THE EFFECT OF TOBACCO TAR ON THE BRONCHIAL MUCOSA OF DOGS

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The impressive evidence for the relationship between cigarette smoking and lung cancer is based for the most part on epidemiological data. Although the carcinogenicity of tobacco tar for the skin of mice has been demonstrated, it has seemed desirable to seek experimental support for the epidemiological evidence in attempts to induce lung tumors comparable to human bronchogenic carcinoma. The bronchial epithelium, with its protective watery mucous coat and its ability to rapidly clear inhaled materials, is a unique tissue, and materials carcinogenic at other sites could well be ineffectual when applied to the respiratory tract. The induction of experimental bronchogenic carcinoma would permit a detailed study of the pathogenesis of this lesion and of those factors that influence its production. The following is a report on the early effects of the application of tobacco tar to the bronchial mucosa of dogs over a period of 11 months.

Material and Methods

The ideal type of experimental exposure would be the one that most closely simulated human exposure. This would involve exposure of animals, through some unique smoking device, to tobacco smoke. While this would ultimately be most desirable in terms of evaluating dosage levels and the effects of the physical state of the tar in carcinogenesis, it seemed reasonable to examine initially the effect of direct application of tar to the bronchial mucosa. This type of application was made possible by the development of the technique of tracheal fenestration. This operative procedure is one in which a skin tube is fashioned so as to form a tracheocutaneous fistula in the neck (Fig. 1). The particularly advantageous feature of this communication is that the walls of the tube are apposed to one another, and the tract remains functionally closed except when a catheter or bronchoscope is passed through it. The ease with which repeated bronchoscopy may be performed through these tracheal windows permits the direct application of tar to selected areas of mucosa. The bronchoscope was introduced after instillation of 2 cc. of 0.5% tetracaine hydrochloride (Pontocaine Hydrochloride). Tobacco tar was applied by means of a special applicator (Fig. 2A to C). This consisted of a 1-cc. tuberculin syringe for which a frame was designed to deliver predetermined amounts. Attached to the syringe was a 17-gauge metal tube with a flattened and widened tip, one surface of which was perforated. Tobacco tar used was that produced in the smoking machine at the Institute of Industrial Medicine of the New York University-Bellevue Medical Center, New York, N.Y.

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The applicator and the syringe frame used in the experiments were designed by Morris Tarr, Middle Village, N.Y.

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N.Y. The technique of production of this tar and its characteristics have been previously described. In all instances the tar was applied to the medial surface of the left main bronchus. Control procedures in this exploratory experiment consisted simply of bronchoscopy, during which the tip of the bronchoscope was rubbed against the medial surface of the left main bronchus in animals otherwise unexposed to tobacco tar or other material.

Initial attempts at bronchial painting with tar involved the use of 0.1 cc. of tar applied in repeated doses 3 to 5 times a week. Such application resulted in the early death of all 8 dogs thus treated. The deaths appeared to conform to a pattern consistent with nicotine poisoning. Accordingly, the treatment protocol was altered so that dogs received 0.05 cc. of tar in the first 3 to 6 applications, after which it was possible to increase the dose to 0.1 cc. again, given 3 to 5 times weekly. Seven dogs on this schedule survived well. The control manipulations were performed on 6 additional dogs. The groups available for study then consisted of (1) 8 dogs that died within 25 days of the start of the experiment, presumably because of tar overdosage; (2) 7 dogs that survived from 178 to 320 days; and (3) 6 control animals that survived 65 to 223 days.

All animals were carefully examined prior to the beginning of the experiment. Chest roentgenograms were taken, bronchoscopic survey of the tracheobronchial tree was performed, and biopsy specimens were taken from the mucosal site to which tar was to be applied or which was to undergo control manipulation. Repeat biopsies were performed at 24- to 72-day intervals in the tarred animals and at 35- to 69-day intervals in the control dogs. Trachea and lungs were removed en bloc from animals dying during the course of

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**Fig. 2.** A, Photograph of the applicator and tuberculin syringe with a fixed delivery frame. B, Detail of the syringe and frame. C, Perforated applicator tip, before and after expression of tar.

Fig. 4. Photomicrograph of normal bronchial epithelium, consisting of a single layer of basal cells and pseudo-stratified ciliated and goblet cells.

Fig. 5. Photomicrograph of bronchial lining, demonstrating hyperplasia. There is an increase in the number of cell layers with basal cells particularly prominent. The surface is still ciliated.
the experiment. The lungs were inflated and fixed by the instillation of 10% Formalin into the trachea. Twenty-two sections of the respiratory tract were taken from each animal, from the sites designated in Fig. 3. Both biopsy and autopsy material was dehydrated, embedded in paraffin, and sectioned in the usual fashion. Sections were stained with hematoxylin and eosin.

RESULTS

Within 3 to 6 weeks, the tar-treated mucosal surface became granular and later developed wartlike elevations that narrowed the bronchial orifice considerably. The manipulated mucosal surface of the control dogs remained smooth and glistening, and the bronchial orifice was not narrowed.

The changes in the bronchial mucosa, as revealed by biopsy and autopsy sections that were judged to be of interest and significance, were hyperplasia, transitional metaplasia, and squamous metaplasia. The histological features of normal epithelium are demonstrated in Fig. 4. Hyperplasia consisted of proliferation of basal cells, with an increase in the number of cell layers in the epithelium. The basic cell type was still columnar, and the surface remained ciliated. This is illustrated in Fig. 5. In transitional metaplasia (Fig. 6) there was definite stratification, the basal cells remained small and tended to be columnar, but the outer cells had become large and cuboidal to polygonal in shape. No intercellular bridges and no keratinization were seen. In general, mucosa so affected resembled lining epithelium of the urinary tract, hence the use of the term "transitional." Squamous metaplasia represented a true epidermoid transformation. (Fig. 7). There was orderly progression from a cuboidal basal layer to large polygonal cells, between some of which intercellular bridges could be made out, and then to a flattened surface layer that frequently demonstrated early keratinization. In no instance was there sufficient atypism in the form of disorganization, increased mitotic activity, nuclear cytoplasmic disproportion, or other nuclear abnormality to justify an additional category of change. It should be pointed out that it was our impression that hyperplasia, transitional metaplasia, and squamous metaplasia, in that order, represented changes of increasing significance and were perhaps consecutive stages in mucosal transformation. Accordingly, only the most advanced lesion was charted for any one area of mucosa.

The pathological alterations observed in the serial biopsies of those groups of dogs in which repeated biopsies could be performed are summarized in Table 1. These groups consist of the 7 dogs treated for longer periods of time and the 6 control dogs. All treated dogs demonstrated striking squamous metaplasia, while this change was not observed in any of the control animals. Similarly, 6 treated dogs had areas of transitional metaplasia, but this was seen in only 1 of the control animals. Hyperplasia was seen in some biopsies of 3 treated and 2 control animals.

Figure 8 is a schematic representation of the autopsy findings. Autopsy group 1 consisted of 4 of the 8 dogs that died soon after tar application. Of the other 4 animals in this short term group, 3 had received 1 treatment and had died within 20 minutes. The specimens from 2 of these showed no alterations, and the third was lost. Another animal had 5 treatments before death, but the poor state of mucosal preservation precluded histological examination.

Autopsy group 2 consisted of 2 long term-treated dogs that died accidentally 178 and 303 days respectively after the start of the experiment. Autopsy group 3 consisted of 2 control dogs that died accidentally during the course of the experiment at 65 and 188 days after its start. These 2 animals were subjected to 36 and 105 bronchoscopies and control manipulations respectively.

The autopsy findings in group 1 demonstrated the rapidity with which changes developed after application of tar. Thus, squamous metaplasia was seen in 2 dogs that survived 17 and 25 days and received 6 and 17 treatments respectively. In an additional 2 animals surviving for 2 and 3 days and receiving 2 treatments each, transitional metaplasia was observed.

Findings at autopsy in the 2 long term-treated dogs (group 2) were those of extensive squamous metaplasia. These changes were consistent with the biopsy observations in this group but illustrate, too, that the apparent effect of treatment is not limited to the area of direct application.

In the control group (group 3) the animal that had undergone 105 control procedures showed mild hyperplasia. The dog that had undergone 36 manipulations demonstrated
FIG. 6. Photomicrograph of transitional metaplasia of bronchial mucosa. There is stratification of polygonal cells. The ciliated cells and goblet cells have been completely replaced.

FIG. 7. Photomicrograph of squamous metaplasia. The mucosal lining has assumed an epidermoid appearance, and there is keratinization on the surface.
transitional metaplasia at autopsy. This was the animal in which transitional metaplasia had been observed in the biopsy sections. No other control animal showed metaplasia in either biopsy or autopsy sections. The significance of this unique change will be considered in the discussion to follow.

**DISCUSSION**

This pilot study has demonstrated the practicability of repeated bronchoscopic applications of test materials to the bronchial mucosa of dogs through a surgically fashioned permanent tracheal window. In the course of this pilot study, tobacco tar was demonstrated to be remarkably effective in inducing metaplastic changes in the mucosa. The specificity of tobacco tar in this regard was, of course, not examined, and the observations may simply reflect a rather nonspecific reaction to irritant injury. Extension of the technique to other irritants and to known polycyclic hydrocarbon carcinogens is currently under way.

The characteristics of the metaplasia were of considerable interest. The biopsy results and the autopsies on the dogs that died early in the experiment demonstrated the rapidity with which metaplasia developed. Continued painting resulted in no further progression of

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**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. treatments or manipulations</th>
<th>Hyperplasia</th>
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*Plus indicates presence of change in 1 or more biopsies; minus, absence of change in all biopsies.*
the lesion, for even in those animals treated for 11 months no atypicality was noted and certainly nothing suggestive of so-called precancerous alteration was seen. Surviving dogs are still under treatment, and it may well be that further transformation will require much more extended tar application. Even as an isolated observation, however, the presence of metaplasia after application of tobacco tar is of interest. This is a material to which human bronchi are exposed, and Auerbach and his associates\textsuperscript{1} have called attention to the apparent association between such metaplastic change and cigarette smoking. Recent experimental observations made in the course of induction of lung cancer in other species\textsuperscript{4} have led to the suggestion that metaplastic epithelium furnishes a suitable substrate for the action of carcinogens. This may be on the basis of increased local stasis and increased permeability to carcinogens. Agents that induce metaplasia may promote carcinogenesis, or the same agent may induce both metaplasia and neoplasia.

**REFERENCES**


