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Pancreatic Collagenase, A New Enzyme.* (25271)

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Ziffren and Hosie(1) claimed that pancreatic juice possessed ability to digest collagen fibers. Straumford and Hummel(2) showed that this activity was inhibited by soybean trypsin inhibitor. More recently, Oken and Boucek(3) claimed that impure pancreatic elastase solubilized collagen fibers, whereas the crystalline enzyme did not. Investigations into collagenolytic enzymes have always been questionable because of the difficulty of assaying an enzyme using an insoluble substrate. Recently Gallop(4) has shown that only bacterial collagenase will reduce the viscosity of procollagen derived from swim-bladder tunica of the carp. We studied the effect of pancreatic extracts and enzymes upon viscosity of acid soluble collagen of calf skin.

Material and methods. Purified acid soluble collagen was prepared from 0.5 M acetic acid extracts according to Gross and Kirk(5). This material was lyophilized, and solutions of 0.2 M acetate buffer (pH 5.5) were made to contain 15 mg/ml of procollagen. These solutions were homogeneous with respect to electrophoresis and in amino acid and hexosamine content were as reported by Gross and Kirk (5). Crystalline trypsin, elastase, chymotrypsin and relatively purified hyaluronidase were obtained from Worthington. Human pancreatic juice was obtained from a patient with freely flowing fistula. Aqueous extracts of "Bridase," a whole, desiccated, defatted, activated preparation of swine pancreas provided by Mr. Levin of VioBin, were prepared by extracting 25 to 100 mg of "Bridase" with 5 ml of 0.2 M acetate buffer (pH 5.5) in a glass homogenizer at 4°C. After centrifugation, about 45% of total weight of tissue had been solubilized into the clear supernatant. Concentration of this material was expressed in terms of mg of "Bridase" initially mixed with 1 ml of solvent. Assay of enzyme activity was as follows: 4 ml of substrate solution (15 mg/ml of acetate buffer) was warmed to 20°C, added to an Ubbelohde viscosimeter in which water had a flow time of about 70 seconds. To this viscosimeter was added 1 ml of buffer containing 1 mg of various enzymes described above, or 1 ml of "Bridase" extract. After manual mixing for a few minutes, the solution was incubated for 75 minutes and flow time determined. The difference between relative viscosity of this solution and that of the control previously found, using boiled enzyme solutions, was expressed as percent of initial viscosity reduced by 75 minutes incubation with the enzyme.

Results. The effect of 1 mg each of crystalline trypsin, chymotrypsin and elastase, as well as the effect of 1 mg of hyaluronidase and 1 ml of human pancreatic juice upon relative viscosity of acid soluble collagen was determined. Similarly, the effect of the aqueous extract of 15 mg/ml of "Bridase," with and without addition of 1 mg each of either soybean, ovomucoid and lima bean trypsin inhibitor was also measured, and the results expressed in terms of per cent viscosity reduced is shown in Table I. The pancreatic extract was uniquely able to reduce 50% of the viscosity of acid soluble collagen. This activity was destroyed by boiling and was independent of addition of 3 kinds of trypsin inhibitor. Amount of inhibitor added was in excess as shown by the method of Rhodes *et al.*(6).

Fig. 1 shows that after 75 minutes incuba-

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TABLE I. Effect of Various Enzyme Preparations upon Relative Viscosity of Acid Soluble Collagen.

Incubation mixture (75 min.)	Relative viscosity Exp.	Control	Viscosity reduced, %
Substrate +			
Buffer	14.57	14.57	0
Trypsin (1 mg)	"	14.58	0
Chymotrypsin (1 mg)	"	14.57	0
Elastase (1 mg)	14.53	14.50	0
Hyaluronidase (1 mg)	14.58	14.58	0
Pancreatic juice (1 ml)	"	"	0
"Bridase" (15 mg)	7.39	14.50	50
" + soybean inhib. (1 mg)	7.35	14.60	"
" + lima bean inhib. (1 mg)	7.30	14.61	"
" + ovomucoid inhib. (1 mg)	7.33	14.56	49

tion with both a 15 mg/ml and a 20 mg/ml solution of "Bridase," most of the viscosity loss produced by this preparation was complete. With lesser amounts of "Bridase," longer periods of time were required for complete reaction. The data presented in Fig. 1 were obtained in the presence of soybean trypsin inhibitor. Previously, pancreatic washings have been shown to be unable to reduce the viscosity of acid soluble collagen solutions. It may also be seen from this figure that the % viscosity reduced is directly proportional to concentration of the "Bridase" used.

The effect of various concentrations of "Bridase" after 75 minutes incubation at 20°C with acid soluble collagen upon % viscosity

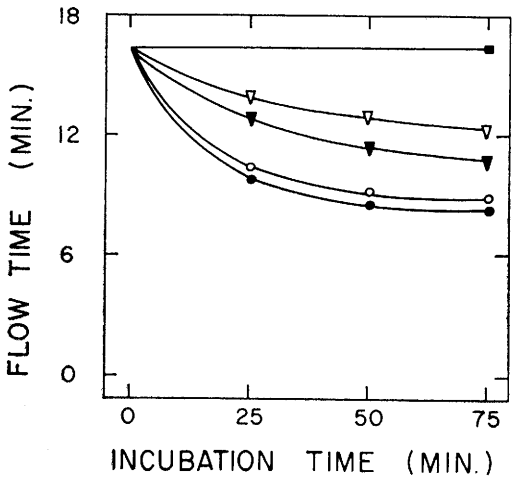

FIG. 1. Flow time (min.) of acid soluble collagen solutions after various periods of incubation with 20 (●), 15 (○), 10 (▲) and 5 (△) mg of "Bridase." Effect of a previously boiled preparation of 15 mg/ml of "Bridase" is shown by the top (■) line.

reduced is shown in Table II. Prolonged exposure of acid soluble collagen solutions to 37°C results in their polymerization to form collagen fibers(5,7). After incubation of the collagen solution with the enzyme as described above, these solutions were then heated to 37°C for 5 minutes. The absorbance of re-

TABLE II. Viscosity Reduction and Fiber Formation Inhibition of Acid Soluble Collagen by Treatment with "Collagenase."

Incubation mixture (75 min.)	Viscosity reduction %	Fiber reduction %
Substrate alone (12 mg/ml)	0	0
+ 5 mg Bridase	26	33
+ 10 " "	39	38
+ 15 " "	49	49
+ 20 " "	52	51
+ *Idem* + soybean inhib.	51	50

sulting fibers was determined in a "Spectronic-20" spectrophotometer. These results, expressed in terms of percentage difference between absorbance of control and enzymatically pretreated substrate solutions, are given in Table II. The loss of viscosity described above was paralleled by a decrease in the fiber-forming property of the substrate solutions. Soybean trypsin inhibitor was without effect upon the fiber forming inhibition of "Bridase" extracts.

The effects of "Bridase" upon viscosity of serum albumin, casein, egg albumin and Knox I.V. gelatin in the presence of excess trypsin inhibitor were also studied. No reduction in viscosity of these proteins was observed.

Conclusions and summary. From these

data, it is apparent that whole activated pancreas, unlike pancreatic juice, contains a thermo-labile material which can reduce by 50% both viscosity and ability to form fibers of acid soluble collagen solutions. This activity is not affected by various trypsin inhibitors, nor is it duplicated by trypsin, elastase, chymotrypsin or hyaluronidase alone. This activity may be due to a new enzyme in the pancreas, a collagenase. In view of lack of activity of this enzyme upon serum and egg albumin, casein and gelatin, this enzyme would seem to be reasonably specific for acid soluble collagen.

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Mobilization of Neutrophils into Peritoneal Fluid Following Intraperitoneal Injection of Bacterial Endotoxins.* (25272)

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Contrary to frequent reports that a 0.9% NaCl solution provokes local neutrophilia when injected intraperitoneally, we found that this does not occur in rats if the saline solution is sterile and non-pyrogenic. Moreover, a solution to which a small amount of a bacterial polysaccharide complex derived from Pseudomonas (Piromen) had been added, produced a marked neutrophilia when injected intraperitoneally(1). Therefore, it was of interest to determine whether or not cellular reactions of peritoneal fluid can be used as a sensitive indicator to detect presence of small amounts of certain bacterial substances.

Methods. Male rats of Sprague-Dawley strain (Holtzman Co.) and weighing 180-220 g were used. All rats received an intraperitoneal injection of 10 ml sterile, non-pyrogenic saline (Baxter Lab.). Solutions were injected at room temperature and the rats allowed food and water *ad lib.* during experiment. Animals were challenged by graded amounts of bacterial extracts added to the saline solutions immediately before injection. Three bacterial extracts were used: 1) Piromen,† a Pseudomonas polysaccharide complex (Baxter Lab.), 2) Staphylococcus A lipopolysaccharide (Difco Lab.), and 3) *E. coli* 026: B6 lipopolysaccharide (Difco Lab.). Control animals received only sterile saline solution, free of pyrogens. After 5 hours rats were sacrificed, then 10 ml heparinized 0.9% saline solution was injected intraperitoneally. This was done because most of the original 10 ml of solution is absorbed from the cavity by 5 hrs. Animal's abdomens were massaged gently for one minute. Then an incision was made into the peritoneal cavity and the fluid containing a suspension of cells was collected with medicine dropper. The fluid was centrifuged to concentrate the cells which were then resuspended in homologous serum. Smears were made using a brush technic(2), and stained by modified Wright-Giemsa stain(3). In the unstimulated state the peritoneal fluid contains mononuclear cells (macrophages and lymphocytes), eosinophils and mast cells, but is virtually devoid of neutrophils. Percentages of various cells, excluding mast cells, were computed. On the basis of previous experience, it was decided that appearance of 5% or more neutrophils in the peritoneal fluid

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