Solubilization of Pig Lymphocyte Plasma Membrane and Partial Separation of Some of the Components

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The plasma membrane of small lymphocytes was prepared from pig mesenteric lymph nodes as described by Allan & Crumpton (1970), and was resuspended in water at a concentration of about 6mg of protein/ml. Increasing amounts of sodium deoxycholate dissolved in 0.9ml of water were added to 0.1ml samples of the membrane suspension and the extent of solubilization was measured after centrifuging at $100\,000g_{av}$ for 1h. About 70% of the membrane protein and 95% of the leucyl-naphthylamidase activity were soluble in 0.5% sodium deoxycholate at 0°C, whereas only about 5% of the 5'-nucleotidase activity dissolved. On increasing the sodium deoxycholate concentration to 2% and the temperature to 23°C, a further 25% of the protein was solubilized together with over 90% of the 5'-nucleotidase activity. Use of this differential procedure allowed almost complete separation of these enzyme activities and gave a threefold increase in the specific activity of 5'-nucleotidase.

The soluble material obtained by using 2% sodium deoxycholate at 23°C (95% of the total membrane protein) was fractionated on a sucrose density gradient [10–30% (w/v) sucrose–1% sodium deoxycholate] by centrifuging at $160\,000g_{av}$ for 17h at 0°C (20ml tubes; MSE 65 centrifuge; 8×25 Ti angle rotor). The phospholipid remained near the top of the gradient and was resolved from the majority of the membrane protein, which formed a fairly symmetrical peak sedimenting slightly slower than ovalbumin (mol.wt. 45 000). The membrane receptors for the lymphoagglutinating and lymphocyte-transforming activities of phytohaemagglutinin (D. Allan, J. Auger & M. J. Crumpton, unpublished work) were localized in the leading edge of the protein peak. The 5'-nucleotidase and leucyl-naphthylamidase activities were resolved from each other and from the major protein peak; the former enzyme sedimented at the same rate as immunoglobulin G (mol.wt. 150 000) whereas the sedimentation rate of the latter enzyme was consistent with a molecular weight of greater than 200 000. A small translucent pellet containing less than 3% of the total membrane protein was usually present. Significant amounts of sodium deoxycholate were apparently not bound by the membrane protein, since sodium [^{14}C]deoxycholate added to solubilized membrane remained entirely at the top of the gradient after centrifugation.

A similar separation to that described above was achieved by using a column of Sephadex G-200 in

1% sodium deoxycholate. However, although the apparent molecular weight of 5'-nucleotidase and leucyl-naphthylamidase were similar to those determined by sucrose-density-gradient centrifugation, the majority of the membrane protein appeared to possess a much higher molecular weight than that indicated previously. Protein that had an apparent molecular weight of about 40 000 by sucrose-density-gradient centrifugation was resolved on Sephadex G-200 into two peaks whose elution volumes corresponded to molecular weights of 95 000 and 200 000. The disparity between the values for the molecular weights of the major membrane proteins obtained by using the above methods may indicate that the molecules are asymmetric.

Allan, D. & Crumpton, M. J. (1970). *Biochem. J.* **120**, 133.

Macromolecular Compounds of Bilirubin in Human Bile

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Gall-bladder or hepatic bile was diluted with 5–9 vol. of ethanol and, after standing in the dark, the residue after decantation was centrifuged at about 8500g. Two washings with ethanol gave a light-coloured supernatant; the coloured residue was freeze-dried in the dark to give 'bilimucin', which was dissolved in NaHCO_3 – Na_2CO_3 buffer, pH 10 and I 0.05 (buffer).

Comparison at 420nm with an ethanolic bilirubin solution diluted with buffer suggested that 'bilimucin' samples had a bilirubin content between 3 and 10% of dry weight. Determination by a diazo method gave lower results.

'Bilimucin' (20–100mg) in buffer (2ml) was fractionated in buffer on columns of Sephadex G-100 and G-200; 3ml fractions were collected and the E_{420} was measured. On Sephadex G-200 three principal fractions were evident: A, just beyond the exclusion volume (mol.wt. about 6×10^5); B, at about half column volume (mol.wt. about 10^5); C, close to column volume. On Sephadex G-100, results varied with different samples. In all cases one small fraction appeared at exclusion volume. In some samples the major fraction was eluted at considerably less than column volume; in other cases the corresponding fraction was close to column volume.

Dialysis of 'bilimucin' (10mg) in buffer (4ml) against buffer (10ml) gave little colour in the diffusate; sodium lauryl sulphate (10mg) in the sac did not increase the colour.

Chloroform did not extract colour from 'bilimucin' and t.l.c. in pentyl acetate-acetic acid-water (5:5:2, by vol.) gave a continuous streak and no discrete spots corresponding to bile salts.

The ethanolic liquors from the original precipitation in one case were evaporated in N_2 at reduced pressure in the dark at about 55°C. From a Sephadex G-100 column, the coloured compounds in the residue were eluted at column volume.

'Bilimucin' appears to be a macromolecular complex in which bilirubin might be linked to mucopolysaccharide.

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Glycosaminoglycans of Hen's-Egg White

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The glycosaminoglycans of hen's-egg white have been the subject of limited study. Their presence has been indicated by carbazole assay (Wood & Anastassiadis, 1965) and by the partial identification of heparan sulphate (Lovell, Schorah & Curran, 1966). Our own study of the distribution of glycosaminoglycans in egg white was undertaken in the hope of isolating a sizeable heparan sulphate fraction.

Egg white (1.7 kg) was digested with papain and the glycosaminoglycan fraction was recovered by ethanol precipitation after trichloroacetic acid treatment and dialysis (by the method described by Schiller, Slover & Dorfman, 1961). The product contained a total of 19.7 mg of hexosamine, 18.1 mg of neutral sugar and 2.6 mg of uronic acid. The hexosamine was a mixture of glucosamine (60.5%) and galactosamine (39.5%). These results suggest that glycoprotein and possibly keratan sulphate constitute a major portion of the isolated material. Upon fractionation of the isolated glycosaminoglycans with stepwise elution with NaCl from Dowex 1-X2 (Cl^- form), only 10.4% of the recovered uronic acid was eluted with 1.25 M-NaCl, where heparan sulphate might be expected. Over the entire elution range from 0.5 M- to 4.0 M-NaCl, neutral sugar-containing material greatly exceeded that containing uronic acid, which was concentrated in the 1.5 M- and 2.0 M-NaCl fractions. Further analyses have been carried out on the fractions, although definitive identification of glycosaminoglycans has been made difficult, and possibly of little value, by the finding of such low concentrations.

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Lovell, D., Schorah, C. J. & Curran, R. C. (1966). *Nature, Lond.*, **212**, 860.

Schiller, S., Slover, G. A. & Dorfman, A. (1961). *J. biol. Chem.* **236**, 983.

Wood, D. F. & Anastassiadis, P. A. (1965). *Can. J. Biochem.* **43**, 1839.

The Oxidation of Exogenous Reduced Nicotinamide-Adenine Dinucleotide by Plant Mitochondria

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It is generally accepted that mitochondria isolated from plant material will readily oxidize exogenous $NADH_2$ via the usual electron-transport chain (Ikuma & Bonner, 1967; Srivastava & Sarkissian, 1970). Mitochondria isolated from Jerusalem-artichoke tubers oxidized malate, exogenous $NADH_2$ and succinate, which was coupled to oxidative phosphorylation to yield P/O ratios of 3.0, 1.7 and 1.8 respectively. The oxidation of malate was largely inhibited by rotenone (1 μ g/mg of protein) whereas the oxidation of both succinate and exogenous $NADH_2$ was unaffected by this inhibitor. The oxidation of all three substrates was completely inhibited by antimycin A (100 μ g/mg of protein). These data suggest that the pathway of electron transport between endogenous $NADH_2$ (malate) and exogenous $NADH_2$ to cytochrome *b* is different, and the ability of these mitochondria to utilize exogenous $NADH_2$ cannot simply be accounted for by suggesting that their membrane system is permeable to added $NADH_2$. Other results suggesting the operation of different routes for the oxidation of exogenous and endogenous $NADH_2$ have been obtained in mitochondria isolated from the spadix tissue of *Arum maculatum*. In the absence of antimycin A or potassium cyanide *Arum* mitochondria oxidized malate, exogenous $NADH_2$ and succinate coupled to a low degree of oxidative phosphorylation, yielding P/O ratios of 0.44, 0.14 and 0.26 respectively. When antimycin A (2 μ g/mg of protein) is added to these mitochondria they are able to utilize an alternative, non-phosphorylating, electron-transport system between cytochrome *b* and oxygen (Hackett & Haas, 1958; Bonner & Bendall, 1968), which allows electrons to by-pass the terminal two sites of ATP synthesis associated with the usual electron-transport chain. The addition of antimycin was observed to remove completely the phosphorylation that normally accompanied the oxidation of succinate and exogenous $NADH_2$. When malate was supplied as the electron donor residual phosphorylation was obtained, indicating the continued operation of the first site of phosphory-