## A Comparison of the Properties of Pure Human Pepsins 1, 2, 3 and 5

By N. B. ROBERTS and W. H. TAYLOR (*The United Liverpool Hospitals, Department of Chemical Pathology, Ashton Street, Liverpool L3 5RT, U.K.*)

Pepsins 1, 2, 3 and 5 were isolated from the histamine-stimulated gastric juice of individual patients by the method of Etherington & Taylor (1971). Qualitative agar-gel electrophoresis confirmed the homogeneity of each isolated enzyme. The yields were small, ranging from 10 to 100 $\mu$g.

Pepsin 1, the pepsin migrating most rapidly towards the anode (Etherington & Taylor, 1967), is secreted in increased amounts in patients with peptic ulcer (Taylor, 1970). Its pH–activity curve, determined with preparations from three individuals, showed a relatively sharp single maximum at pH1.9 with bovine haemoglobin as substrate. Pepsin 2 gave a single maximum, but at pH2.1, with each of two preparations. Pepsin 3, the principal human pepsin, showed a broader pH–activity curve with a plateau-type maximum in the pH range 2.4–2.8. Pepsin 5 showed a plateau of fairly constant activity over the pH range 2.0–3.6, but with the highest point occurring between pH2.8 and 3.4.

The sites of action of all four pepsins on the B-chain of oxidized insulin were investigated at pH1.7 and pH3.5. At pH1.7 pepsins 1 and 2 showed similar specificities. The major sites of action were at Phe-25–Tyr-26, Leu-15–Tyr-16, Tyr-16–Leu-17 and Leu-11–Val-12. These sites were generally similar to those of pepsin 3, but the specificity of pepsin 5 differed (Etherington & Taylor, 1971). At pH3.5 the overall rate of peptide-bond cleavage for pepsins 1 and 2 was much diminished, as would be predicted from the pH–activity curves. For pepsins 3 and 5 the rates were relatively unaffected. The specificity of each of the four enzymes did not differ significantly at the two different pH values.

Molecular-weight determinations by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis (Weber & Osborn, 1969; Dunker & Rueckert, 1969) gave values of 43800 for pepsin 1, 39950 for pepsin 2 and 37150 for pepsin 3. Each value is the mean for three different preparations. A single observation for pepsin 5 gave a value of 34600.

Pepsin 1 is more readily inactivated above pH6.0 than are the other pepsins.

There is thus a relationship between the electrophoretic mobility of the pepsins, the position of the pH maxima and increasing molecular weight.

Dunker, A. K. & Rueckert, R. R. (1969) *J. Biol. Chem.* **244**, 5074–5080

Etherington, D. J. & Taylor, W. H. (1967) *Nature (London)* **216**, 279–280

Etherington, D. J. & Taylor, W. H. (1971) *Biochim. Biophys. Acta* **236**, 92–98

Taylor, W. H. (1970) *Nature (London)* **227**, 76–77

Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412

## Isoenzymes of Acid Phosphatase in Human Endometrium

By N. A. SUMNER and M. G. BRUSH (*Department of Gynaecology, St. Thomas's Hospital Medical School, London S.E.1, U.K.*)

Many observers have noted a rise in acid phosphatase (EC 3.1.3.2) activity in human endometrium during the second half of the menstrual cycle. This increase is thought to reflect the influence of progesterone.

Fresh endometrial specimens, obtained from patients undergoing diagnostic curettage or from hysterectomy material, were used in a study of the activity and isoenzyme pattern of acid phosphatase throughout the menstrual cycle.

Endometrium was homogenized in ice-cold 0.25M-sucrose solution. A portion of the homogenate was treated with Triton X-100 (0.1%) and allowed to stand for 15min at 4°C. Then both samples were centrifuged at 900*g* for 10min. Diluted samples of the resulting supernatant were used for assay of the total acid phosphatase activity with 4-methylumbelliferyl phosphate (0.2mM) as substrate by the method of Robinson & Willcox (1969).

Polyacrylamide-gel electrophoresis at pH9.5 was carried out essentially as described by Smith *et al.* (1968). Samples were applied in 40% sucrose solution. Acid phosphatase activity was detected by using an azo-dye coupling technique with $\alpha$-naphthyl phosphate as substrate. In a few experiments 4-methylumbelliferyl phosphate was used and yielded similar results.

NaF (10mM) and L(+)-tartrate (25mM), known inhibitors of certain acid phosphatases, were used in some experiments.

A soluble fraction was prepared in a few instances by centrifuging a 900*g* supernatant, from a 0.25M-sucrose homogenate, at 105000*g* for 1h.

Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin (Sigma Chemical Co., St. Louis, Mo., U.S.A.) as standard.

Variations in acid phosphatase activity from tissue to tissue were similar whether related to wet weight or mg of protein. NaF inhibition was 80–100% and that by L(+)-tartrate was 30–50%.

Three distinct patterns emerged as a result of electrophoresis of 900*g* supernatants. (*a*) A single origin band: Triton X-100 (0.5%) effected the complete penetration of this band into the gel.