PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 142–145 (1975)

Homotransplantation of Larynxes of Cigarette Smoke-Exposed Syrian Hamsters[1] (38760)

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In 20% of BIO inbred Syrian hamsters, carcinoma of the laryngeal respiratory epithelium developed after 70–100 wk of daily exposure to smoke generated from IRI Kentucky Reference cigarettes. Even larger numbers of smoke-exposed animals developed less severe epithelial changes in the area of the larynx bordering the vocal cords and including the ventricle of Morgagni (1). Under similar experimental conditions in smaller numbers of BIO 87.20 hamsters, laryngeal carcinoma occurred in 4% of the animals, epithelial changes lesser than carcinoma in 13%, and metaplasia in 16%. Similar rates for laryngeal epithelial changes and carcinoma had previously been observed by German investigators using German Reference cigarettes and noninbred hamsters (2).

The earliest malignant lesion induced by cigarette smoke in the hamster larynx was observed 73 wk after the start of inhalation, when the aged animal was close to death from degenerative disease attendant upon old age; therefore, little time remained for growth of the neoplastic lesion and metastatic spread in this animal (1). Actually, no metastases of laryngeal cancer were found in these experiments, although metastases of a smoke-induced nasopharyngeal sarcoma did occur. It was postulated that transplantation of a cigarette smoke-induced early laryngeal carcinoma might lead to further growth of the tumor in a new host.

BIO 15.16 inbred hamsters, which had proved to be the most receptive hosts for homo- and isotransplantation (3), were chosen for the present study. Because of the microscopic nature of the induced laryngeal cancers, it was impossible to select by previous microscopic study the best possible lesions for transplantation; rather, the largest possible number of smoke-exposed larynxes was taken for transplantation into as many BIO 15.16 cheek pouches as possible. At the end of our smoke inhalation experiments, 15 larynxes of smoke-exposed BIO 15.16 animals and 34 larynxes of BIO 87.20 smoke-exposed animals were available as starting material.

Methods. Animals and procedures. Exposure of animals to smoke. These experiments are described in detail elsewhere (1). In brief: 102 male BIO 15.16 hamsters and an equal number of male BIO 87.20s were exposed 5 consecutive days each week to 19.3% cigarette smoke generated in a modified Walton–Morrissey reverse smoker, starting at age 90 days. Smoke exposure sessions took place twice a day for 8 min, during which time the animals received a 15-sec exposure each minute to smoke generated by 35 ml puffs of 2-sec duration, followed by exposure to room air for 43 sec before the next puff. All animals saved for transplantation of laryngeal tissue received this treatment for 76–90 wk, at the end of which time they were killed. Sham-smoked animals underwent the same manipulation as the smoke-exposed animals, except that there were no cigarettes in the machine.

Transplantation of laryngeal tissue. BIO 15.16 males were chosen as hosts because of their great tolerance to transplanted tissue. These animals, all in good health, were 60–90 days old at the time of transplantation.

The larynxes of the smoke-exposed animals were removed and cut horizontally in sections of approximately 0.5 mm thickness. Such ring-shaped sections, often containing parts of the vocal cords and the ventricle of Morgagni, were inserted through a small incision made in the everted cheek pouch of the lightly anesthetized BIO 15.16 hosts.

[1] This work was supported in part by USPHS General Research Support Grant No. S01-FR-05525 (Division of Research Facilities and Resources).

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TABLE I

Strain	Donor animals: No.	Donor animals: Treatment	Host animals: Histology of transplants: Surviving squamous epithelium	
15.16	15	Smoke-exposed	15	1
15.16	—	Sham-smoked	—	
15.16	—	Cage-held	—	
87.20	34	Smoke-exposed	67	13
87.20	17	Sham-smoked	17	0
87.20	9	Cage-held	9	0

In the case of the BIO 15.16 smoke-exposed animals, only 15 segments from 15 larynxes of smoke-exposed animals were available for transplantation. All sham-smoked and untreated cage-held control BIO 15.16 animals had been sacrificed prior to our decision to carry out transplantation studies.

Among the BIO smoke-exposed animals and their controls, 34 smoke-exposed animals yielded 67 transplantable larynx sections, and 17 sham-smoked animals gave 17 larynx sections. Also, nine cage-held control animals yielded nine transplants.

At weekly intervals, the transplants were inspected by everting the cheek pouch of the lightly etherized host animal. Of the 108 transplants in as many host animals, 24 had to be retransferred into new hosts because of signs of infection or rejection. In only three cases was this done more than once. After the transplants had been left *in situ* for at least 10 mo, the host animals were killed and the transplants and cheek pouch envelopes were removed and fixed in Tellyesniczky's fluid for histological examination of hematoxylin-eosin-stained sections.

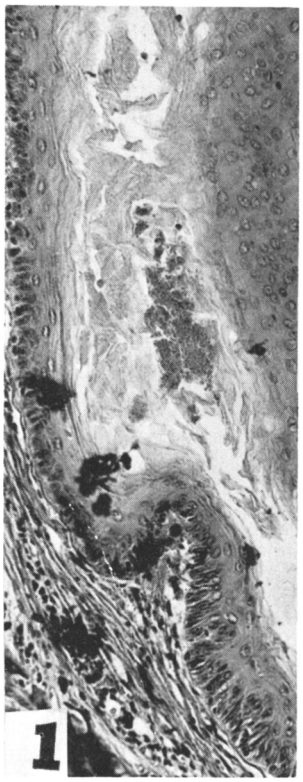
Results. Histological examination. With the exception of a very small number of transplants (or retransplants) lost by rejection or infection, all transplants had become well vascularized and had grown larger than when originally inserted into the cheek pouch. Common to all transplants, whether from smoke-exposed larynxes or from controls, was the formation of occasional multilocular cysts which were usually lined by a single layer of low cuboidal cells. In the case of all transplants from nonsmoke-exposed animals, the epithelium lining these cysts was usually atrophic, sometimes cuboidal, but never multistratified squamous. In most of the sections from smoke-exposed animals, the cysts contained desquamated epithelial cells, and squamous epithelium was often observed (Figs. 1 and 2). Six specimens (9%) from smoke-exposed BIO 87.20 hamsters contained squamous epithelium with some degree of epithelial hyperplasia or nuclear atypia (Figs. 3 and 4). One section showed a nest of squamous cells apparently in the middle of cartilage which might well be an artifact. There was no clearcut evidence of malignant transformation (Fig. 5). In the case of the BIO 87.20 hamsters, statistical evaluation by Fisher's exact test (4) showed that the incidence of squamous epithelium in the smoke-exposed animals was significantly higher than in controls not exposed to smoke at a probability level of less than 1% (see Table I).

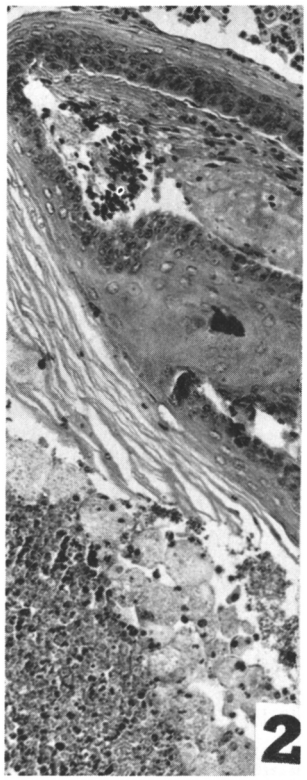
All specimens (those from smoke-exposed animals as well as controls) contained well preserved cartilage, often with areas of calcification or osseous metaplasia. In a few cases, the cartilage pattern was characteristic of the upper larynx, the region where hyperplastic changes occurred in the intact, smoke-exposed hamsters. In some specimens, the cartilage pattern was that of normal tracheal rings. In most specimens it was impossible to determine the level of the upper respiratory tract whence the transplant originated. Two specimens included a well preserved, normal thyroid gland (Fig. 5). There was a limited amount of surrounding connective tissue, which was not remarkable.

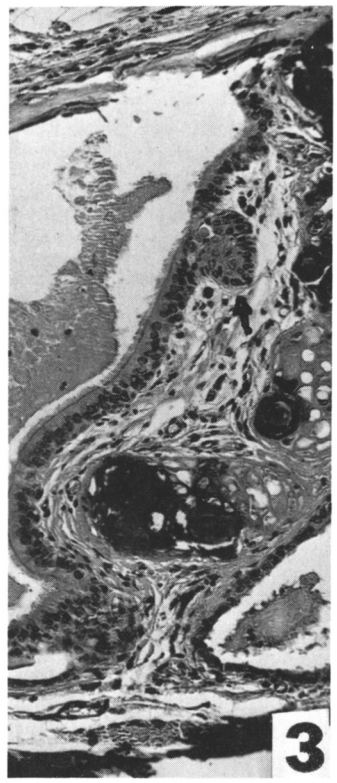
Any organ of the host which showed gross anomalies was studied histologically. The commonest abnormalities seen were renal amyloidosis and reactive hyperplasia of the lymph nodes. The remaining pathology observed was comparable to that generally seen in aging hamsters of the BIO 15.16 strain.

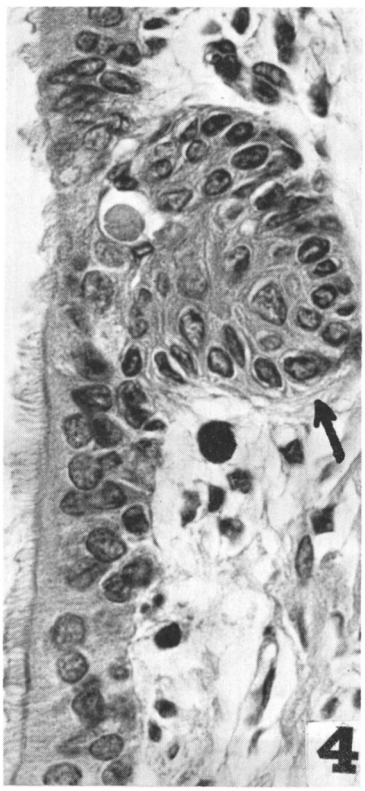
Discussion. Hyperplasia of the laryngeal epithelium caused by cigarette smoke persists for up to one year in a host animal without further exposure to smoke. No hyperplastic epithelium was seen in transplants of tracheas which had not been exposed to smoke prior to implantation.

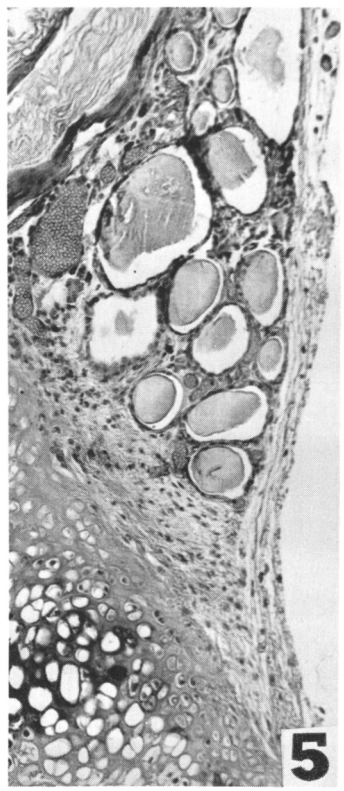
It was also observed that other adult normal tissues such as thyroid, laryngeal and











FIGS. 1–5.

tracheal cartilage and normal tracheo-laryngeal epithelium survive under these conditions; hence, mere survival of a metaplastic tissue is no proof of its malignancy. However, it is of interest that even in the absence of a further stimulus such as smoke, a transplanted altered tissue may retain its anomalous characteristics.

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Received September 6, 1974 P.S.E.B.M. 1975, Vol. 149.

FIG. 1. This illustrates a metaplastic tracheal tube from a smoke-exposed BIO 87.20 male hamster (cartilage on extreme lower left) in a transplant having resided 1 yr in the cheek pouch of a female BIO 15.16 hamster (HE, ×200).

FIG. 2. Section from tracheal implant from smoke-exposed BIO 87.20 male hamster placed 1 yr earlier into cheek pouch of male BIO 15.16 host (HE, ×200). In the midst of a chronic inflammatory exudate (which was contained in a large cyst), there is a well preserved tubular structure lined with a tall columnar epithelium which in places has changed into a metaplastic squamous epithelium.

FIG. 3. Section from a tracheal transplant from a smoke-exposed male BIO 87.20 hamster in cheek pouch of a female BIO 15.16 hamster after 1 yr (HE, ×200). The tracheal tube, bordered by cartilage shows portions with missing or atrophic epithelium (top) as well as a ciliated, tall columnar epithelium and a nodular aggregate of metaplastic cells bulging into the submucosa (at arrow).

FIG. 4. Same field as Fig. 5 (HE, ×770), clearly showing cilia and epithelial changes described in legend 3, above.

FIG. 5. Section from implant of trachea or larynx from sham-smoked BIO 15.16 male hamster having remained for 10 mo in cheek pouch of BIO 15.16 male host (HE, × 200), showing surviving thyroid gland and cartilage. The laryngeal epithelium has atrophied.