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Radioimmunoassay of Plasma Elastase (38235)

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(Introduced by M. John Boyd)

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In 1960 Yalow and Berson (1) developed the first radioimmunoassay technique for insulin. Since then this method has been adapted to many other hormones and nonhormone substances. Temler and Felber (2, 3) were the first investigators to apply the radioimmunoassay technique to the determination of pancreatic enzymes and to detect minute amounts of these enzymes in biological fluids.

The important features of this method are the high specificity and sensitivity. The procedure is based on the competitive binding of a specific antibody to an unlabeled antigen (enzyme) and tracer amounts of the same antigen (enzyme) labeled with a radioisotope. There does not appear to be any previous reports in the literature involving the measurement of elastase by radioimmunoassay. This work, based on a radioimmunoassay, has been adapted to the measurement of canine elastase, as part of a study of circulating pancreatic enzymes and their determination by radioisotopic techniques.

Materials and Methods. Elastase was extracted from fresh canine pancreata by the method of Balo and Banga (4) as modified by Hall (5). Further purification was accomplished by means of a Sephadex column 100 × 5 cm filled with Sephadex G-75 (regular), previously equilibrated with 0.067 *M* phosphate buffer at pH 8.0. The fractions containing the enzyme were pooled together, dialysed against distilled water and lyophilized. The solid obtained was dissolved in 0.1 *M* glycine buffer pH 9.6 and applied on a Brinkmann free flow electrophoresis, with a voltage of 900 V and 95 mA. The enzyme preparation obtained by this method contained 25 units/mg of protein. Elastase was iodinated with ^{125}I following the method published by Greenwood *et al.* (6), and modified by McConahey and Dixon (7). Separation of the labeled enzyme from the free iodine was performed by gel filtration using Sephadex G-50 (regular) in a 50 × 1 cm column. The radioactive labeled elastase obtained had a specific activity of 300–500 μCi/mg of protein.

The Rabbit antielastase (RAE) antibodies were produced in female white New Zealand rabbits by successive injections at 1 week intervals for 5 weeks with 2 mg of the enzyme emulsified in complete Freund's adjuvant. The second antibody utilized in the system, Goat antirabbit globulin serum (GARG), was obtained by repeated injections into a female goat at weekly intervals for 5 weeks of 25 mg of rabbit globulin emulsified in complete Freund's adjuvant. The radioimmunoassay method for the elastase determination was a modification of the double antibody technique for insulin described previously by Morgan and Lazarow (8). The buffer utilized for dilution of antibodies, enzymes and unknown samples was 0.5 *M* borate buffer, pH 8.0, containing 5% Bovine Serum Albumin, Fraction V (Metrix, Chicago, Ill.) and 0.01% merthiolate. Figure 1 is a flow sheet showing the essential features of the assay method that has been used in these experiments.

The reaction of the first and second antibodies was conducted at 4°. The first reaction involved the mixture and incubation at 4° for 48 hr of unlabeled enzyme or unknown sample (1 ml), diluted antiserum

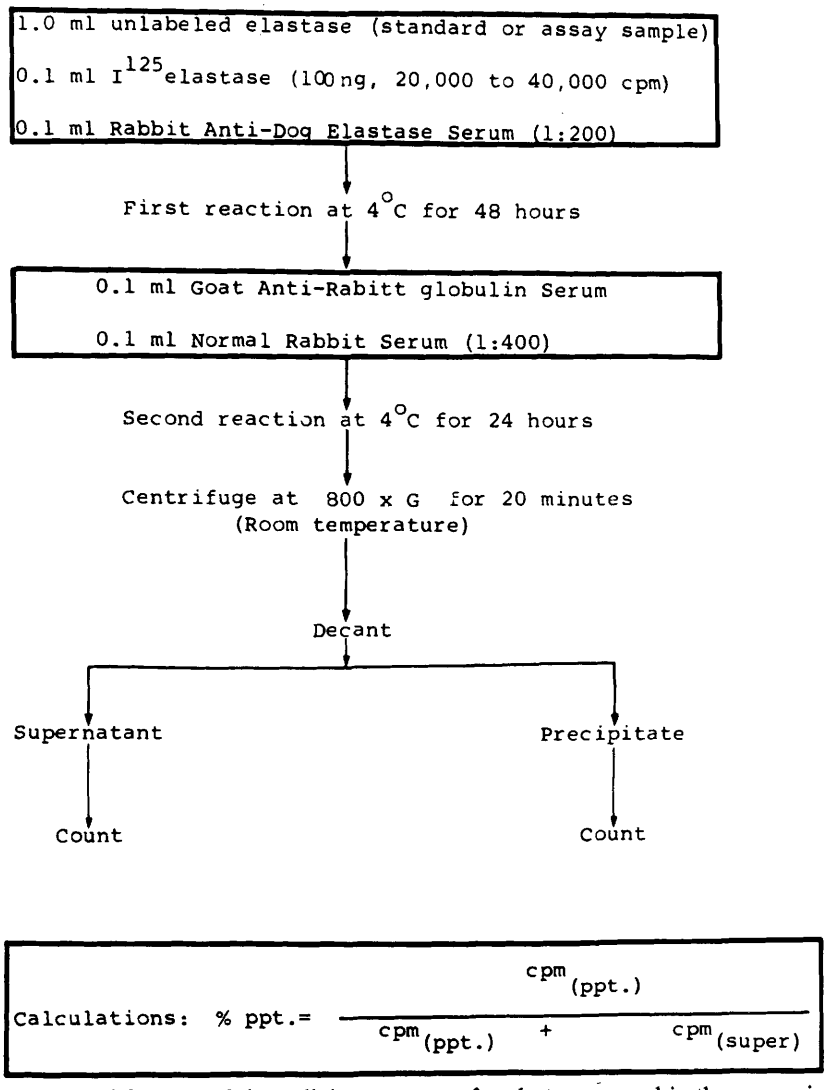

FIG. 1. Essential features of the radioimmunoassay for elastase as used in these experiments.

(RAE) (0.1 ml) and the labeled enzyme (0.1 ml). This was performed in rimmed glass test tubes (13 × 100 mm). The effect of temperature on the first reaction was studied by incubating the mixture at 25° and 37° as well as at 4°. The second reaction occurred after addition of 0.1 ml of the second antibody (GARG) and 0.1 ml of diluted normal rabbit serum. The latter component of this system was added to increase the mass of protein precipitated and served to increase the effectiveness of the assay. After incubation for 24 hr at 4°, this reaction mixture was centrifuged at 800*g* for 20 min at room temperature. After centrifugation, the supernatant fluid from each tube was gently decanted into clean tubes and both tubes were then stoppered and counted successively in a Packard Auto-Gamma Spectrometer. The enzymatic activity of the canine elastase was measured by the method Rinderknecht *et al.* (9) using fluorescein-elastin as substrate. Blood was drawn from ten fasting dogs before and after the induction of experimental pancreatitis produced by the injection of autol-

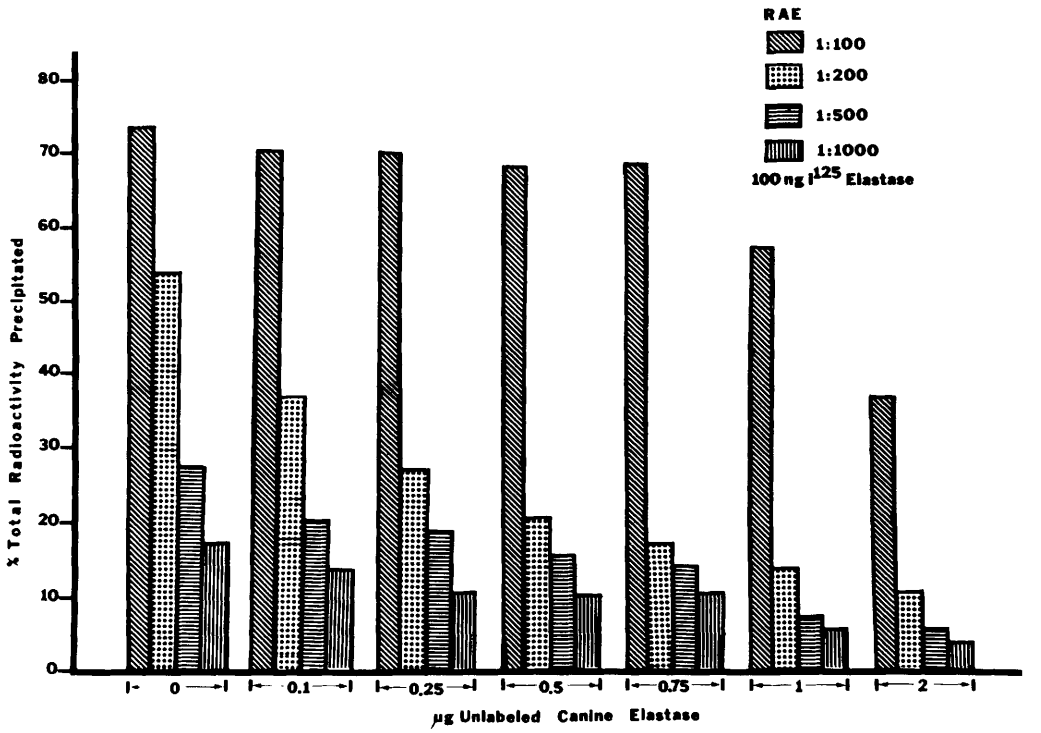

FIG. 3. Effect of different dilutions of the first antibody on the percent of total radioactivity precipitated at 6 different levels of known concentrations of canine elastase.

trol levels. Between 30 and 60 min the elastase levels decreased but remained at about twice the control levels. The elastase as determined by radioimmunoassay remained above the control levels throughout the course of the experiment. After the induction of pancreatitis, at the time when the circulating elastase levels were elevated as determined by radioimmunoassay, the elastase as determined by enzymatic activity decreased from the moment of the induction to the end of the experiment.

Discussion. The advantage of using radioimmunoassay techniques for the determination of enzymes is that the method possesses at the same time high accuracy, sensitivity and specificity. Levels of 0.1–2.0 µg/ml of elastase in canine plasma have been de-

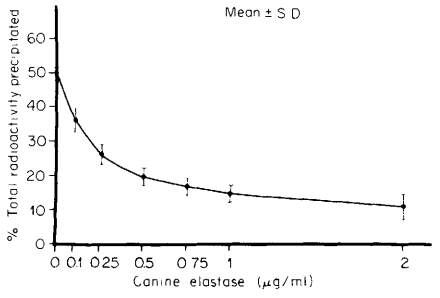

FIG. 4. Standard curve derived from the results of 20 studies of total radioactivity precipitated employing 8 different lots of ^{125}I-labeled canine elastase.

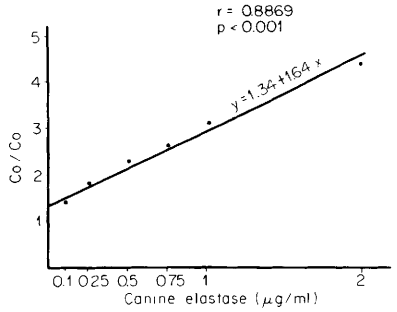

FIG. 5. Mean of the standard curves calculated as Co/Ce. Results expressed as the percent precipitated in "0" standard elastase (Co) divided by the percent precipitated in each respective standard elastase (Ce).

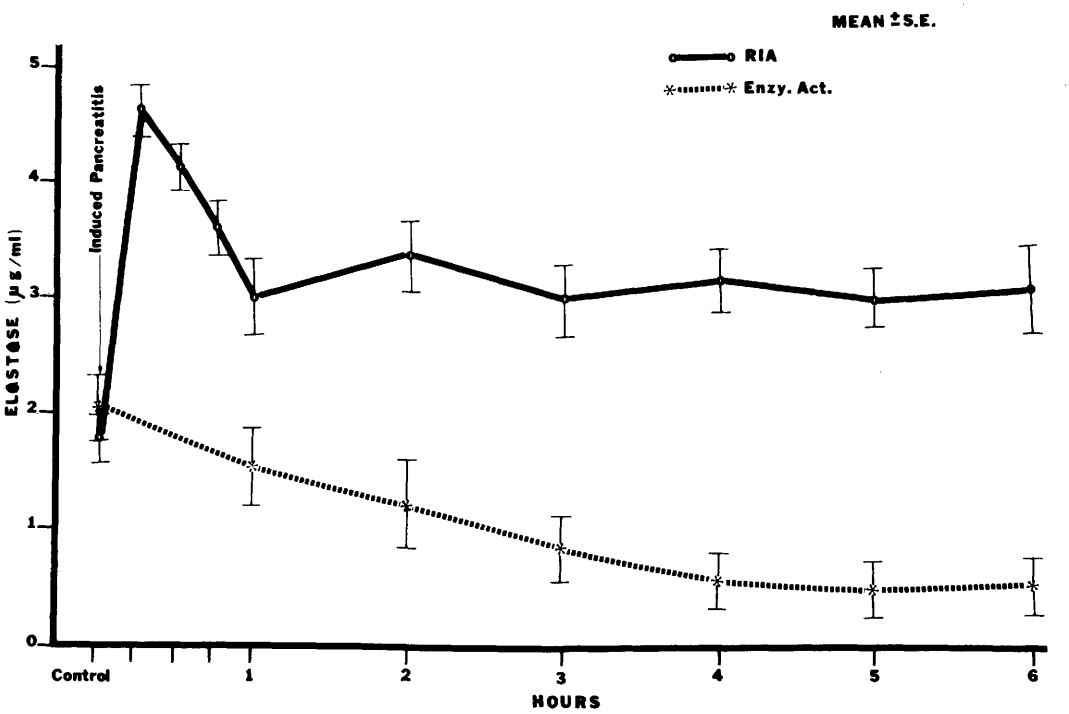

FIG. 6. Effect of experimental pancreatitis induced in 10 dogs on the determination of elastase by radioimmunoassay and elastolytic activity by fluorometric method (9).

tected. Slight modifications of the procedure could certainly lower the limit of sensitivity even further should it be required. A study of the different parameters that affect this method was performed to obtain the optimal conditions needed to meet our requirements.

An important feature of this determination is that it is based on the ability of an antibody to recognize the specific structure of an enzyme rather than the ability of an enzyme to break down a substrate. This becomes important in the study of isoenzymes. The determination also made possible the measurement of enzyme concentrations even in the presence of their inhibitors. The results of radioimmunoassay of elastase standard without inhibitor was compared with the assay of elastase standard to which plasma containing high concentrations of elastase inhibitor was added. The amount of elastase determined after the addition of the plasma containing elastase inhibitor was the same as that determined before the addition of plasma containing inhibitor. These results suggest that the active site of the enzyme is not the one involved in the immunological reaction, and the determination of the enzyme by radioimmunoassay is unaffected by the presence of inhibitors.

The finding that incubation at temperatures above 4° decreased the percent binding of ^{125}I-labeled elastase agrees with findings of previous investigators. Berson and Yalow (12) found that increasing the temperature during the incubation period favored dissociation of the antigen–antibody complex.

The cross-reactions with other pancreatic enzymes (trypsin and chymotrypsin) were studied and no cross-reactivity was observed. The recognition of the antibody must be very specific for a small portion of the structure since changing to other species reduced, but did not abolish the reaction. Canine elastase gave moderate reaction against human elastase and weak to poor reaction against porcine elastase. These latter findings are consistent with the concept that there is a structural similarity between the same enzyme of different species. On the other hand, the structure of different

enzymes from the same species seems to vary enough so that immunologic recognition is not possible.

During experimental pancreatitis in dogs the elastase levels determined in the plasma by radioimmunoassay were higher than those determined by enzymatic assay. Furthermore the enzymatic activity of elastase in the plasma decreased with time after the onset of pancreatitis. This decrease in enzymatic activity of elastase measured by flurometric assay can possibly be explained by the augmentation of levels of circulating inhibitors produced or activated during the induction of pancreatitis in these dogs.

This investigation demonstrates the feasibility of applying the radioimmunoassay technique to the determination of a pancreatic enzyme such as elastase. It appears to give higher specificity and sensitivity than the methods previously utilized.

Summary. A radioimmunoassay system was developed for the measurement of canine elastase. The method is able to measure elastase in plasma in concentrations ranging from 0.1 to 2.0 μg/ml. No cross-reactivity was found with trypsin or chymotrypsin. The elastase levels in canine plasma were measured during experimental pancreatitis. Within 15 min after the induction of pancreatitis, the elastase levels as measured by radioimmunoassay increased to about 2.5 times their control levels. They remained above the control levels throughout the course of the experiment. On the other hand the elastolytic activity decreased after pancreatectomy.

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