COMPARATIVE STUDY OF THE PROTEOLYTIC ACTIVITY IN THE TISSUES OF THE INTACT AND DEAFFERENTATED LUNG

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The author's investigation of the lung tissue of cats and dogs following extirpation of the spinal ganglia at the level C_7-D_5 showed that the collagen fibers of the connective-tissue stroma undergo destruction and disintegration. Similar changes in the collagen fibers were also observed when the tissue reactions of the skin to deafferentation were studied [1-3]. Since processes similar in their course to chronic aseptic inflammation take place in a deafferentated region, with marked infiltration of neutrophils and macrophages, the observed disturbances in the connective-tissue stroma have been associated with increased proteolytic activity in this zone [1].

In order to verify this hypothesis, the proteolytic activity was studied in the tissues of the intact and deafferentated lung. The method of determination of proteolytic activity by means of substrate (gelating) films [4], as modified by the author, was used.

EXPERIMENTAL METHOD

After preliminary experiments using various photographic films and photographic plates marketed by Soviet industry (type MZ-2, mikrat "200" diapositive plates, etc.,) it was eventually decided to use nuclear plates of type MR, characterized by an emulsion layer of minimal thickness (8-15 μ) and by the very small dimensions of the microcrystals of the silver halides.

The degree of proteolysis was determined by the originators of this method after fixation of the tissues for 24-72 h with formaldehyde vapor at 4°. The material for the present investigation consisted of sections about 30 μ thick and 5 · 10 mm in size, cut on a freezing microtome from fresh, unfixed tissue.*

The illuminated plates were developed in any developer and fixed. After washing with distilled water the plates were gently dried and the prepared section placed on the emulsion layer. Sections of the lung of the intact animals and the animals undergoing the operation were fixed to the same plate to create identical conditions of analysis. The plates together with the affixed sections were placed in a moist chamber and incubated for 18-20 h at 37°.† To prevent the section from drying, from time to time they were slightly moistened by a soft brush soaked in acetate buffer, pH 5.0.

The subsequent treatment of the material was as described in the original formula [4]. After incubation, the plates were rinsed in distilled water. The tissue components possessing proteolytic activity liquefied the gelatin of the emulsion layer in the corresponding areas, and the silver granules present in that layer were washed away. The plates were then dried at room temperature, and the sections fixed in 96° alcohol. The localization of the proteolytic enzyme was determined from the size, the number, and the degree of translucency of the areas of the emulsioned layer. The relative area of translucency of this layer was measured after affixing the sections of the lung of the experimental and intact animals. The area of the plate to which the section was glued was conventionally divided into six approximately equal fractions, and each was photographed on reversible film in identical conditions of exposure and intensity of illumination. The degree of transmission of light by the different parts of the transparency was determined by a densitometer. The number of measurements on each transparency was 36. The mean

* In the author's opinion, during the investigation of tissues with relatively low proteolytic activity, it is advisable to use unfixed material because fixation with formalin inevitably leads to partial depression of the enzyme activity.
† With shorter periods of incubation, satisfactory results cannot be obtained in the study of tissues with low proteolytic activity.

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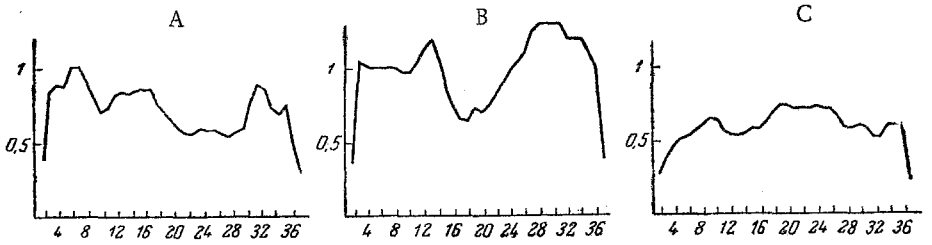

Curve of protease activity in the lung tissues of cats on normal conditions (A), and 3 days (B) and 20 days (C) after removal of the spinal sensory ganglia. Along the axis of abscissas—number of measurements, along the axis of ordinates—densitometric readings of the degree of transmission of light by the emulsion layer after attachment of the section. In B the area of translucency of the gelatin layer (bounded by the curve and the axis of abscissas on the graph) is larger than in normal, but in C it is smaller.

values were calculated from the densitometer readings after measurement of each of the six fractions of the section, and a curve was plotted showing the transmission of light by the emulsion layer for the total area of the section. Next, by means of a planimeter, the relative area of translucency was determined, bounded by the curve and the axis of abscissas.

EXPERIMENTAL RESULTS

The study of the lung tissue of the intact animals by the substrate film method revealed a high level of proteolytic activity in the adventitia of the blood vessels and bronchii, while the activity of the proteases in the lung parenchyma was weaker (see figure, A).

In the lung tissue of the animals after removal of the spinal ganglia, during the first days after the operation (up to 5 days) the proteolytic activity was approximately 31.4% higher than in the controls (see figure, B). Later (until the 7th-9th day) no significant differences could be found by the method described between the lung tissue of the intact and the denervated animals. However, at the end of the second week after the operation, the intensity of proteolysis in the deafferentated lung fell appreciably, and later the decrease became more considerable (see figure, C). The area of translucency of the emulsion layer in this case was 21% below that in the control.

It may be concluded from the results obtained that destruction of the fibrous structures during the first days after removal of the spinal ganglia may be due to increased proteolysis (possibly on account of the activity of the proteolytic enzymes of the leukocytes). Destruction of the collagen fibers at later periods after the operation is difficult to attribute to increased proteolytic activity, for the results obtained demonstrate a marked fall in the level of proteolysis in the deafferentated lung by comparison with the controls. The latter is probably associated with the predominance of sclerotic processes over processes of destruction at this period, and this is confirmed by the result of a morphological study of the tissue of the deafferentated lung. However, the possibility is not ruled out that the changes in the fibrous stroma in the late periods after operation may be due to a disturbance of the trophic function of the nerve as a result of deafferentation.

SUMMARY

A study has been carried out on proteolytic activity in the tissues of an intact and deafferentiated lung in cats and dogs. A modification of Adams' and Tugan's method has been developed (applicable to Soviet-made photographic materials and in account with the characteristics of tissues with a relatively small content of proteolytic enzymes) for determining proteolytic activity with the aid of substrate films.

It has been shown that in the early days after the removal of cerebrospinal sensory ganglia, the proteolytic activity in the lung tissues is increased, but by the 7th-9th day it falls down to normal, whereupon a progressive decrease in the activity of proteases is observed.

A decrease in the activity of proteolytic enzymes in the tissues of a deafferentated lung is attributed to predominance of sclerotic processes in the later periods after an operation.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
