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Enzymatic Lysis in Vitro of Hyalin Deposits in Human Kidney

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ABSTRACT: Vascular hyalin was readily dissolved in vitro from sections of the formalin-preserved, paraffin-embedded kidney of a hypertensive patient, by means of an enzyme (BJ-B-66) isolated from Bac. cereus. The enzyme attacked other hyalins and tissue components as well. The enzyme is active at body temperature and pH, and appears substantially nontoxic to rats and hamsters.

Hyalin accumulation occurs in many pathological conditions. It is particularly significant in the kidneys of hypertensive and diabetic patients, where it may be found in the arterioles and glomeruli. The condition is progressive.

The following experiment was performed to explore the therapeutic possibilities.

EXPERIMENTAL

Frozen sections (20 microns) were made from a hypertensive patient's kidney in which there was a relatively large amount of hyalin in the arterioles. Two serial sections were mounted on each microscopic slide. Serial sections were exposed to a proteolytic concentrate (BJ-B-66) with a proteolytic activity of $^{A}495 = 310$/ml, by the Congocoll method of Nelson, Ciaccio and Hess (1). This concentrate of a cell-free, fermentor broth of a strain of Bac. cereus had been passed twice through an Amicon PM-10 filter retaining molecules larger than 10,000 molecular weight.

A drop of this concentrate was placed on one of the sections on each microscopic slide. Each experimental section treated with the concentrate was ultimately compared with the serial "control" section on the same slide. Thus the control and the experimental sections were handled in an identical manner except that the experimental section was incubated with a drop of concentrate while the control section (Fig. 1) was treated with a drop of the buffer (Tris buffer, pH 7.2) used to dilute the enzyme in making the concentrate. Both sections were incubated at 35°C and 100% humidity for the periods indicated in Figures 2-4.

After time intervals ranging from 15 minutes to 1.5 hours, the enzyme action was interrupted by immersion of the slides in 10% formaldehyde for 30 seconds, followed by washing with water, drying in air, and staining by the PAS method. The sections were preserved in acrylate medium.

A 3-ml dose of the enzyme concentrate, having a Congocoll activity value of $^{A}260 = 310$/ml and a molecular weight of 5700 ± 20% (2), was injected intraperitoneally into a 430-gm rat. No reaction was observed. Crude cell-free enzyme broths from Bac. cereus, having proteolytic activities in the range of $^{A}260 = 10$-30 (of which 15-30 per cent had a molecular weight of about 6000), were fed to hamsters as a replacement of 25 per cent of their water supply. No reaction was observed during a four-week period.

RESULTS AND DISCUSSION

The histological results are demonstrated in Figures 2-4. It may be seen that enzyme BJ-B-66 attacks not only the hyalin but also a fairly uniform amount of proteinaceous and hyalinaceous material in dead tissues. From a therapeutic standpoint, the general nature of this attack is not disturbing, since dosage can

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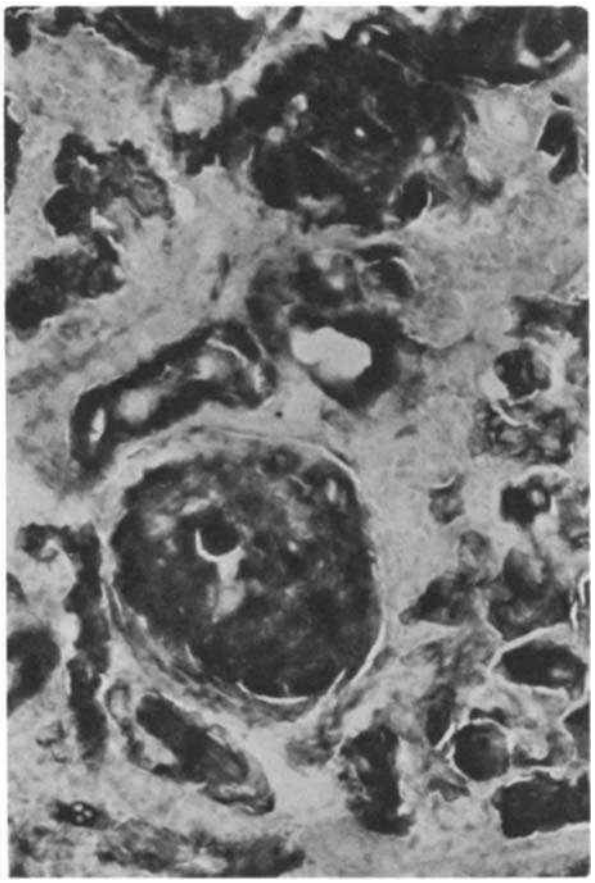

Fig. 1. Control.

be moderated to keep the attrition of essential components well below the level of normal replacement reactions. The abnormal accretions would appear to be removable in this manner.

Such a generalized action might be attained by the use of some already-known enzymes. However, all of these enzymes have more than double the molecular weight of the present one, and therefore would be far more subject to steric hindrances in densely knit structures such as those occurring in biological crosslinkage. Moreover, the present enzyme has the advantage of apparent nontoxicity and, in contradistinction to pepsin and trypsin, an optimal pH of about 7. It is possible that several of the current enzymes may similarly attack hyalinized kidney slices in vitro. Only clinical tests can determine the merits of any enzyme for the treatment of hyalin deposits or related amyloid disorders.

The enzyme reported here would appear to be prime candidate material for such clinical tests because:

1. Its unprecedently low molecular weight permits penetration into dense molecular structures that present steric hindrance to larger enzyme molecules.
2. It is active at neutral pH.
3. No adverse effect has been so far apparent in experiments with rats and hamsters.
4. There have been no reports of success in dissolving gerogenic hyalin or amyloid deposits with the use of previously known enzymes.
5. The potent effect of the present enzyme on extracellular hyalin and its lack of significant local or systemic reactions when given intraperitoneally to a living animal may make it possible to use this enzyme for solubilizing vascular hyalins in the living organism. Since it acts on hyalin in a degree comparable to its action on dead tissue and more delicate cell components, any in vivo action on hyalin might tend to be equal to or greater than the in vitro action. The more resistant living cells might not be affected significantly.

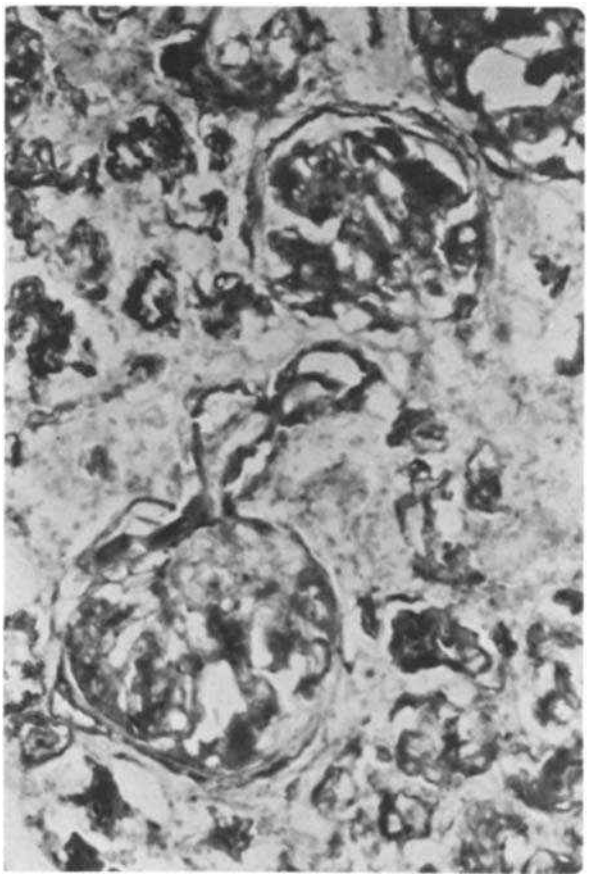

Fig. 2. After exposure for 15 minutes to enzyme BJ-B-66.

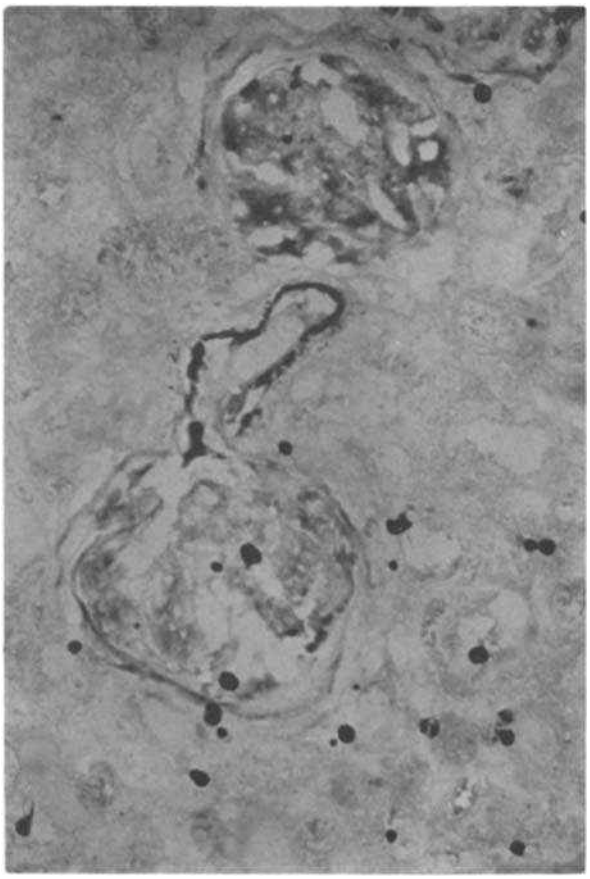

Fig. 3. After exposure for 1.5 hours to enzyme BJ-B-66.

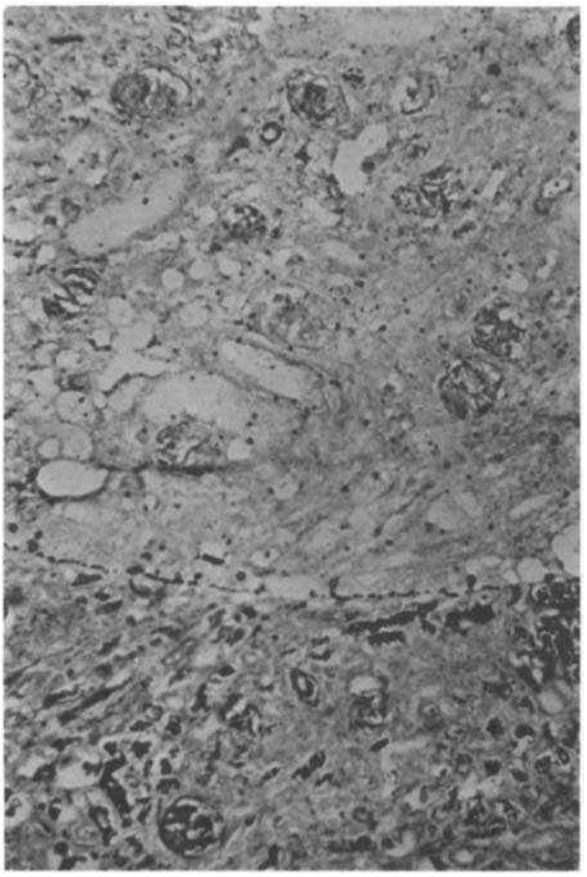

Fig. 4. Upper two-thirds of section — after exposure for 1.5 hours to enzyme BJ-B-66. Lower third of section — no enzyme.

Acknowledgment

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