COMMUNICATIONS

Differential Assay of Human Hexosaminidases A and B

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Interest in the two forms (A and B) of *N*-acetyl- β -glucosaminidase first described by Robinson & Stirling (1968) has increased as a result of observations by Sandhoff (1969) and Okada & O'Brien (1969) that the A form is absent in Tay-Sachs disease and by Dance, Price, Cattell, Lansdell & Richards (1970) that the appearance of the B form in urine is a sensitive indicator of kidney damage. A rapid and reliable method for determining relative activities of the two forms is required, and we have compared several methods based on differences in their physical properties.

Comparison of the isoenzymes by starch-gel electrophoresis can be made quantitative by using 4-methylumbelliferyl *N*-acetyl- β -glucosaminide as the substrate and scanning the pattern of fluorescent zones. Excessive substrate hydrolysis must be avoided.

On selective denaturation by gentle heat the A form is destroyed most rapidly, and the denaturation curves of tissue homogenates are biphasic at pH 5.5 at 50°C. Extrapolation of the second gradient to zero time gives an estimate of the form B present, or assays based on denaturation for the precise half-life of form A can be used, assuming that form B is unaffected. These methods may be subject to gross error, since the A form is apparently converted in the process into the more stable B form. In our original study on the human kidney enzyme (Dance, Price, Robinson & Stirling, 1969) we assayed the two forms after separation on DEAE-cellulose columns with an NaCl eluent at pH 6.0. A simplified version of this procedure is rapid, effective and useful for small volumes of tissue homogenates or pathological urine. The sample (typically 1 ml of fivefold-diluted urine or 0.5% tissue homogenate) is mixed with 1 ml of a 10% suspension of Whatman DE32 ion-exchange cellulose, pre-equilibrated in 10 mM-phosphate buffer, pH 6.0. The suspension is continuously inverted for 15 min and then centrifuged. The activity of the supernatant (B form) is compared with that of the original sample (A and B). To check the recovery of activity the A form adhering to the cellulose can be displaced in 0.2 M-NaCl.

In our hands this last method is the easiest and

most reproducible, it requires the smallest amount of sample and can be carried out in the shortest time.

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The Lipases Produced by *Anaerovibrio lipolytica* in Continuous Culture

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Earlier publications (Hobson & Summers, 1966; Henderson, Hobson & Summers, 1968) have described the phenomenon, in continuous cultures of the rumen bacterium *Anaerovibrio lipolytica*, of two growth rates at which maximal amounts of lipase activity can be detected in the cell-free culture medium. As part of an attempt to explain this phenomenon, samples of enzyme produced at each of these growth rates have been purified and their properties compared. The purification process involved precipitation and adsorption of the enzyme from large volumes of medium by using ammonium sulphate and cellulose powder. The enzyme was eluted from the cellulose powder with phosphate buffer and after dialysis and ultrafiltration further purified on columns of Sephadex G-100 and Sephadex G-200. The enzymes collected at each of the growth rates studied were excluded from Sephadex G-200. Electrophoresis of these purified samples showed the presence of more than one component in each enzyme preparation.

The characteristics compared in studies of lipases purified on Sephadex G-200 were: effect of pH on activity, effect of temperature on activity and the rates of hydrolysis of different triglyceride substrates.

The pH at which optimum activity was obtained was 7.4 in both cases, with only a small difference in the shapes of the curves obtained when enzyme activity was plotted against incubation pH.

The two enzyme preparations differed in the temperature at which maximum hydrolysis of