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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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-

lation between endogenous NADH_2 and cytochrome *b*. Thus it appears that electron transport from exogenous NADH_2 is again different from endogenous NADH_2 and is not coupled to the first site of oxidative phosphorylation. It seems reasonable to suggest that the rotenone-insensitive oxidation of exogenous NADH_2 found in Jerusalem-artichoke mitochondria may also not be coupled to oxidative phosphorylation at the first coupling site, resulting in the lower P/O ratio. These results could be explained on the basis of the chemiosmotic theory if the dehydrogenase responsible for the oxidation of exogenous NADH_2 is located on the outside of the coupling membrane, where it would fail to translocate protons during the first loop of electron transport (Mitchell, 1967). The results of initial experiments with ferriyanide as a non-penetrating electron acceptor are consistent with the localization of the dehydrogenase on the outside of the coupling membrane.

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Polyamine Oxidase in Higher Plants

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Although the polyamines spermidine and spermine are widespread in higher plants (Smith, 1970a), specific enzymes oxidizing these substances in plant tissue have not been reported.

By using gas chromatography, peaks indistinguishable from 1,3-diaminopropane and 1-(3-aminopropyl)pyrroline were found in extracts of both seedling and mature leaves of barley (Smith, 1970b), suggesting the presence of a spermine oxidase (spermine- O_2 oxidoreductase, EC 1.5.3.3), similar to an enzyme demonstrated in the bacterium *Neisseria perflava* (Weaver & Herbst, 1958).

Reputedly authentic 1-(3-aminopropyl)pyrroline has been prepared by the catalytic oxidation of spermine (Wrede, 1926; Wrede, Fanselow & Strack, 1926) and by the enzymic oxidation of spermidine (Hasse & Schührer, 1962). Presumed 1,3-diaminopropane and 1-(3-aminopropyl)pyrroline formed on oxidation of spermine by the barley and maize enzymes were indistinguishable from the

synthetic amines by thin-layer and gas-liquid chromatography.

Leaves of barley and maize seedlings were extracted into water (2vol.), acidified to pH 4.0 with HCl, centrifuged (1000g, 15min) and readjusted to pH 6.0 with NaOH. Ammonium sulphate was then added to 50% saturation and the precipitate was dialysed against buffer. Activity was determined by oxygen uptake in the presence of catalase, added to destroy the hydrogen peroxide formed, and also by gas chromatography of the products, 1,3-diaminopropane and 1-(3-aminopropyl)pyrroline. For barley, the optimum pH was 4.5 with spermine as substrate, but with spermidine and 3,3'-diaminodipropylamine the optimum was about pH 7.0. The activity with the latter substrates at pH 7.0 was less than 5% of that with spermine at pH 4.5. Stoichiometry of the reaction is difficult to establish, since oxygen uptake exceeds the expected value.

Spermidine and spermine (10mM) were fed separately to intact 7-day-old barley leaves for 24h and the amine fraction (Smith, 1970a) was subjected to gas chromatography. Spermidine gave an increase in 1,3-diaminopropane, putrescine and 1-(3-aminopropyl)pyrroline, and spermine gave an increase in 1,3-diaminopropane and 1-(3-aminopropyl)pyrroline.

Enzyme activity in maize seedlings was localized histochemically. Tissue sections were immersed in a reagent containing soluble starch (1.3%), KI (20mM) and spermine tetrahydrochloride (8mM). Development of a blue colour due to enzymically generated hydrogen peroxide indicated spermine oxidase activity. Most activity was found in the vascular tissue (excluding the xylem) in the leaves, roots and especially the mesocotyl. No colour developed in controls omitting any one component, nor would putrescine substitute for spermine.

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