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).

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Adams, E. & Smith, E. L. (1951). *J. biol. Chem.* **191**, 651.
Markham, R. & Smith, J. D. (1952). *Biochem. J.* **52**, 558.
Meyer, R. K. & Clifton, K. H. (1956). *Archs Biochem. Biophys.* **62**, 198.

Perdue, J. F. & McShan, W. H. (1962). *J. Cell Biol.* **15**, 159.

Todd, P. E. E. & Trikojus, V. M. (1960). *Biochim. biophys. Acta*, **45**, 234.

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.

be relatively insensitive to variation of substrate structure.

Values of K_m , but not of V_{max} , showed relationships with substrate structure, increasing in the order *para*, *ortho*, *meta*. This suggests that electronic rather than steric factors are the dominant influence on K_m values. The energy of the highest occupied molecular orbital has been calculated for nine of the substrates studied (Pettersson, 1970b). These values correlate with $\log K_m$, higher orbital energies being associated with lower K_m values. K_m values are also affected by negatively charged groups attached to the benzene ring; the more highly charged the group, the higher the K_m value.

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Curzon, G. (1961). *Biochem. J.* **79**, 656.

Curzon, G. (1967). *Biochem. J.* **103**, 289.

Løvstad, R. A. & Walaas, E. (1967). *Abstr. FEBS 4th Meet.* no. 428, p. 107.

Osaki, S. & Walaas, O. (1967). *J. biol. Chem.* **242**, 2653.

Pettersson, G. (1970a). *Acta chem. scand.* **24**, 1809.

Pettersson, G. (1970b). *Acta chem. scand.* **24**, 1838.

Walaas, E., Løvstad, R. A. & Walaas, O. (1967). *Archs Biochem. Biophys.* **121**, 480.

Immunochemical Studies on Human Calcitonin M Leading to Information on the Shape of the Molecule

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Antigen-antibody interactions are sensitive to conformational changes in the antigen (Brown, 1962, 1963; Sela, Schechter, Schechter & Borek, 1967; Goodman, 1969). We have used changes in such interactions (assessed qualitatively) to derive information on the conformation of human calcitonin M.

Two highly specific antisera to the hormone were obtained from a single rabbit. The first was taken 8 months after immunization and boosting with crude extracts of human thyroid-medullary carcinoma: the second was obtained after a further 9 months of boosting with the pure synthetic hormone. By using the radioimmunoassay system described by Clark, Boyd, Byfield & Foster (1969), the reactions of the hormone and its derivatives and fragments with these antisera were studied. It was shown that antiserum I reacts with two major sites, one within the sequence 11-18 of the 32-residue peptide amide and the second located around residue 28. Antiserum II reacts mainly with

a site around residue 28, which may be different from the site in this region recognized by the first antiserum. Neither antiserum reacts appreciably with the *N*-terminal end of the peptide. This may be correlated with the fact that only residues 1, 3, 4, 5, 6, 7, 9, 28 and 32 are conserved in the five species known and that immunogenicity often resides in non-conserved regions (Schwyzer, 1968).

An analysis of the data suggests that the conformation of the *C*-terminal antigenic sites is influenced by the *N*-terminal region. Studies on oxidized derivatives locate the site of influence near the methionine⁸ residue, but the 1-7 disulphide bond, the residues of the ring and the terminal amino group do not appear to be involved.

All the data suggest that human calcitonin M is not a random coil but has some degree of preferred conformation. It is suggested that the peptide is folded to bring the *N*- and *C*-termini into close proximity with non-covalent interaction between separate parts of the linear sequence, perhaps near residues 8 and 28.

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Brown, R. K. (1962). *J. biol. Chem.* **237**, 1162.

Brown, R. K. (1963). *Ann. N.Y. Acad. Sci.* **103**, 754.

Clark, M. B., Boyd, G. W., Byfield, P. G. H. & Foster, G. V. (1969). *Lancet*, ii, 74.

Goodman, J. W. (1969). *Immunochemistry*, **6**, 139.

Schwyzer, R. (1968). In *Protein and Polypeptide Hormones*, p. 201. Ed. by Margoulies M. Amsterdam: Excerpta Medica Foundation.

Sela, M., Schechter, B., Schechter, I. & Borek, F. (1967). *Cold Spring Harb. Symp. quant. Biol.* **32**, 537.

Patterns of Serum γ G-Globulin Heterogeneity in Health, Rheumatoid Arthritis and Cancer

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Several workers have suggested that the serum immunoglobulin concentrations of the individual in normal health may be genetically determined, a view supported by recent studies on monozygotic and dizygotic twins (Kalf & Hijmans, 1969). Differences in the relative proportions of the immunoglobulins may indicate differences in the type and extent of the immune response of each individual and correlate with certain disease patterns. The increased incidence of lymphoma in patients with