identified. The identity of the tri-*O*-methylglucosides has been confirmed by mass spectroscopy.

On oxidation with 15mM-NaIO₄ the periodate uptake was 0.68mol/mol of anhydroglucose units. The polyaldehyde was reduced with NaBH₄ to give a water-insoluble polyol. The products of hydrolysis with dilute acid (0.5M-HCl at 37°C) were glycerol and a series of glycerol glycosides. Hydrolysis with 2M-HCl at 100°C gave glycerol and glucose in the ratio 3:5, determined enzymically and by g.l.c. of their alditol acetates. Methylation analysis of the Smith degradation product gave the following methyl ethers of methyl α -D-glucopyranoside: 2,3,4,6-tetra- (19%), 2,3,4-tri- (9%) and 2,4,6-tri- (72%).

On the basis of these results it is proposed that the (water-)insoluble glucan is a heteroglucan containing both α -(1 $\rightarrow$ 3)- and α -(1 $\rightarrow$ 6)-linkages, and the high proportion of α -(1 $\rightarrow$ 3)-linkages may explain the insolubility of the glucan in water and the resistance to hydrolysis by dextranase.

Carlsson, J. & Egelberg, J. (1965) *Odontol. Revy* 16, 112
Guggenheim, B. & Schroeder, H. E. (1967) *Helv. Odontol. Acta* 11, 131

Particulate Malate Oxidation in Strictly Aerobic Bacteria: the Respiratory System of *Moraxella lwoffii*

By MERFYN JONES and H. K. KING (*Department of Biochemistry, University College of Wales, Aberystwyth, Cards. SY23 3DD, U.K.*)

Moraxella lwoffii has two malate dehydrogenases (Jones, 1969; Jones & King, 1970). One is a very active soluble NAD-linked dehydrogenase, and the other is very firmly bound to the membranes and associated with the electron-transport system. On passage of a sample of disintegrated cells through a Sephadex G-200 column the two malate dehydrogenases are well separated.

More than 90% of the cytochromes are located in the particulate fraction obtained by centrifugation at 140000g, as shown by analysis. The room-temperature-oxidized versus Na₂S₂O₄-reduced difference spectra of samples of whole and disintegrated cells indicate absorbance maxima at 427, 524 and 558nm and an increase in absorbance at about 625nm. Liquid-N₂ (77°K) spectra of the same samples carried out with a Perkin-Elmer 356 double-beam dual-wavelength spectrophotometer showed an absorbance maximum at 428nm, shoulders at 440 and 521nm and maxima at 528, 535, 556, 561 and 625.5nm, indicating the presence of an *a*-type cytochrome (625.5 and 440nm), a *b*-type cytochrome (561, 535 and 428nm), a *c*-type cytochrome (554, 521 and 428nm) and a *b*- or *c*-type cytochrome (556, 528 and 428nm). Results so far show only one

type of cytochrome *a*. The absorbance maxima suggest it to be an *a*₂-type cytochrome, although further work is necessary to confirm this.

Reduction of oxidized samples by malate also reduces this cytochrome. Experiments are continuing to confirm the idea that in *Moraxella lwoffii* the particulate malate dehydrogenase, succinate dehydrogenase and NADH dehydrogenase have common or similar flavoprotein prosthetic groups linking into the cytochrome chain via ubiquinone.

Jones, M. (1969) M.Sc. Thesis, University of Wales
Jones, M. & King, H. K. (1970) *Biochem. J.* 118, 27P

Defluorination of Fluoroacetate by Lettuce

By P. F. V. WARD and N. S. HUSKISSON (*Department of Biochemistry, Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, U.K.*)

Although fluoroacetate is toxic to most animals, it is relatively non-toxic to plants. It has been isolated from forage crops grown in a fluoride-rich environment (Yu & Miller, 1970), and it seemed worth while to investigate how the compound is metabolized by plants.

A lettuce (*Latuca sativa* L. var. *capitata*) was placed in a closed desiccator with its roots immersed in water. Air was bubbled through the water, and the effluent gas from the desiccator was dried with CaCl₂ and bubbled through 2-methoxyethanol-ethanolamine (2:1, v/v) to trap CO₂. The desiccator was constantly illuminated. Plants were incubated in this apparatus in one of three ways: (1) for 43h with sodium [1-¹⁴C]acetate (1mg/kg wet wt.); (2) for 24h with sodium fluoroacetate (50mg/kg wet wt.) followed by 43h with sodium [1-¹⁴C]acetate (1mg/kg wet wt.); (3) for 43h with sodium fluoro[1-¹⁴C]acetate (50mg/kg wet wt.) (Ward & Huskisson, 1966).

In the [¹⁴C]acetate-treated lettuces 15–20% of the radioactivity was expired as CO₂, and this was unaffected by the presence of non-radioactive fluoroacetate. In the fluoro[¹⁴C]acetate-treated plants only 0.5% of the radioactivity appeared in the CO₂. Citrate concentrations in fluoroacetate-treated plants were about 28mol/g dry wt., almost double those of untreated plants.

The distribution of radioactivity was examined in the fluoro[¹⁴C]acetate-treated plants. It could all be extracted with ethanol-water mixtures (Canvin & Beevers, 1961). Up to 50% of it was associated with unchanged fluoroacetate, about 2% with fluorocitrate and the remainder with two low-molecular-weight acidic compounds. These compounds were purified by ion-exchange and paper chromatography and found to be devoid of fluorine, but contained nitrogen and sulphur. Hydrolysis of one of the compounds

yielded the other compound plus glutamic acid and glycine in equimolar amounts. Mass-spectroscopic analysis showed that the two radioactive compounds were *S*-carboxymethylglutathione and *S*-carboxymethylcysteine.

It appears that lettuce can remove fluorine from fluoroacetate in much the same way that animals remove halogens from some halogenated aliphatic compounds (Thomson *et al.*, 1963). Unlike fluoropyruvate, fluoroacetate does not react directly with cysteine or glutathione; a catalyst such as an enzyme is required.

Canvin, D. T. & Beevers, H. (1961) *J. Biol. Chem.* **236**, 988-995

Thomson, A. E. R., Barnsley, E. A. & Young, L. (1963) *Biochem. J.* **86**, 145-152

Ward, P. F. V. & Huskisson, N. S. (1966) *Biochim. Biophys. Acta* **115**, 515-517

Yu, M. & Miller, G. W. (1970) *Environ. Sci. Technol.* **4**, 492-495

Effects of Early Visual Experiences on the Metabolism of Adenosine 3':5'-Cyclic Monophosphate in Chick Brain

By J. HAMBLEY and S. P. R. ROSE (*Department of Biology, Open University, Walton, Bletchley, Bucks., U.K.*) and P. P. G. BATESON (*Sub-Department of Animal Behaviour, University of Cambridge, Cambridge, U.K.*)

As part of a programme of work trying to elucidate the biochemical events occurring when an organism adapts to a new environmental situation, we have been looking at the effects of exposing 1-day-old dark-reared chicks to an imprinting stimulus. Previous work in our two laboratories (Bateson *et al.*, 1969; Rose *et al.*, 1970) has shown an increase in the incorporation of precursors into both RNA and protein in the forebrain roof 76 and 160 min respectively after onset of the stimulus (a flashing light). Before these effects, after 30 min stimulus exposure, an increase in RNA polymerase activity can be detected in the same region (Haywood *et al.*, 1970). In considering what biochemical events possibly precede this activation, we have measured the concentrations of cyclic AMP (adenosine 3':5'-cyclic monophosphate) in brain regions after short periods of exposure to the stimulus.

A protein-binding assay (Brown *et al.*, 1971) has been used to measure cyclic AMP. The binding protein used was isolated from bovine adrenal cortex. Cyclic AMP was extracted by homogenizing brain tissue in 5% (w/v) trichloroacetic acid and extracting the acid supernatant three times with 10 vol. of water-saturated ether.

As reported for other species (Breckenridge, 1964; Schmidt *et al.*, 1970), there were rapid post-mortem increases in the concentration of cyclic AMP in chick brain, and values as high as 350 pmol/mg of protein have been recorded. Consequently in this study animals were killed by immersion in isopentane, maintained near its freezing point in liquid N₂.

Unexposed dark-reared animals killed by freezing yielded the following values for the concentration of cyclic AMP in various brain regions (means $\pm$ S.D.): forebrain roof, 84.8 ± 20.9 pmol/mg of protein; forebrain base, 57.6 ± 23.7 pmol/mg of protein; midbrain, 59.2 ± 24.3 pmol/mg of protein.

After 15 min exposure to the flashing light there were significant changes in the concentration of cyclic AMP in the forebrain roof and midbrain of experimental animals. The forebrain base showed only a slight increase in cyclic AMP. In the forebrain roof there was a decrease of 55.0 ± 20.0 pmol/mg of protein relative to dark-exposed controls, whereas the midbrain, showed an increase of 33.0 ± 14.6 pmol/mg of protein. These differences are significant at the 0.05 level (*t* test).

This enhancement of the concentration of cyclic AMP in the midbrain was also shown by birds receiving only diffuse overhead light for the same length of time (light-exposed controls). Moreover both light-exposed controls and experimental birds showed increased concentrations of cyclic AMP in the midbrain after only 5 min of stimulation. It would seem that there is a rapid non-specific accumulation of this cyclic nucleotide in this brain region in response to visual activity after the birds had been reared in the dark. The optic tectum is associated with the midbrain. The results for forebrain roof show a different time-course. This, as well as differences between experimental and light-exposed control animals, will be discussed.

Bateson, P. P. G., Horn, G. & Rose, S. P. R. (1969) *Nature (London)* **223**, 534

Breckenridge, B. McL. (1964) *Proc. Nat. Acad. Sci. U.S.A.* **52**, 1580

Brown, B. L., Albano, J. D. M., Ekins, R. P., Scherzi, A. M. & Tampion, W. (1971) *Biochem. J.* **121**, 561

Haywood, J., Rose, S. P. R. & Bateson, P. P. G. (1970) *Nature (London)* **228**, 373

Rose, S. P. R., Bateson, P. P. G., Horn, A. L. P. & Horn, G. (1970) *Nature (London)* **225**, 650

Schmidt, M. J., Palmer, E. C., Dettbarn, W. D. & Robison, G. A. (1970) *Develop. Psychobiol.* **3**, 53