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Artificial Tumors in Dog Bronchus and Their Implications in Production of Experimental Emphysema.* (26010)

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Early diagnosis of pulmonary tumors by X-ray studies is difficult, and endobronchial lesions often go undetected until they occlude the bronchial lumen with resultant distal atelectasis. The original purpose of this study was to produce lesions in experimental animals that simulate endobronchial neoplasms and to demonstrate these lesions by a bronchographic technic recently described(1). While the bronchographic study was being carried out, it was observed that with increasing time after the lesions were made, some of these animals showed evidence of chronic, progressive pulmonary disease suggesting emphysema. This report presents results of this initial experiment.

Materials and methods. Two methods of producing intrabronchial lesions in animals were tried, (1) ligation of individual bronchi to produce incomplete stenosis, and (2) injection of a plastic material to produce an endobronchial mass which would partially obstruct a bronchus. The first method was ineffective in that the incomplete obstruction produced by constricting ligatures did not result in a demonstrable lesion and could not be effectively studied by X-ray. This method was discarded and the second procedure investigated. To find a satisfactory plastic substance, studies were carried out on various materials. Preliminary observations were made with 4 different types of liquid plastics, which were injected subcutaneously into hamsters. Three of these plastics caused severe inflammatory reactions, followed by varying degrees of necrosis at site of injection. The fourth material, *i.e.*, an acrylic resin emulsion called Rhoplex AC-33, (product of Rohm & Haas Co. of Philadelphia) produced little inflammation or necrosis and apparently acted as a bland foreign body after hardening *in situ*. This material is a milky liquid containing 46% solids and is miscible with water. The Rhoplex was thickened by addition of a sodium polyacrylate, Acrysol G.S., which is supplied by the same company at 12-13% solids in water solution. The latter is extremely viscous and may be handled more easily if diluted with an equal volume of distilled water. When 10 ml of the resulting mixture is added to 20 ml of Rhoplex AC-33, a thick creamy emulsion is obtained, which flows readily through a 25 gauge needle. This preparation may be easily maintained for long periods at room temperature if kept covered. Large dogs were utilized for production of the bronchial lesions. During thoracotomy, a lobar or segmental bronchus was prepared by clearing adjacent connective tissues, and 0.5 to 1.0 ml of the thickened acrylic resin emulsion was injected transbronchially and deposited at the immediate sub-mucosal level. Care was taken not to puncture bronchial mucosa and enter the bronchial lumen. This can read-

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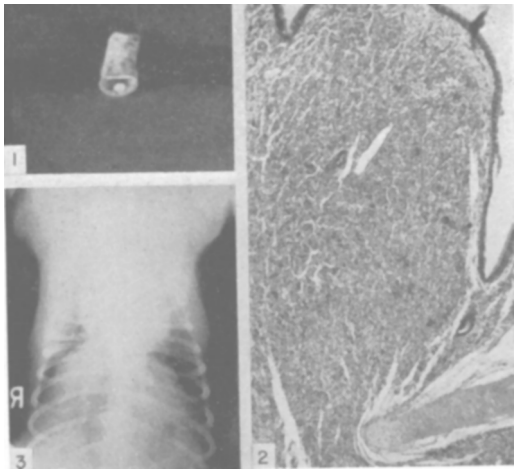

FIG. 1. Transected bronchus showing tumor. × ⅓.
FIG. 2. Cross section of endobronchial tumor. × 9.
FIG. 3. Chest X-ray of dog 22 mo after preparation showing trapped air in left lower lobe.

ily be checked after inserting the needle by drawing back the syringe plunger and checking for appearance of air bubbles. If the mucosa was punctured, the injection was made at a different site. The technic requires some practice but is usually mastered after a few attempts. The emulsion hardens quickly and produces a mass which projects into the bronchial lumen. Ten dogs were prepared as described above. Nine survived the procedure and remained in good health. The one fatality showed, at autopsy, an inadvertent penetration of the bronchial mucosa with deposition of the emulsion within the bronchial lumen and resultant extensive pneumonia. The remaining dogs were utilized frequently to demonstrate the lesions by bronchographic procedures. From 3 to 8 months after the preparations were made, 6 of the dogs were sacrificed periodically so that bronchial level of injection and structure of the lesions could be examined.

Results. Grossly, the endobronchial lesions were similar in appearance and resembled bronchial adenomata (Fig. 1). These pseudo-tumors varied in size but in general, degree of bronchial obstruction was proportional not only to amount of plastic injected but also to elapsed time after injection.

Histologically, the tumor (Fig. 2) was composed primarily of macrophages which contained ingested plastic. The macrophages tended to be arranged in groups separated by thin walled blood vessels and fibrous tissue. In older lesions, a thickened capsule and trabeculae of fibrous connective tissue became more prominent.

About 8 months after thoracotomy the 3 surviving dogs began to show clinical signs of bronchial obstruction as evidenced by periodic bouts of "asthma" (relieved by bronchodilators), enlargement of PA diameters with expiratory fixation of the involved side of the chest, and expiratory phase prolongation. Through a laboratory error, 2 of these dogs were lost to the study approximately 10 months after preparation and autopsies were not performed. One animal is still alive and shows a further progression of these symptoms. An X-ray taken 22 months after preparation of this dog's left lower lobe bronchus shows enlargement of the left chest, widening of the rib interspaces and scant lung markings resembling emphysema (Fig. 3).

Discussion. A method of producing endobronchial tumors has been described, and there is some evidence that these lesions produce obstructive emphysema. Eiseman, Petty and Silen(2) recently described a method of producing experimental emphysema and in addition carefully reviewed the historical background of this problem. Their criticisms of previous attempts to reproduce this disease in the experimental animal are valid, especially the commentary that "acute experiments are unlikely to produce the disease." We have felt for some time that successful production of experimental emphysema is probably dependent on production of a slowly developing, chronic, partial airway obstruction functioning over a prolonged period of time. Haidak and Curd(3) have suggested that injury to a bronchus due to trauma may result in chronic obstruction sufficient to produce expiratory bronchial collapse and thereby pulmonary emphysema. The method of Eiseman *et al.*(2) of inserting a small tube into the trachea to hinder expiration has limitations in that total lung tissue is involved. It is suggested that the slowly growing endobronchial lesion reported here represents a

physiological refinement over their method in that air can be trapped in localized lung areas while adjacent lung tissues will remain relatively collapsed and resilient as is known to occur clinically. In addition, these animals remain in good health for long periods of time and are available for various types of experiments.

Summary. Partially obstructive endobronchial lesions have been produced in dogs by transbronchial injection of a plastic material. These lesions were composed primarily of large accumulations of vacuolated macrophages. The size of these masses was apparently dependent upon their age. The implication of this type of lesion in possible production of experimental emphysema has been discussed.

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Corticosterone Levels in Adrenal Effluent Blood of Some Gallinaceous Birds.* (26011)

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Collection of adrenal effluent blood from mammalian species by cannulation of the adrenal vein(1) is facilitated by the morphological relation of this vessel to the vena cava. Recently Phillips and Jones(2) made direct collections of adrenal venous blood from the capon by a procedure that involved obstruction of the blood flow through the vena cava and formation of a pocket within this vein into which flowed adrenal effluent blood. This method, while satisfactory for some types of experimentation, is laborious for routine collection, is unsuitable for repetitive sampling, and is acutely traumatic.

In domestic fowl the adrenal veins are located on the ventral surface of the glands; the left adrenal vein passes transversely from the adrenal to the vena cava whereas the right adrenal vein passes dorso-laterally to the caval vein(3). The nature of the venous drainage from the *left gland* permits collection of adrenal effluent blood from the bird. However, the intimate apposition of the ovary to the left adrenal gland restricts sampling from this vein to the *male* galliform. We routinely make direct collections of adrenal venous blood by a simple, expedient manipulation that allows the animal to be used for study at a subsequent period.

Materials and methods. The male gallinaceous birds used in this study were 3 breeds of domestic fowl which were 10 to 20 weeks old and 1.5 to 2.5 kg in body weight; 5 to 12 month old ring-necked pheasants† about 1 kg in weight; and broad-breasted bronze turkeys† about 6 months old and approximately 12 to 14 kg in weight.

The birds were anesthetized by intravenous infusion of Nembutal. Peripheral blood was withdrawn either from the cephalic vein or from the heart. The body cavity was entered through incisions made in the skin and intercostal musculature between 6th and 7th ribs. A rib spreader was inserted and expanded to allow better access to the region of the adrenal gland. The presence of recrudesced gonads often necessitated retraction of the left testis for exposure of the adrenal vein. If sexually mature males are to be studied, it would be helpful unilaterally to castrate the animals.

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