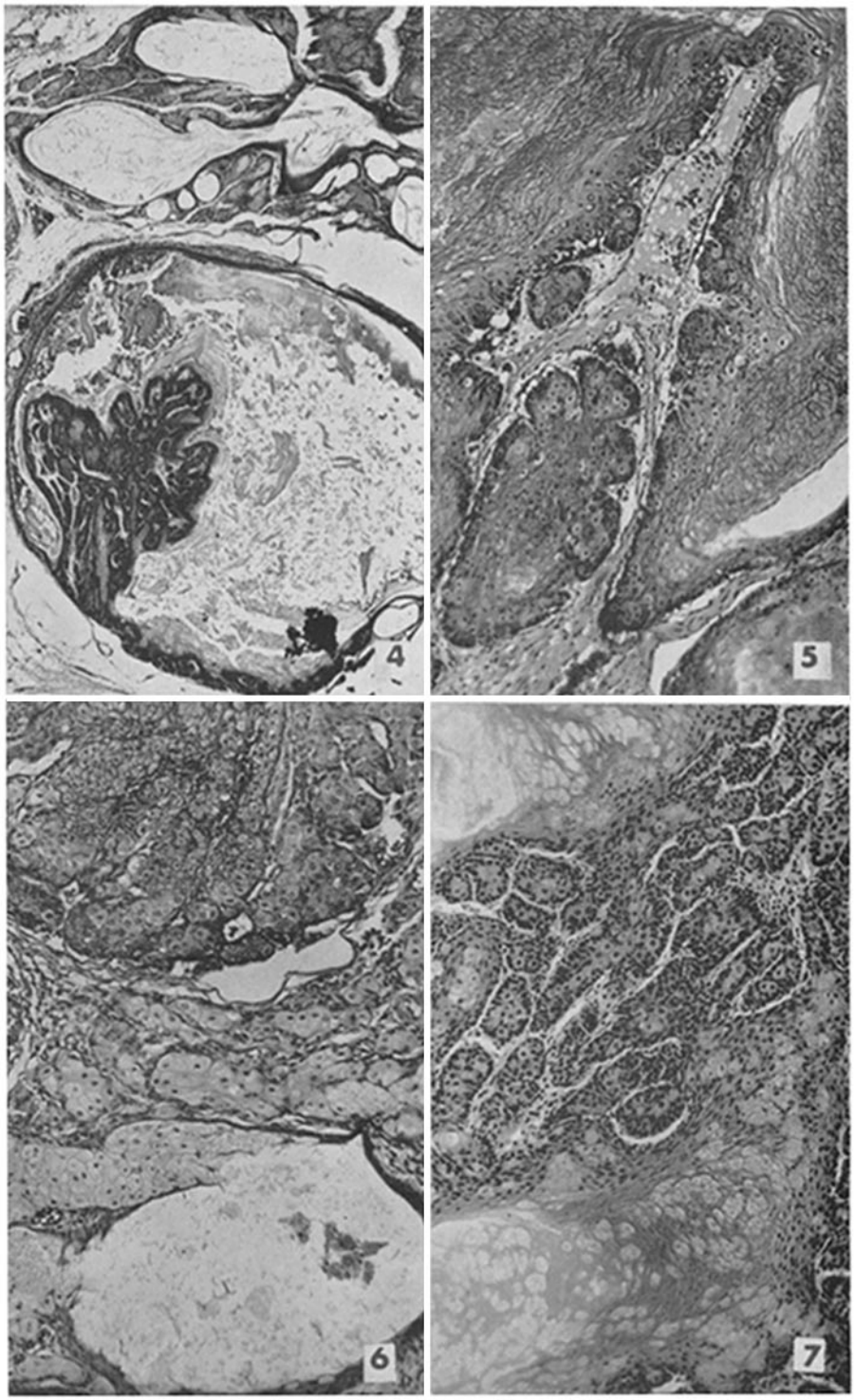


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PLATE 3

FIG. 8. Keratinizing tumor, showing an island of exfoliated "mummified" cells within the keratin. Erosion of bone is evident on the right. Hematoxylin and eosin. × 100.

FIG. 9. Squamous carcinoma showing a marked tendency to parakeratosis. Hematoxylin and eosin. × 100.

FIG. 10. Tumor showing keratinizing squamous character on the left and sebaceous structure on the right of a large capillary. Hematoxylin and eosin. × 100.

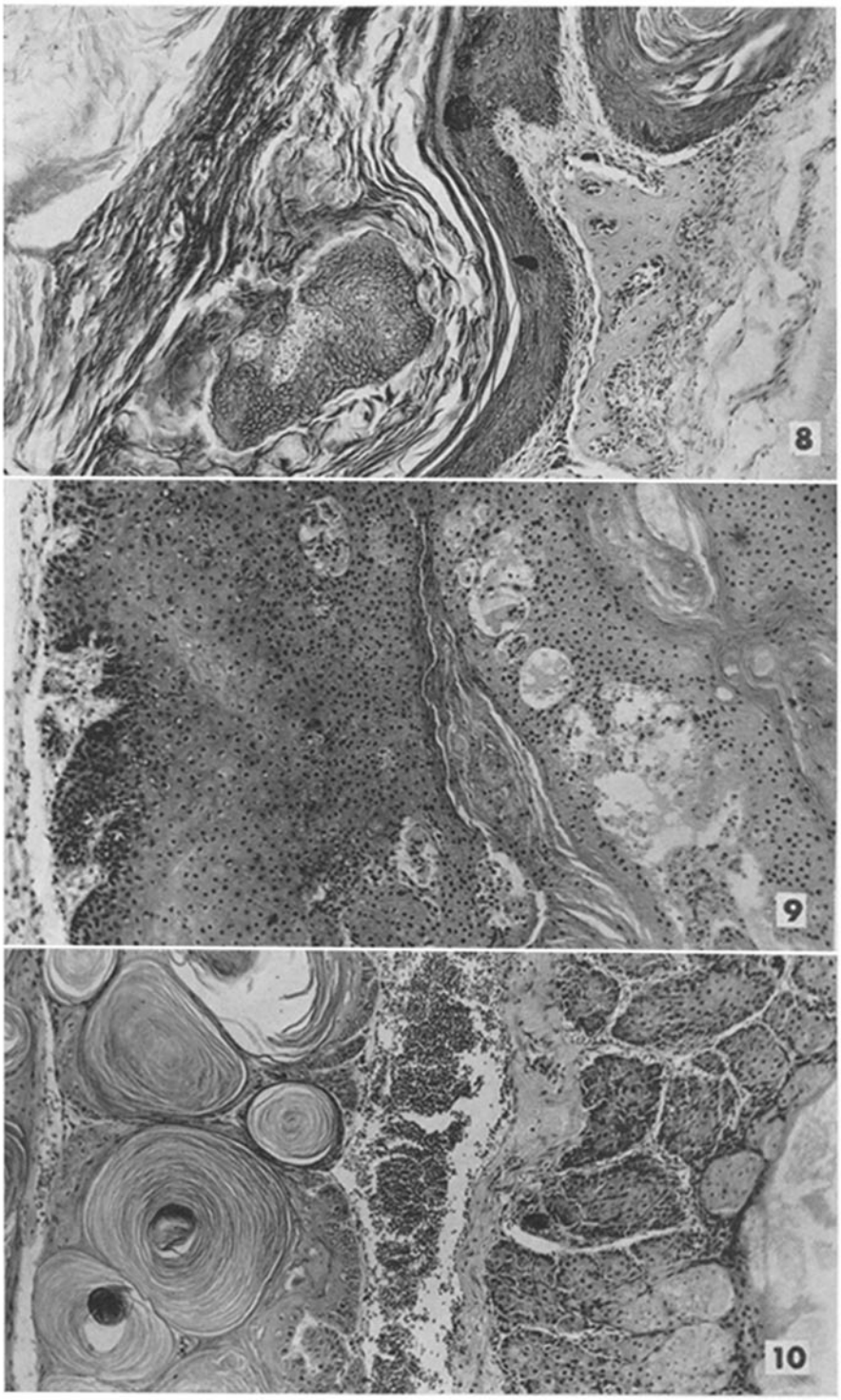

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PLATE 4

FIG. 11. Poorly differentiated area of tumor showing pleomorphism, nuclear hyperchromatism, and infection. A mitotic figure can be seen in right foreground. Hematoxylin and eosin. × 100.

FIG. 12. Peripheral portion of tumor showing infiltration of adjoining connective tissue. Hematoxylin and eosin. × 100.

FIG. 13. Permeation of perineural lymphatics by tumor. Erosion of bone is evident at the lower left. Hematoxylin and eosin. × 100.

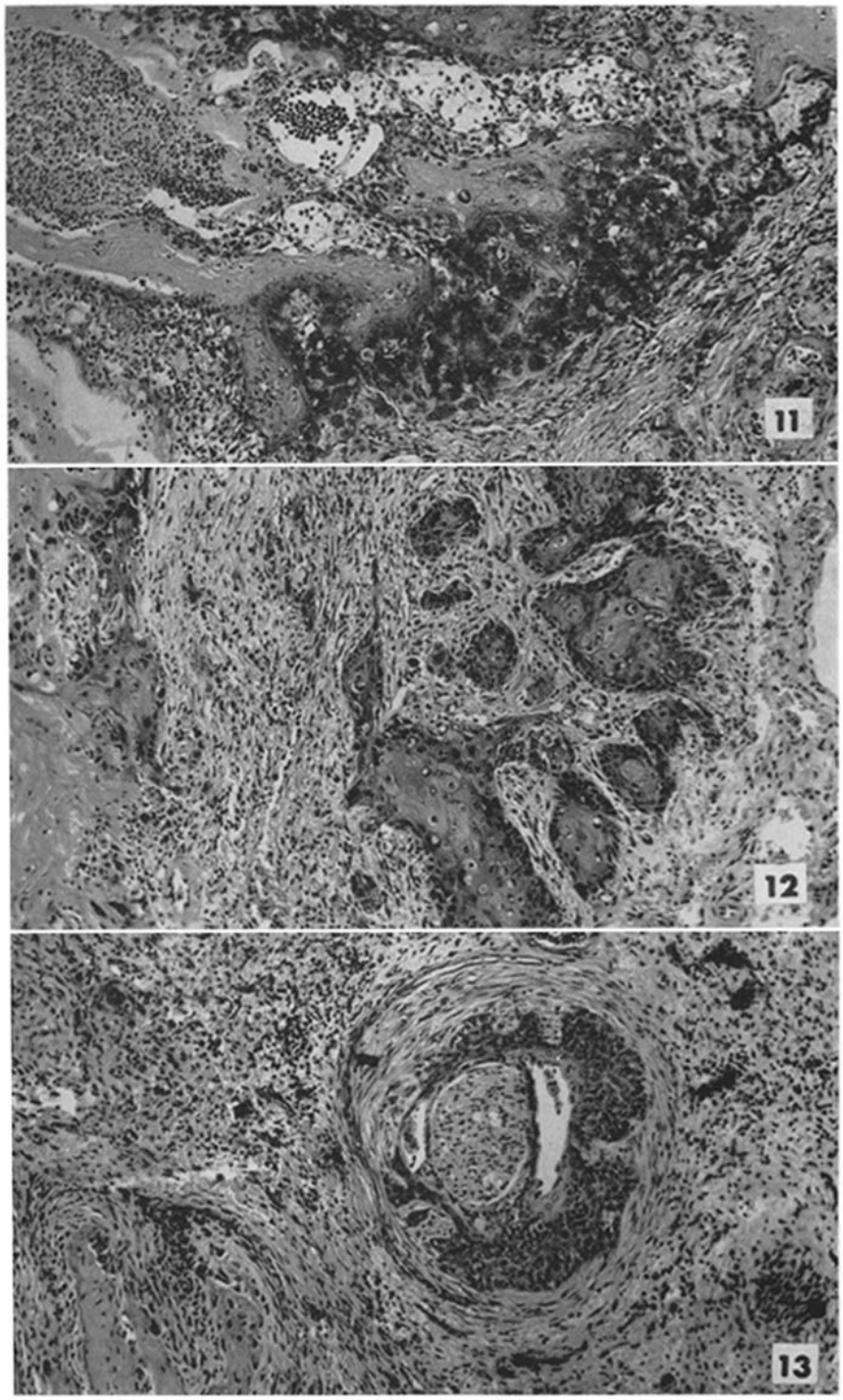

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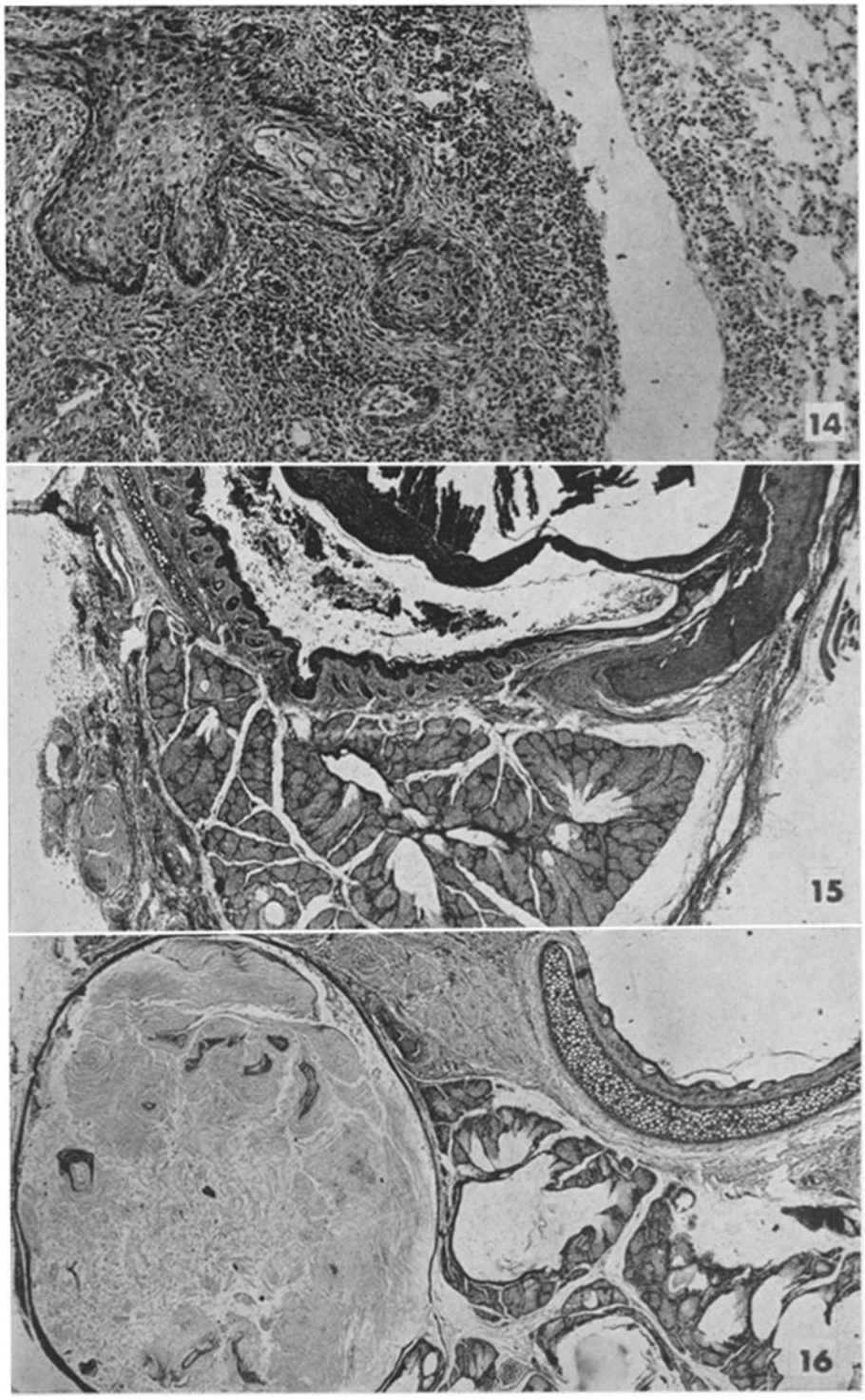
PLATE 5

FIG. 14. Metastatic tumor in lung with bulging of the nodule into the bronchial lumen. The bronchial epithelium has been shed. Masson trichrome. × 100.

FIG. 15. Chronic otitis media in an old rat which did not receive 2-AAF. Purulent exudate in the middle ear has produced bulging of the tympanic membrane into the auditory canal. There is moderate duct dilatation and stasis in the sebaceous gland. Hematoxylin and eosin. × 100.

FIG. 16. Marked dilatation of ducts with cyst formation in the compound sebaceous gland of a rat which received 2-AAF, but did not develop a tumor. Hematoxylin and eosin. × 100.



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