variants ('fast', 'slow' and 'intermediate') in their native forms and after acetylation, which increases the anodal migration of alkaline phosphatase without altering the characteristics of the residual enzyme activity (Moss, 1970).

All three forms are accelerated by acetylation: on starch-gel electrophoresis at pH 8.6 (tris-citrate-borate buffer) the slow isoenzyme is more accelerated than the fast variant, the modified forms having closely similar though not identical mobilities. The intermediate form behaves similarly to the fast isoenzyme at this pH value. Succinylation confirms the results of acetylation. The ratios of the mobilities of the acetylated and native forms are dependent on pH over the range 6.0–7.5 (phosphate buffers) and 8.0–10.4 (glycine buffers). The shape of these curves suggests the presence of modifiable charged groups with a pK_a in the region of pH 6.8, and that these groups make a greater contribution to the net charge of the intermediate isoenzyme in the pH range 6–8 than to that of the other two variants. Further groups with pK_a values about 9.0, which can be acetylated, are indicated, and the slow variant appears to possess a greater proportion of these groups than the fast and intermediate isoenzymes, accounting for its lower mobility and the more pronounced effect of acetylation.

Although these observations account for some of the electrophoretic differences between the three placental phosphatase isoenzymes, small residual differences in the mobilities of the acetylated forms suggest that further variations in structure also exist involving groups that are not acetylated. The groups in the protein or carbohydrate portions of the enzyme molecule that are modified by acetylation cannot be identified from these data alone, but extension of the method to include reagents of greater specificity may permit this, provided that such reagents do not also abolish enzymic activity.

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Characterization of an Alkaline Phosphatase from Rat Heart

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An alkaline phosphatase has been detected histochemically in sections of rat heart, activity being particularly marked in the tissues of the right atrium. The amount of this enzyme is increased after injections of catecholamines or isoprenaline

(Müller & Pearse, 1969). The enzyme activity has now been extracted from the atria of isoprenaline-treated and control male rats and certain of its properties have been investigated.

Homogenization of atria in sucrose solution or extraction of the tissue with butan-1-ol or autolysis give essentially equal recoveries of phosphatase activity. The activity in the left atrium of unstressed control animals averages about $0.003 \mu\text{mol}$ of *p*-nitrophenyl phosphate hydrolysed/min per mg of tissue by the method of Bowers & McComb (1966), compared with $0.007 \mu\text{mol}/\text{min}$ per mg in the right atrium. After subcutaneous injection of isoprenaline (40 mg/kg body wt.), right-atrial activity rises from three- to ten-fold, depending on the age of the animals, to values averaging $0.025 \mu\text{mol}/\text{min}$ per mg, but that of the left atrium reaches only $0.006 \mu\text{mol}/\text{min}$ per mg.

The atrial phosphatase has a pH optimum in the region of 9.7 at 5 mM substrate concentration and is activated by Mg^{2+} ions. It is not inhibited by L-phenylalanine (Fishman, Green & Inglis, 1962) and is thus of non-intestinal type by this criterion. Starch-gel electrophoresis shows only one major zone of alkaline phosphatase activity in each of the right and left atria. The zones in extracts of the two tissues have similar electrophoretic mobilities. The increase in activity after injection of isoprenaline does not appear to be accompanied by the appearance of additional enzyme zones.

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Protease and Ribonuclease Activities in Bovine Pituitary Lobes

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The acid and alkaline proteinase activity first described by Adams & Smith (1951) in bovine anterior and posterior pituitary lobes has been reinvestigated by an assay in which the trichloroacetic acid-soluble digestion products of denatured haemoglobin are estimated by measuring both the extinction at 280 nm and the colour produced by Folin-Ciocalteu reagent. The pH-dependence curve for fresh and acetone-dried posterior, and fresh anterior, bovine pituitary lobes confirms the presence of a cathepsin with a pH optimum at 3.8, but an optimum between pH 8.3 and 9.7 is given by u.v.-absorbing material that is Folin-negative and therefore not the result of proteolytic activity.

Controls showed that the u.v.-absorbing products

at alkaline pH were derived from an endogenous substrate and had an absorption spectrum similar to that of nucleotides.

Paper chromatography of the digestion products in propan-2-ol-water-ammonia (7:3, by vol., with 0.35 ml of NH_3 solution added per litre of gas space) produced a pattern of u.v.-absorbing spots similar to that described by Markham & Smith (1952) for the products of digestion of RNA by bovine pancreatic ribonuclease A. The fast component was eluted and when incubated with bovine pancreatic ribonuclease A gave rise to a slower-running spot, consistent with the conversion of 2':3'-cyclic nucleoside phosphate into nucleoside 3'-phosphate.

The presence of a 'cyclizing' ribonuclease active at alkaline pH was confirmed by the inhibitory effects of bentonite, phosphate and arsenate; the activity was unaffected by boiling at acid pH, EDTA and various metal ions.

The pH-dependence of the ribonuclease activity of fresh and acetone-dried posterior pituitary lobes with exogenous RNA gave two optima, one between pH 5.2 and 6.9 and the other between 8.0 and 9.3. The position of these optima was dependent on ionic strength. Boiling the enzyme preparation at acid pH markedly decreased the acid ribonuclease activity but had little effect on the alkaline ribonuclease activity.

The demonstration of 'alkaline protease' activity in purified anterior-pituitary granules (Perdue & McShan, 1962) suggested a study of the subcellular localization of alkaline ribonuclease activity by the differential centrifugation of homogenates of posterior pituitary lobes. Activity was, however, confined to the supernatant and/or microsomal fraction.

This work indicates that it is necessary to reconsider the possible part played by so-called 'alkaline-proteases' in the synthesis and release of anterior-pituitary hormones (Meyer & Clifton, 1956; Perdue & McShan, 1962) and their presence in other endocrine organs (Todd & Trikojus, 1960).

This work was supported by research and training award (to J.C.P) grants from the Medical Research Council.

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Relationships between Kinetic Properties and Structure of Caeruloplasmin Substrates

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Curved reciprocal plots of substrate concentration against reaction velocity have been found for caeruloplasmin by using DPD* (Curzon, 1967; Walaas, Løvstad & Walaas, 1967) and dopamine (Løvstad & Walaas, 1967) as substrates. This was explained in terms of two kinetically different active sites either on the same or on different enzyme molecules. However, evidence has now been obtained that the curved plot for DPD is due to DPD^+ , the free-radical oxidation product of DPD, also being a substrate. Thus in the presence of ascorbic acid, which rapidly reduces DPD^+ back to DPD, a straight-line reciprocal plot was obtained. This is consistent with work by Pettersson (1970a), who showed by calculation that the curved reciprocal plots can be explained by the substrate activity of DPD^+ . The substrate activity of DPD^+ also explains the initial time-dependent increase of reaction rate found with low concentrations of DPD as substrate (Curzon, 1967). This was abolished by adding DPD^+ or ascorbic acid, the rate with ascorbic acid being lower than that with DPD^+ . The increasing activity as DPD^+ accumulates is due to the lower K_m of DPD^+ compared with DPD (Pettersson, 1970a).

Because products may act as substrates and also because trace iron contamination affects enzyme activity (Curzon, 1961), previous comparisons of caeruloplasmin substrates are difficult to interpret. Therefore a series of substrates were compared, avoiding these sources of interference by measuring activities in the presence of ascorbic acid and EDTA. K_m and $V_{\max}$ values were determined with human caeruloplasmin at 25°C for 33 phenylenediamines, aminophenols and polyphenols in 10 mM-acetate buffer, pH 5.5, with 100 μM -EDTA and 50 μM -ascorbic acid present, by measuring oxygen uptake at different substrate concentrations. All reciprocal plots were straight lines. Although the K_m values varied over a 10^4 -fold range the $V_{\max}$ values were within only a sixfold range and 29 of these were within a twofold range. This is consistent with the work of Osaki & Walaas (1967), who, using Fe^{2+} as substrate, obtained results suggesting that the rate-determining process in catalytic action was a conformational change of the enzyme before its reoxidation by molecular oxygen. If this applied generally then the $V_{\max}$ values would

* Abbreviation: DPD, *NN*-dimethyl-*p*-phenylenediamine.