physiological pH succinate oxidation is limited by its entry.

Short exposure to pH 6.3 with succinate and uncoupler present did not significantly increase membrane permeability to a sulphonated azo-dye. Lowering the pH to 6.3 changes the quantities of ionic species $\text{H}_2\text{succinate}:\text{Hsuccinate}^{1-}:\text{succinate}^{2-}$: from 0.1mM:2.5mM:37.4mM to 0.4mM:6.2mM:33.4mM and the faster succinate entry is probably due to the increased proportion of uncharged molecules, which can permeate the relatively hydrophobic osmotic barrier by a diffusion pathway. It appears that the membranes of these cells, grown in suspension in modified Waymouth medium (Sinclair, Reid & Mitchell, 1963), are virtually impermeable to succinate²⁻, which predominates at physiological pH. This is in contrast with the apparent ability of succinate²⁻ to permeate the mitochondrial membrane by a carrier mechanism (Chappell & Haahrhoff, 1967).

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Indirect Evidence that Light-Induction of Adenosine Triphosphate Hydrolysis by Chloroplasts Depends on Electron-Transfer Reactions

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The dark hydrolysis of ATP by spinach or lettuce chloroplasts, after activation by light in the presence of cysteine (80mM), is stimulated by ammonium chloride (5mM) added after illumination; the ATP hydrolysis then becomes progressively slower, thus confirming earlier observations with dithiothreitol (Carmeli & Avron, 1969; Carmeli, 1969; cf. also Marchant, 1969). A distinct difference is found between the use of *N*-methylphenazonium methyl sulphate on the one hand and FMN on the other as added redox substance. If the reaction mixture is not stirred during the illumination period (5min) when *N*-methylphenazonium methyl sulphate was used the initial stimulated rate is high and falls off relatively slowly, whereas with FMN the initial rate is lower and falls off rapidly. If on the other hand the mixture is continuously stirred during the light phase, the initial high rate with *N*-methylphenazonium methyl sulphate now falls off more rapidly, whereas with FMN the initial rate is now considerably higher and no longer falls off so rapidly. This higher rate with FMN is even

observed if a few seconds' agitation is given during the latter part of the illumination period. Neither stirring nor agitation are necessary for these higher rates with FMN if the illumination period is shortened to 2min.

The effects with FMN can be attributed to the need for either oxygen or oxidized FMN for electron transfer, the FMN having become reduced during early illumination and not then available in the oxidized form unless the mixture is stirred. With *N*-methylphenazonium methyl sulphate a possible explanation is that stirring in from the surface layers of the oxidized cofactor itself, or a derivative of it, is responsible for the decreased response to ammonium chloride subsequently, assuming that maintenance of efficient cyclic electron transfer throughout illumination depends on the nature and redox state of this cofactor (cf. Zweig & Avron, 1965). Both of these explanations would imply that a maintained stimulation by ammonium chloride depends on prior electron transport.

Further support for the involvement of electron transfer in the light-induction is given by the absence of inhibition by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (0.1mM), with or without ammonium chloride, when *N*-methylphenazonium methyl sulphate is used (cf. Carmeli & Avron, 1966), but very clear inhibition with FMN, as is found for photophosphorylation.

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Preparation of Human Pathological γ -Macroglobulins by Rate Zonal Centrifugation in a BXV Ti Rotor

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Zonal rotors (Anderson, 1966) have mostly been used for the preparation of cell particles, but both Fisher & Canning (1966) and Anderson *et al.* (1967) used the separation of a macroglobulin peak from rat plasma as a test of resolution for zonal rotors. Their results suggested that the technique could be

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 22 h at 25 000 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. 25 000. 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 0.2 mg/ml for bone sialoprotein and 0.6 mg/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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