

that the accumulation of such a range of phosphatases at this site might break down both its structural integrity and its selective permeability.

These results suggest that the fundamental defect in progressive muscular dystrophy may be not in the muscle fibre but in the connective tissue which supports it.

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### Chemical Changes in Human Bronchial Epithelium and their Relation to Bronchial Cancer

A RECENT histological study<sup>1</sup> of the changes that occur in human bronchial epithelium has confirmed in part earlier American findings<sup>2</sup> and shown that considerable proliferation may occur in the cells of the basal layer without any other histological abnormality. As it seemed possible that some of these changes might be related to the development of bronchial cancer, a histochemical investigation has been begun. Since this work was not concerned with the distribution of free lipids, formalin-fixed tissue embedded in paraffin was used. The most striking result has been the very marked increase in the content or availability of lipid, probably bound phospholipid<sup>3</sup>, as shown by colouring with sudan black and staining by the acid haematein method<sup>4</sup>. This effect was strongest in the nuclei of the basal cells and was particularly well seen in the condition known as basal cell hyperplasia. In contrast, scarcely any lipid could be demonstrated in the cells of foci of established squamous metaplasia.

Since basal cell hyperplasia and other proliferative changes are common in smokers who exceed 40 cigarettes a day, it seemed worth-while to see if similar histochemical disorders occurred in histologically normal areas of bronchial epithelium in such persons. By these methods an increased lipid content has now been demonstrated in the basal cells, particularly in the nuclei, of otherwise normal bronchial epithelium from such smokers; in chemically normal epithelium from non-smokers only the nucleoli in the basal cells are stained.

Sections were also treated with an aqueous (hydro-tropic) solution of 3,4-benzpyrene, and the distribution of this fluorescent carcinogen was followed by fluorescence microscopy. Those cells which gave a positive reaction with the acid haematein method also showed selective absorption and concentration of benzpyrene, especially into the nuclei.

If these observations on fixed cells can be related to events in the living epithelium, they would suggest the possibility that bronchial cancer is produced in two main stages. In the first stage, damage to areas of epithelium, possibly by cigarette smoking, might alter them in such a way as to increase their affinity for lipid-soluble substances, leading to preferential

absorption of carcinogenic hydrocarbons in the second stage. These events need not necessarily occur in every focus of proliferation, and the development of bronchial cancer might also be affected by local differences in the concentration of carcinogens which accumulate in the vicinity of the foci. The circumstances influencing the local concentration of carcinogens are probably complex and include such factors as stagnation of mucus and loss of cilia.

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## BIOLOGY

### Selective Staining of Visceral Efferents in the Rat Brain Stem by a Modified Koelle Technique

THE histochemical technique for cholinesterases introduced by Koelle and Friedenwald<sup>1</sup> has been used in numerous studies of nervous tissue. Much of this work has been concerned primarily either with the histochemistry of the method or with the cytological distribution of enzyme revealed. This method has, we feel, also great potentialities in the field of neuro-anatomy—as a means of selectively staining particular groups of neurones which cannot otherwise be distinguished by more conventional methods. Some attempts along these lines have been made in studies of peripheral innervation<sup>2,3</sup>, but it is in studies of the central nervous system that there is particular need for a selective staining method. This communication is therefore concerned not so much with enumerating our detailed findings in this preliminary investigation as with emphasizing the potential value of this technique in solving many neurological problems which have so far resisted attack by more conventional methods.

The sensitivity of the Koelle technique can be controlled by varying the pH of the incubation medium and the time of incubation. At an acid pH the method is highly selective for sites of high enzyme activity. This fact was made use of by Snell and Garrett<sup>4</sup> in their study of the cholinergic innervation to the rat salivary glands—the secretor-motor fibres, both pre- and post-ganglionic<sup>3</sup>, being selectively shown up when a sufficiently acid incubation medium was used. This work suggested that it might be possible with some such technique to follow the salivatory pathway back to the salivatory nuclei in the hind