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A SIMPLE METHOD FOR DETERMINATION OF PEPSINOGEN IN BOVINE SERUM AND PLASMA

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THODE JENSEN, P.: *A simple method for determination of pepsinogen in bovine serum and plasma.* Acta vet. scand. 1977, *18*, 10—14. — Calf serum pepsinogen can be determined by radial diffusion in a casein-containing agarose gel prepared at pH 8.2 and afterwards immersed in a $BaCl_2$-containing buffer, pH 2.4, whereby the casein is precipitated homogeneously. Serum samples are placed in wells in the gel, and at the low pH pepsinogen present in the samples will be activated and the casein digested, whereby transparent, circular zones are formed in the otherwise opaque gel. The diameter of these zones can be measured directly and used as an expression of the serum pepsinogen level. On analysis of a number of sera from calves with or without ostertagiasis a positive correlation was found between the results obtained by this method and conventional absorption photometry ($r = 0.87$, $n = 55$).

serum pepsinogen analysis; ostertagiasis.

Determination of serum pepsinogen is a valuable and commonly used diagnostic tool in ostertagiasis (*Jennings et al.* 1966, *Thomas & Waller* 1975, *Jørgensen et al.* 1976). In the methods so far used for serum pepsinogen determination, pepsinogen is activated to pepsin with hydrochloric acid and the digestive effect of the pepsin on serum proteins or denatured hemoglobin measured by absorption photometry (*Ross et al.* 1967).

An agarose gel diffusion method for determination of pepsin in biological fluids (e.g. gastric juice) was described by *Doleschel & Auerswald* in 1973. The method is based on radial diffusion of the enzyme into a thin layer of agarose gel prepared at a low pH value and containing barium caseinate flocks. In 1972 *Carlsson & Karlsson* described another gel diffusion technique for protease determination at low pH values in which a casein

substrate is also used. The agarose-casein plates are made at neutral or basic pH and then immersed in buffer of low pH, whereby the casein will precipitate in small aggregates homogeneuosly distributed in the gel. When the casein is digested by the enzyme, transparent circular zones are formed in the opaque gel. The diameter of these zones can be measured directly.

In the present paper a simple, sensitive, and inexpensive radial diffusion method for serum pepsinogen determination is described.

MATERIALS AND METHODS

Serum samples. Calf sera stored at —20°C and previously analysed for pepsinogen by the method of *Ross et al.* (1967).

Pepsin and pepsinogen. Porcine pepsin, twice crystallized (Sigma P 7012) and porcine pepsinogen, without pepsin activity (Sigma P 0258) were used for preparing standard solutions and for evaluating the sensitivity of the technique.

Bovine serum albumin. Cohn fraction V (Sigma A-4503).

Agarose-casein plates. Two g of agarose ("Indubiose" A 37 l'Industrie Biologique Française S.A.) is dissolved in 100 ml of boiling Tris buffer (0.1 M, pH 8.2). After cooling to about 50°C, 50 ml of a 0.5 % casein solution (Casein, Merck 2244, in the same buffer) is added. Twenty ml of this mixture is poured onto a level glass plate (10×10 ×0.1 cm) coated beforehand with a thin agarose film. After solidification the substrate plate is immersed for at least 2 hrs. in glycine buffer pH 2.4 (2 g glycine, 1.5 g NaCl, 15 g $BaCl_2$, 15 ml 1 N-HCl, distilled water to 1 l). On acidification the casein precipitates homogeneously in the gel, which thereby becomes whitish. The plates can be stored at 4°C in a moist chamber.

Performance of the test

By means of a gel puncher with a suction device, wells 4.0 mm in diameter are made in the agarose-casein gel, and 15 µl of standard solution or serum placed in each well. The plates are incubated in a moist chamber at 37°C or at room temperature for 10 to 40 hrs. The plates are read against dark background, and the appearance of circular transparent zones in the white gel will indicate digestion of the casein as a result of pepsinogen in the samples having been activated to pepsin by the low pH in the gel.

RESULTS

The following results were obtained after incubation for 18 hrs. at 37°C. On analysis of a number of sera from calves with or without ostertagiasis a linear relationship was demonstrated be-

tween the results obtained by this test, expressed as the diameter of transparent zones, and the results obtained on the same sera by the conventional method, expressed as units of tyrosin/l (procedure according to *Ross et al.* 1967). A significant correlation coefficient of 0.87 ($n = 55$) was found. Furthermore, with amounts ranging from 10 ng to 1000 ng of enzyme or zymogen, a linear relationship was observed between the logarithms of the enzyme or zymogen concentrations and the diameters of the lysed areas ($r = 0.997$, $n = 16$). Amounts exceeding 1000 ng were not examined. The lower sensitivity limit of the test was approx. 5 ng both for porcine pepsin and porcine pepsinogen. The coefficient of variation within individual plates was 1.4 % ($\bar{x} = 8.9$ mm ∞ 85 ng porcine pepsinogen, $n = 10$) for samples with relatively high values (4.2 units tyrosin/l) and 2.4 % ($\bar{x} = 6.3$ mm ∞ 12 ng porcine pepsinogen, $n = 10$) for samples with low values (1.5 units tyrosin/l). The influence of variations in serum protein concentration was tested by adding different amounts of bovine serum albumin (0—5 %) to 24 samples containing the same amount of porcine pepsinogen (35 ng). No effect of the addition of protein could be discovered.

With serum samples or solutions containing albumin the lysed zones were not quite as clear and well-defined as with pure pepsin or pepsinogen solutions.

DISCUSSION

The radial diffusion technique with pepsinogen activation in situ followed by pepsin hydrolysis and clearing of the opaque casein precipitate was found to be a very sensitive and simple method for determination of serum pepsinogen, especially as compared to the photometric methods. In order to get the necessary contrast between the surrounding gel and the incompletely cleared zones of hydrolysis which appear on testing serum samples, it is very important to use an agarose-casein gel of homogeneous opacity. A homogeneous precipitation of caseinate in the gel can be obtained by preparing gel plates with a casein solution at a basic pH and afterwards immersing them in a Ba^{2+}-containing buffer with a pH between 2.3 and 4.5. Since barium caseinate is insoluble within this pH range, it is almost impossible to get a homogeneous dispersion of it in the gel if it is mixed directly with agarose and buffer as done by *Doleschel & Auers-*

wald (1973). That procedure will give a very heterogeneous precipitation with formation of flocks of casein.

The presence of Ba^{2+} ions in the immersion buffer makes it possible to obtain a homogeneously opaque agarose-casein plate in which the pH is sufficiently low to activate pepsinogen, and in which pepsin activity can be quantitated under almost optimal conditions.

Without the presence of Ba^{2+} ions the caseinate would resolve at pH 2.4, whereby a direct evaluation of the pepsin activity would become very difficult. When testing serum samples, reading of the plates after staining as described by *Carlsson & Karlsson* (1972) is not possible, because the acid denaturation of other serum proteins diffusing from the wells into the gel will prevent a washing out of these proteins from the plates.

For clinical use the radial diffusion technique described above has several advantages: It is easy and inexpensive to perform and requires but a simple equipment. Furthermore it is highly sensitive, and only small volumes of serum or plasma are needed. From a practical laboratory point of view it is also important that the substrate plates can be stored in a refrigerator ready for use. Contrary to what is the case when the photometric technique is used, albumin does not seem to have much influence on the results. It is possible to use the test quantitatively with a pepsin or pepsinogen standard, or semiquantitatively simply by visual inspection of the plates and with normal serum as a control.

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SAMMENDRAG

En simpel metode til bestemmelse af pepsinogen i bovint serum og plasma.

Pepsinogen i kalveserum kan bestemmes ved radial diffusion i en kaseinholdig agarose gel, der fremstilles ved pH 8,2 og dernæst henstilles til buffer skift i en $BaCl_2$ holdig buffer, pH 2,4; herved vil kaseinet præcipitere homogent i gelen. Serumprøver fyldes i udstansede huller i gelen, hvor det lave pH vil aktivere pepsinogenet til pepsin, der dernæst vil spalte kaseinet og give cirkulære opklaringer. Diameteren af de opklarede zoner kan måles direkte og anvendes som et mål for serumpepsinogenkoncentrationen. Ved undersøgelse af et antal sera fra kalve med og uden ostertagiose fandtes en positiv korrelation mellem måleresultaterne opnået ved denne metode og ved almindelig absorptionsfotometri.

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