In a recent paper (5) the early changes produced in the liver by administration of the carcinogen 2-acetylaminofluorene were described; a few additional facts concerning this organ are given here.

In the course of this experiment the behavior of the epithelium of Zymbal’s gland has also been studied. This sebaceous gland of the external auditory meatus (first described by Zymbal [11]) is a frequent site for tumor formation in rats treated with 2-acetylaminofluorene. Similar carcinomas produced by benzidine are reported by Spitz et al. (10), and their development from the time they became palpable externally is described in their paper. The really early stages of tumorigenesis have not hitherto been studied in detail. The results presented here throw some light on the site of origin of the cancers, and afford an interesting contrast with the findings in the liver.

**Experimental Methods**

**Animals.**—The animals used in these experiments were male albino rats of the Wistar strain. Administration of the carcinogen was in all cases commenced after the animals had reached a weight of 180 gm. Twelve of the 40 rats started on experiment died between 4 and 7 weeks. On autopsy these rats generally had severe liver lesions and, in some cases, also lesions in the lungs. The animals surviving this period lived until tumors appeared, except for a few that were sacrificed earlier.

**Preparation and administration of 2-acetylaminofluorene.**—The 2-acetylaminofluorene (AAF) used in these experiments was prepared in the organic chemistry laboratory of the Institut du Radium by a method described previously (5). The chemical was incorporated in a crude diet, complete in all food factors, at a level of 0.05 per cent. This diet had the following composition: wheat flour, 50; maize flour, 10; skim milk powder, 10; molasses, 5; meat meal, 2; dried alfalfa, 4; peanut husks, 3; dried brewers’ yeast, 4; wheat germ, 3; fish meal, 3; vegetable oil, 1; cod liver oil, 1.5; bone meal, 0.5; bran, 3; and NaCl, 0.5. This was available ad libitum. In one series of experiments in which only the Zymbal’s glands were studied in detail, an equal quantity of 4-acetylaminodiphenyl was also incorporated; this had been shown to be noncarcinogenic (6) or only slightly carcinogenic, except for the mammary gland in the female (8).

**Methods of study.**—The Zymbal’s glands were studied by biopsy and in animals dying or sacrificed; details are given in Table 1. The removal of this gland by biopsy is complicated somewhat by its position under a venous plexus and in close relation to the arterial and nervous structures passing through the base of the skull. It was found possible, however, to remove it by an approach posterior to the external ear with a negligible mortality, and investigation after the death of the animal usually showed complete removal. However, as discussed later, it was found that the gland showed the first evidence of action by the carcinogen much later than the liver, and the biopsy material was essentially normal; animals dying of the tumors provided the earliest changes in these glands. In all cases the glands were imbedded whole and cut in serial sections so that the entire organ could be studied.
RESULTS

There was a certain mortality among these animals near the end of the 4th week, probably due to the marked destruction of the liver, which was at a maximum about this time. Otherwise the animals remained in reasonable condition for the duration of the experiment. The body weights, which were rising slowly at the beginning of the experiment, ceased to increase for the first 6 weeks of drug administration. After this there was some tendency to increase again for 2 or 3 months, but then the weight curve tended downward until the end of the experiment.

TABLE 1

| Data on Zymbal’s Glands of Rats Fed 2-Acetylaminofluorene | (Compounds fed for 24 weeks; data included only for animals surviving at least 18 wks.) |

| | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  |
| Start of experiment (no.) | 18  | 18  | 20  | 21  | 25  | 26  | 32  | 35  |
| Right | Left | Right | Left | Right | Left | Right | Left |
| Weight of Zymbal’s glands (mg.) | 5.0  | 7.0  | 4.7  | 7.5  | 8.0  | 12.0 | 3.5  | 5.0  |
| Presence of otitis media | +  | +  | +  | +  | +  | +  | +  | +  |
| Tumors on foie of hyperplasia | +  | +  | +  | +  | +  | +  | +  | +  |

Liver lesions.—The early changes in the liver have been described in the earlier paper quoted above. At the end of this experiment it was noted that, in all rats dead after 3 months or more of AAF feeding, there was some degree of hyper trophy and hyperplasia of the liver. Only two animals had livers weighing less than 20 gm. (normal 12–15 gm.), and in most animals this figure was considerably surpassed, reaching 40 gm. in two animals without liver tumors and 60 gm. in one animal with a small tumor of 2–3 gm. only. Sections showed little or no increase in cell size in these livers, so that marked hyperplasia is clearly present.

Lesions of Zymbal’s glands.—These lesions were observed in two series of rats, one of which received only 2-acetylaminofluorene and the other a mixture of this with 4-acetylaminodiphenyl. The purpose of the latter group was to study any possible antagonism of effect between these compounds. The negative results of this aspect of the experiment have been published elsewhere (7), and the findings on Zymbal’s glands are included here.

It will be seen from Table 1 that eleven foci of abnormal growth were found in the two series, of which three had caused significant enlargement of the gland. In these three cases there was some destruction of the normal gland. In the smallest foci, invasion and destruction were absent, but the histological and cytological character of the cells formed a strong contrast to the entirely normal tissues surrounding them. From these a sequence of enlargement and development to the invasive tumors could be traced.

In regard to the smaller foci and their origin, it was found that there was a general pattern among them. The earliest area of abnormal growth was seen in rat 7 and involved only the sebaceous cells; as in all cases, the whole of one small lobule was affected, the pattern of this being basically unaffected; but all the cells showed a basophilia of the cytoplasm and a hyperchromatism and irregularity of the nucleus in strong contrast to the normal. A further stage was seen in rats 5 and 15; in these there was change in the sebaceous tissue of one lobule and in the squamous tissue of the corresponding duct, the two abnormal epithelia being continuous. In some animals, however, e.g., no. 18, a separate focus of squamous hyperplasia in a duct could be seen in addition to an early glandular focus.

The larger tumors, which were barely visible to the naked eye, also showed a mixed appearance; there was intermingling of just recognizable sebaceous elements with clearly squamous carcinoma containing epithelial pearls. As the tumor enlarged, however, the squamous epithelium—whether by overgrowth or by metaplasia of the cells of sebaceous origin—became the most prominent feature. This accounts for the earlier descriptions of these tumors as squamous carcinomas, although the occasional persistence of sebaceous type tissue did lead to the identification of their origin by Skoryna et al. (9). Bielschowsky (1) noted adenomatous and squamous changes together in one gland. The larger tumors corre-
sponded in structure with the descriptions given by these authors.

In view of the opinion of Skoryna et al. (9) that stasis in the glands (secondary in most cases to otitis media) was an important causative factor, special attention was paid to the appearance and weight of the glands and to the incidence of otitis media at autopsy. Slight dilatation of the ducts was seen not infrequently, but it was as frequent in nontumorous glands as in those bearing tumors. Moreover, the lobules affected by the early changes described showed no greater dilatation of their ducts than the normal lobules nearby. None of the present tumors showed evidence of origin in cysts. The weights of the glands tabulated in Table 1 show considerable variation, but there appears to be no consistent change in the presence of otitis. Table 2 shows that there is no correlation between the occurrence of tumors and of otitis.

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tr>
<td><strong>THE RELATIONSHIP OF OTITIS MEDIA AND TUMORS OF ZYMBAL'S GLANDS IN RATS FED 2-ACETYLAMINOFLUORENE</strong></td>
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<tr>
<td><strong>INCIDENCE OF OTITIS</strong></td>
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**DISCUSSION**

These results, as was hoped, have directed attention to a number of points which may indicate profitable lines of study of the mode of action of acetylaminofluorene. The hypertrophy and hyperplasia of the liver during the carcinogenesis has been mentioned. Similar figures have been given by Griffin et al. (3), and Gutmann and Peters (4) report some degree of hypertrophy of the liver even in young female rats given 2-AAF. It seems, therefore, that in the case of the liver this overgrowth may play a part in the development or “promotion” of the carcinogenic process. Whether or not this is so, it forms such a striking example of the escape of the organ from those forces which normally restrain its growth that the mechanism of its production must be worthy of consideration.

It is a great contrast to turn from this to the picture presented by the Zymbal's glands. Here hypertrophy and hyperplasia appear to play no part in the preparation of the epithelium for tumor production. The earliest change, still affecting only a small lobule, seemed already well on the way to tumor formation, while the surrounding gland appeared normal. The contrast between the two organs is probably to be explained by the difference in the stability of the normal cells, those of the liver not seen in mitosis in the ordinary way. The lack of preliminary change in the sebaceous gland has, however, proved very helpful in the present instance, since it has enabled the earliest foci of changes in the epithelium to be clearly distinguished and followed to the larger tumors. The most striking observation on the early changes is the way in which the whole of a lobule seems to be involved. The change appears to affect all the cells, in some cases even the squamous duct cells, without any immediate distortion of the glandular pattern. This picture is more easily to be explained by a simultaneous or near-simultaneous change in many cells than by a mutation of only one cell. Davids (2) has reached similar conclusions in his study of early intraocular melanomas.

The more advanced tumors do not support the view of Skoryna et al. (9) that “the tumours were invariably observed to begin in the epithelium of cystic lobules.” On the contrary, in the present series only three squamous proliferations were observed in slightly cystic ducts. The other tumors arose in apparently normal glandular lobules. Otitis media, although common, did not seem to be related in any way to the tumors. The reason for the frequent appearance of tumors in these glands is not clearer than it was, but the observations on the glandular origin of most of them may serve as a guide to further investigations, possibly of a biochemical nature.

**SUMMARY**

1. Investigation of the changes occurring in Zymbal's glands showed that generalized hyperplasia was absent. The earliest changes involved the whole of a small sebaceous lobule in marked hyperplasia, with changes in both nuclear and cytoplasmic characters, and this progressed with both squamous and sebaceous characters forming an expansively growing tumor. Foci of squamous hyperplasia occurred less frequently. Tumors were not related to cystic changes in the ducts or to otitis media.

2. The theoretical significance of the absence of hyperplasia in these glands, as compared with that seen in the liver of the same animals, is discussed.

**REFERENCES**

3. Griffin, A. C.; Cook, H.; and Cunningham, L. Tissue


The Early Changes Produced in the Auditory Sebaceous Gland (Zymbal's Gland) of the Rat by 2-Acetylaminofluorene


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