tissue homogenization has been reported previously[4,8], but in this case the cells were not disrupted by sectioning but by freeze drying the slices and then bursting the cells by immersion in buffer.

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## Detection of Enzymes in Agar Electropherograms

To demonstrate the proteolytic enzymes in an electropherogram, Uriel *et al.*[1,2] dipped an electropherogram into a substrate solution of an appropriate *p*H and, after an incubation period of some hours, the electropherogram was stained with amido black. Light spots indicated the presence of proteolytic enzymes. In their immunological work, Raunio and Gabriel[3] used a second slide covered with substrate in agar to find the precipitation lines that had proteolytic activity. Here, too, the disappearance of substrate through enzymatic activity is demonstrated by staining the protein. With these publications in mind, we developed a simple and rapid method for localizing enzymatic activities in agar electropherograms.

We carried out the electrophoresis of a protein extract on a microscope slide. The substrate solution in 1 per cent agar was poured on to a second slide. After electrophoresis the slide with the agar substrate gel was placed, with the gel layer down, on to the electropherogram. After an incubation time of 5–20 min, at 37° C, the two slides were separated and the remaining substrate was precipitated by an appropriate fixing agent. Transparent spots indicated where enzymatic activity had occurred.

The advantage of precipitating rather than staining the substrate lies in the possibility of constantly observing the process. A short fixation only precipitates the uppermost layer of the substrate gel and thus reveals even very slight activity. On the other hand, the narrowing of the transparent spots after a long fixation time allows a more exact localization of the stronger enzymatic activities.

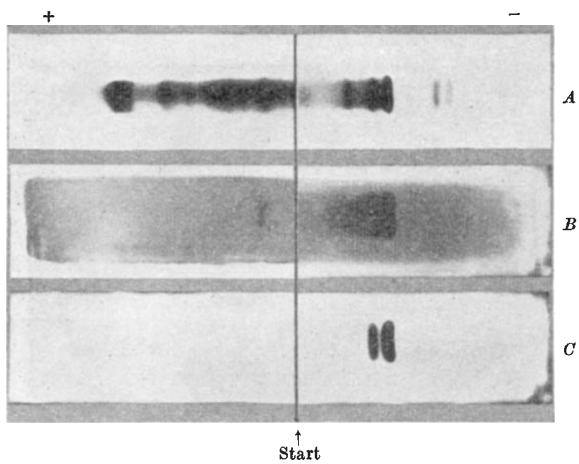


Fig. 1. *A*, The stained electropherogram of rat pancreas extract electrophoresis on microscope slides, covered with 2 ml. 1 per cent agarose in veronal buffer *p*H 8·6 ($\mu$ = 0·033), 35 min, 18 m.amp (approx. 20 V/cm); *B*, the substrate slide, after 5 min fixation in ethanol-acetic acid; *C*, the substrate slide, after 30 min fixation

This method can be used for the demonstration of several enzymes. It has been applied to the localization of amylase in electropherograms of a rat pancreas extract. The extract was obtained by grinding rat pancreas with sand under 0·14 M NaCl in a mortar. The extract was concentrated and subjected to electrophoresis on agarose[4] in a veronal buffer, *p*H 8·6.

For the substrate slide, 1 ml. 1 per cent hydrolysed starch in 1 per cent agar in the same buffer was used and after 15 min incubation at 37° C the starch was precipitated by immersing the slide in 70 per cent ethanol containing 5 per cent AcOH.

The presence of amylase activity is betrayed by the transparent spots on a milky white background, as seen in Fig. 1.

Three components with amylase activity could be distinguished in the rat pancreas extract. One of them occurred in such a low concentration that its effect was only visible after a short fixation time. The transparent spot disappeared after a prolonged fixation time.

This method, which can easily be adapted to the detection of other enzymes because of its flexibility, has helped us to locate proteolytic enzymes and nucleases. In the latter case, we used 0·5 N HCl as the precipitant.

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## A New Mould D-Amino-acid Oxidase

D-amino-acid oxidase has been found in mammalian tissues[1], bacteria[2,3] and moulds[4–7], and the enzyme has been used for many purposes, such as the determination of D-amino-acids, the optical resolution of DL-amino-acids and the preparation of α-keto acids.

On D-amino-acid oxidase from moulds, the constitutive existence of its activity has been reported in *Neurospora crassa* by Horowitz[4], in *Neurospora sitophila* by Burton[5] and in *Penicillium chrysogenum* by Emerson *et al.*[6]. Besides these reports, adaptive formation of D-glutamic acid oxidase activity, which also exhibits some activity towards D-aspartic acid in *Aspergillus ustus*, was reported by Mizushima *et al.*[7].

We also investigated extensively the D-amino-acid oxidase activity in moulds and found that an enzyme activity, which is highly active towards a wide variety of D-amino-acids, was formed in *Aspergillus flavus* and *parasiticus*.

The screening experiment of the moulds which could utilize D-amino-acids was carried out first. To the basal medium containing 2 per cent glucose, 0·2 per cent $K_2HPO_4$ and 0·1 per cent $MgSO_4 \cdot 7H_2O$, was added D-methionine, D-phenylalanine, D-tryptophan or D-valine as a sole source of nitrogen at a final concentration of 0·5 per cent. 43 typical strains of moulds were cultured with shaking at 30° for 3–6 days in test-tubes containing the foregoing screening media. Most of the tested moulds grew by utilizing the D-amino-acids.

Oxidase activity towards several D-amino-acids of the 8 strains, which showed better growth among those tested, was measured by a Warburg manometer. As a result, *Aspergillus flavus* and *parasiticus* were found to show the highest activities.

Thus with these two moulds, cultural conditions for the formation of D-amino-acid oxidase were investigated. Cultures of the moulds were prepared in 500-ml. shaking flasks containing 100 ml. of the respective media prepared by supplementing 0·2 per cent L-, D-, or DL-