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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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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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

hypogammaglobulinaemia (Miller, 1968), and a connexion between autoimmune and malignant disease (Lea, 1964) supports this hypothesis.

γ G-globulin, the most specialized of the immunoglobulin classes, comprises four subclasses which differ antigenically and in biological function. It is possible that patients with either autoimmune disease or certain types of cancer may have characteristic and recognizable patterns of γ G-globulin heterogeneity. This communication reports an initial survey of 'heterogeneity patterns', which may result from (a) variations in subclass proportions, (b) aggregation phenomena, (c) differences of molecular configuration and (d) the Gm and Inv antigens. Catabolic experiments carried out in healthy individuals suggest that the rate of γ G-globulin elimination is reproducible and characteristic of the individual. The non-linear elimination curves can be largely explained as the resultant breakdown of different proportions of γ G₁ and γ G₂ molecules, but differences in configuration within the subclasses modify these patterns. Configurational abnormalities were also demonstrated by the unique electrophoretic patterns obtained from papain hydrolysates of γ G-globulins isolated from the sera of rheumatoid patients. The γ G-globulins of healthy individuals and of melanoma and myeloma patients did not show these patterns (Watkins, Unger & Mahon, 1970). There is evidence that the hinge region of the molecule is involved in these configurational differences.

The Gm antigens of 25 healthy individuals and of 14 rheumatoid and 39 melanoma patients described the most frequently occurring phenotypes in the U.K. In contrast there was an increased incidence of the Inv factors 1, 2 in the melanoma group (9/39) compared with the rheumatoid (1/14) and control (1/25) groups. The numbers are too small for real significance, but the incidence might be indicative of a specific genetic trait in individuals with melanoma.

These investigations are being extended.

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Extraction of Polymeric Collagen from Human Skin

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The polymeric collagen fraction of tissues has been isolated by the α -amylase (Steven, 1964), EDTA (Steven, 1967) and lyotropic-relaxation (Veis, Bhatnagar, Shuttleworth & Mussell, 1970) methods. These three methods have been compared by Veis *et al.* (1970) using bovine corium. However, Veis *et al.* (1970) concentrated on the preparation of a unit-fibril fraction and departed, in important respects, from the methods of Steven (1964, 1967). We describe the isolation of polymeric collagen fractions from 50 mm² samples of human skin, a size that could reasonably be obtained by biopsy, by modifications of these methods and the comparison of the properties of the polymeric collagen fractions.

Contiguous human skin samples (30–150 mg wet wt.), obtained *post mortem*, were extracted successively with 5% (w/v) NaCl, 0.2M-sodium phosphate buffer, pH 5.4, and water, and incubated at 18°C with either crude *Bacillus subtilis* α -amylase (Steven, 1964), EDTA (Steven, 1967) or water (Veis *et al.* 1970). The insoluble residues were washed exhaustively with water, homogenized in 100 ml of 0.2M-acetic acid for 10 s with an Ultra-Turrax disintegrator and the suspension was centrifuged at 300g for 10 min; the resulting residues were re-extracted with a further 100 ml of 0.2M-acetic acid and similarly centrifuged. The polymeric collagen in the combined acetic acid supernatants was isolated as a fibrous mass by adding 1.0M-NaOH to pH 6.0–7.0 and washed repeatedly with water; 3.0–20 mg of polymeric collagen was obtained, sufficient for chemical investigation by the methods of Steven (1966) and for electron microscopy.

The yields of polymeric collagen from six samples were 40–90% of the total collagen but did not depend on the method of extraction. The three methods gave polymeric collagen fractions with similar chemical stabilities, though the samples prepared after α -amylase treatment differed on electron microscopy from those prepared after aqueous treatment.

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