Technical methods

A fast technique for the separation and detection of amylase isoenzymes using a chromogenic substrate

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The technique utilizes the principle of the release of a soluble blue dye (Cibachron blue F3A) from an insoluble dye-starch polymer (Ceska, Hultman, and Ingelman, 1969) suspended in agar for the demonstration of amylase activity. Such a polymer is obtainable as Phadebas tablets.

The isoenzymes are separated by electrophoresis on cellulose acetate in a discontinuous tris-barbitone buffer system.

A similar method has been described by Rosalki (1970) using amylose azure (Calbiochem) as substrate, which is less sensitive than the method described below, and does not resolve the subcomponents.

Materials

1 Cellulose acetate (CAM): Celagram (Shandon Scientific Co. Ltd.).
2 Shandon Multi-Microband system SAE-2680 with 10 sample multiapplicator and plate.
3 Electrophoresis buffers as described by Kohn (1969) for the separation of haemoglobins.
4 Phadebas amylase test tablets (Pharmacia G.B. Ltd).
5 ID agar (Oxoid)
6 Shallow trays approximately 8 × 15 cm internal dimension.
7 30% bovine albumin (Stayne Laboratories Ltd).

Method

1 PREPARATION OF SUBSTRATE

Phadebas tablets are mixed in distilled water (one tablet/ml), and the resulting suspension is added to an equal volume of melted 2% agar.

The agar + Phadebas suspension is quickly and thoroughly mixed, poured into the trays, avoiding air bubbles, to a depth of approximately 2 mm and allowed to set on a level surface. When set the trays may be stacked in a moist chamber, and kept at 4°C until needed (stable at least one month).

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2 SEPARATION OF ISOENZYMES

Equal volumes of anodic and cathodic buffer are added to their respective tank compartments.

For preliminary CAM impregnation equal volumes of the buffers are mixed, and bovine albumin is added to make a 1% solution in buffer. Albumin has a protective effect on amylase and improves the separation and colour reaction.

The CAM is floated on this solution, and when wet is removed, blotted to remove any surface droplets and the samples (approximately 0·8μl) evenly applied using the applicator about 2 cm from the anodic border. (The same quantity is appropriate if an alternative method of application is used.)

When necessary the test samples are diluted in inert serum (prepared by heating at 56°C overnight) to give an activity of approximately 1,000 IU/litre.

The CAM is then transferred to the electrophoresis tank and samples are separated at 0·5 mA/cm width of CAM with a bridge gap of 7 cm for 90 minutes.

3 DEMONSTRATION OF ISOENZYMES

On completion of the run, the CAM is removed from the tank and laid on the surface of the blue-starch agar, care being taken to exclude air bubbles.

The tray + CAM is transferred to a moist chamber and incubated at 37°C until the amylase isoenzymes appear as blue bands on the CAM, usually about 15 min, according to activity. Further incubation will lead to diffusion of dye and blurring of the bands.

The CAM is removed from the gel and air dried, after which the colour is stable.

Results and Interpretation

The bands are distinct, with no background stain, and represent specific amylase activity. No non-specific dye binding has been observed. Activity can be detected in the normal range if the incubation time is prolonged.

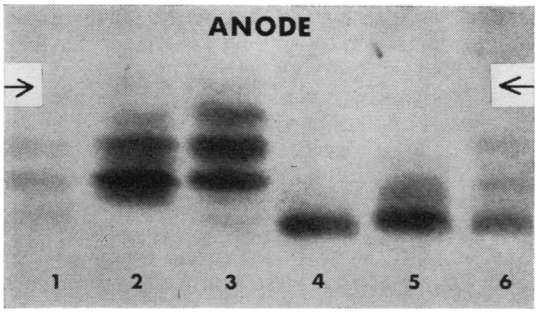

Fig. *Application indicated by arrows.*
From L to R: 1 *normal plasma;* 2 *normal saliva;* 3 *plasma from a case of parotitis;* 4 *pancreatic homogenate;* 5 *plasma from a case of pancreatitis;* 6 *normal urine.*

The findings agree closely with those of Hobbs and Aw (1968).

The isoamylase patterns of normal plasma and urine are similar, though the activity in urine is greater. Three bands are usually found in normal urine, the slower, and usually more distinct, band being the pancreatic component, and the faster those derived from salivary gland. The activities of these isoenzymes in plasma appear to be equal.

In cases of pancreatitis, the slower band becomes much more distinct, and often further activity can be detected between this and the salivary bands. This is a similar pattern to that found in pancreatic extract.

Saliva shows three bands, all faster than those of pancreatic origin, and a similar pattern is seen in the plasma in parotitis, with an increase in the fastest band.

These appearances can all be clearly seen in the accompanying photograph.

I wish to acknowledge the advice of Dr J. Kohn in preparing this paper, the help of the Department of Medical Photography for preparing the photograph, and to thank Pharmacia (GB) Ltd for providing the Phadebas tablets.

References

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A screen test for rubella haemagglutination inhibition antibodies

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When live rubella vaccine became available, it was apparent that there was need for a pre-vaccination screen test to assess the susceptibility of the subject before vaccination. Such a test should be specific and be capable of being applied to large numbers of sera without calling for great increases of staff. In this laboratory all antenatal sera are now screened by the modification of a standard haemagglutination inhibition test described below.

Materials

The test is based on the method of Stewart, Parkman, Hopps, Hope, Douglas, Hamilton, and Meyer (1967). Sera are absorbed in disposable plastic tubes and reagents are added by Eppendorf[1] pipettes delivering 0·1, 0·2, 0·6, and 0·9 ml. Microtitre equipment and rigid plastic U-plates[2] are employed. Twenty-five percent acid-washed kaolin in borate saline for absorptions and borate saline with 0·4% bovine albumin *p*H 9 for diluent are as described by Clarke and Casals (1958). Red cell diluent is the Clarke and Casals phosphate-buffered borate saline with 0·2% bovine albumin *p*H 6·2 with 0·0078% CaCl added as advised by Auletta, Gitnick, Whitmire, and Sever (1968). Commercial rubella haemagglutination antigen is used. Each new vial of lyophilized antigen is titrated and 4 HA units are used in the test. A 20% suspension of washed day-old chick cells is used for absorptions of sera when necessary, and from this the 0·16% suspension for the screen test and titrations is prepared. The chick cells used are never more than five days old and all unused diluted suspensions are discarded at the end of a day's run. Unheated sera are tested since inactivation of sera has been found unnecessary.

Method

THE SCREEN TEST

Serum, 0·2 ml, is placed in a disposable tube and 0·6

[1]From Anderson and Co Ltd, 87-95 Tooley Street, London, SE1.
[2]From Flow Laboratories, Heatherhouse Road, Irvine, Scotland.

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