used to prepare human γ M-globulin but, as sera from macroglobulinaemic patients often have very fast-sedimenting components, the methods for rat plasma could not be used unmodified.

Good separation of human macroglobulins was obtained in the BXV Ti rotor with a linear gradient (1030ml) of sucrose (15–30%, w/w) in Miller-Golder (Miller & Golder, 1950) buffer, pH 7.4 and $I0.2$, overlying a 'cushion' (200ml) of 40% (w/w) sucrose in this buffer. The sample, containing 10% (w/w) of sucrose, was introduced over the gradient followed by 400ml of the buffer to displace the sample to a position of high centrifugal field and to prevent pelleting of lipoproteins at the rotor core. A well-resolved macroglobulin peak was obtained from 10ml of macroglobulinaemia serum by centrifuging for 22h at 25000 rev./min ($\int \omega^2 dt = 55 \times 10^{10}$), but a 20ml sample showed evidence of overloading. When sera contained fast-sedimenting components these were eluted as a shoulder on the high-density side of the macroglobulin peak.

After dialysis against Miller-Golder buffer, and re-concentration the macroglobulin peak contained α_2 M- and γ M-globulins with traces of γ G-globulin and albumin. Euglobulin precipitation with 7.5g of boric acid/l (Masseyeff, Gombert & Josselin, 1965) gave a precipitate containing only γ M-globulin and sedimenting as a single peak in the analytical ultracentrifuge. The yield was 40–50% of the original serum γ M-globulin.

When a large amount of fast-sedimenting protein was present resolution was improved by decreasing the overlay to 300ml and increasing the cushion correspondingly. The extra peak so obtained contained only γ M-globulin but showed both 19S and 27S boundaries in the analytical ultracentrifuge. Further studies on the components of this peak are in progress.

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Calcium Ion Binding by Bone Mucosubstances

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Although a number of observations of Ca^{2+} binding by chondroitin sulphate from cartilage have been described (Urist, Speer, Ibsen & Strates, 1968), it appears that Ca^{2+} binding by mucosubstances in bone has received less attention. Herring (1969) has isolated several glycoprotein fractions from bone, including three chondroitin sulphate-protein complexes and bone sialoprotein, a highly acidic glycoprotein of mol.wt. 25000. In this work, the Ca^{2+} -binding properties of bone sialoprotein, bone chondroitin sulphate and shark chondroitin sulphate, have been compared by using the murexide method and the inhibition of calcium phosphate precipitation *in vitro*.

In the murexide method measurements of free Ca^{2+} permit determination of the formation constant (K_f) and the exchange parameter (K_p) (Urist *et al.* 1968). For chondroitin sulphate these constants are expressed conventionally in terms of the equivalent weight, and here they are expressed similarly for bone sialoprotein, i.e. assuming one Ca^{2+} ion binds to one carboxyl group (Herring, 1969). For bone sialoprotein the values ($\pm$ S.D.) of K_f (82.5 ± 22.2) and K_p (2.26 ± 0.68) were significantly higher than those for protein-free bone chondroitin sulphate (26.8 ± 7.3 and 0.65 ± 0.18 respectively). The latter did not differ significantly from the values for shark chondroitin sulphate of K_f (36.8 ± 6.1) and K_p (0.88 ± 0.14), which were in accordance with the data of Urist *et al.* (1968).

The inhibition *in vitro* of calcium phosphate precipitation (Di Salvo & Schubert, 1967) also showed that Ca^{2+} binds more strongly to bone sialoprotein than to the bone chondroitin sulphate-protein complex. Under the conditions used the concentrations required to effect a 50% inhibition of precipitation were 0.2mg/ml for bone sialoprotein and 0.6mg/ml for the bone chondroitin sulphate-protein complex.

Oldroyd & Herring (1967) have shown that the quantities of bone sialoprotein and chondroitin sulphate in bovine cortical bone are of the same order, and therefore it is possible that this Ca^{2+} binding may be of some significance *in vivo* (Herring, 1969).

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