sensitive split-beam spectrophotometer and of ferrieyanide-ferrocyanide mixtures as redox buffers. For cytochrome-559 in pea chloroplasts a value of $E'_0 + 0.37$ v (25°) was obtained at both pH 6.5 and 7.5. The characteristic potential of potassium ferrieyanide was assumed to be $+0.43$ v. For cytochrome *f* a value for E'_0 of $+0.35$ v was obtained with both pea chloroplasts (pH 7.5) and etiolated barley plastids (pH 6.5). This value for bound cytochrome *f* is in good agreement with that obtained by Davenport & Hill (1952) for the soluble component ($E'_0 + 0.365$ v).

The remarkably high potential of cytochrome-559 suggests its possible role as a donor to P-700. It is incompatible with the suggestion that its position in the electron transport chain is close to photosystem 2 (Bendall & Hill, 1968).

Work is in progress on the measurement of the potential of cytochrome *b₆* in chloroplasts. Observations on the kinetics of reduction of cytochrome *b₆* by dithionite have shown that it is a complex of at least two components with α -bands at 559 and 563 m μ respectively. Neither of these components can be reduced by ascorbate. Thus chloroplasts contain two cytochromes with α -peaks at 559 m μ , but these differ in potential by about 350 mv.

I am indebted to the Science Research Council for purchase of the split-beam spectrophotometer.

Bendall, D. S. & Hill, R. (1968). *Annu. Rev. Plant Physiol.* (in the Press).

Davenport, H. E. & Hill, R. (1952). *Proc. Roy. Soc. B*, **139**, 327.

Reversed Electron Transport in Mitochondria from the Spadix of *Arum maculatum*

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A dramatic feature of the flowering process in *Arum maculatum* is a sharp increase in the rate of respiration of the sterile portion of the spadix as the spathe opens. The rapid respiration, which is completely resistant to inhibition by mm-cyanide (James & Beevers, 1950), leads to an increase in temperature of the tissue of the order of 10° above ambient. Mitochondria isolated from the tissue are similarly resistant to cyanide, and contain, in addition to a normal cytochrome oxidase system, a cyanide-resistant oxidase that is as yet unidentified (Bendall & Hill, 1956; Bendall, 1958; D. S. Bendall, B. T. Storey & W. D. Bonner, unpublished work). It has generally been supposed that the cyanide-resistant pathway represents a non-phosphorylating bypass to the normal respiratory chain and therefore is of special importance in the generation of heat. We have been unable to detect phosphorylation during the oxidation of either succinate or malate.

Recent experiments with double-beam and split-beam spectrophotometers in which we studied the kinetics of reduction of cytochromes have shown that these mitochondria do retain some energy-linked functions. In the aerobic steady state with cyanide present cytochromes *a* and *c* were only partially reduced by succinate. Addition of uncoupler allowed complete reduction, which suggested that reversed electron transport occurs rather than inhibited forward transport. This idea was confirmed by the following type of experiment. Addition of ascorbate and *NNN'*-tetramethyl-*p*-phenylenediamine in the presence of cyanide caused complete reduction of cytochrome *a* and *c* components. Subsequent addition of succinate or malate caused partial oxidation of cytochromes *a* and *c* and partial reduction of cytochrome *b*, a state that persisted until the suspension went anaerobic. The reoxidation was prevented by uncoupling concentrations of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone or 2,4-dinitrophenol added immediately before succinate. A high concentration of ADP was not inhibitory, and reoxidation could not be obtained with ATP replacing succinate or malate.

These observations suggest that the cyanide-resistant pathway is capable of driving electron transport against the thermochemical gradient from cytochrome *c*, although the mitochondria have lost the normal ability to interact with ADP and ATP.

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Bendall, D. S. (1958). *Biochem. J.* **70**, 381.

Bendall, D. S. & Hill, R. (1956). *New Phytol.* **55**, 206.

James, W. O. & Beevers, H. (1950). *New Phytol.* **49**, 353.

The Amino Acid Sequence of Cytochrome *c₃* from *Desulfovibrio vulgaris* (N.C.I.B. 8303)

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Cytochrome *c₃*, discovered and purified by Postgate (1956), is found in *Desulfovibrio vulgaris* (Postgate & Campbell, 1966) and other species of sulphate-reducing bacteria. It is similar to the cytochromes *c* of higher organisms in spectrum and molecular weight, but has a very low redox potential (-0.205 v) and has two haem groups per molecule. The amino acid composition of the protein has been determined (Coval, Horio & Kamen, 1961) and the terminal groups have been identified (Bruschi-Heriaud & Le Gall, 1967).

The amino acid sequence of the protein from

Desulfovibrio vulgaris strain Hildenborough (N.C.I.B. 8303) has been investigated, and the structure shown below deduced from peptides isolated from trypsin, chymotrypsin and thermolysin digests:

Ala-Pro-Lys-Ala-Pro-Ala-Asp-Gly-Leu-Lys-Met-Glu-Ala-Thr-Lys-Gln-Pro-Val-Val-Phe-Asn-His-Ser-Thr-His-Lys-Ser-Val-Lys-Cys-Gly-Asp-Cys-His-His-Pro-Val-Asn-Gly-Lys-Glu-Asp-Tyr-Arg-Lys-Cys-Gly-Thr-Ala-Gly-Cys-His-Asp-Ser-Met-Asp-Lys-Lys-Asp-Lys-Ser-Ala-Lys-Gly-Tyr-Tyr-His-Val-Met-His-Asp-Lys-Asn-Thr-Lys-Phe-Lys-Ser-Cys-Val-Gly-Cys-His-Val-Glu-Val-Ala-Gly-Ala-Asp-Ala-Ala-Lys-Lys-Lys-Asp-Leu-Thr-Gly-Cys-Lys-Lys-Ser-Lys-Cys-His-Glu.

The native protein is very resistant to proteolysis, but if the haem is first removed (by reduction with HgCl_2 in 0.1N-HCl-8M-urea) and the apoprotein then oxidized with performic acid, digestion is satisfactory.

The structure contains two sequences of the type -Cys-X-Y-Cys-His-, typical of the haem attachment sites of other cytochromes *c*, but it also contains two sequences of the type -Cys-A-B-C-D-Cys-His-. It has not been possible to react any of the eight cyst(e)ine groups of the protein with alkylating agents when the haem is present, even after reduction with thioglycolic acid in 8M-urea. The relative positions of the cyst(e)ine and histidine residues suggest that the protein consists of two half-sections, each of which binds a haem group. There is little evidence for direct sequence homology between these halves.

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Bruschi-Heriaud, M. & Le Gall, J. (1967). *Bull. Soc. Chim. biol., Paris*, **49**, 753.

Coval, M. L., Horio, T. & Kamen, M. D. (1961). *Biochim. biophys. Acta*, **51**, 246.

Postgate, J. R. (1958). *J. gen. Microbiol.* **14**, 545.

Postgate, J. R. & Campbell, L. L. (1966). *Bact. Rev.* **30**, 732.

Enzyme Changes in Mice under Hypoxic Conditions

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Hypoxia is one of the natural differences in the environment to which the animal must adapt and there is evidence that under hypoxic conditions oxygen utilization by the tissues is increased (Valdivia, 1956). The $p\text{O}_2$ of venous blood is lower in acclimatized humans at high altitude than at sea

level (Hurtado, 1964) and Hurtado *et al.* (1956) found that acclimatized Amerindians had a lower than normal blood lactate and pyruvate after exercise. Increased muscle myoglobin and increased NADH oxidase activity has been found in man at high altitudes (Reynafarje, 1962). Increases in muscle myoglobin and cytochrome, haemoglobin, heart weight and succinate oxidase have been described in guinea pigs kept at high altitudes (Tappan & Reynafarje, 1957; Tappan, Reynafarje, Potter & Hurtado, 1957). There is, however, little work on the enzyme patterns of the tissues of animals kept under controlled conditions of oxygen tension. The animals used in this study were adult male C57 B1 mice. The experimental animals, six in a cage, were maintained in a decompression chamber at 390mm. Hg pressure for up to 1 week. Litter mates were used as controls. Groups of six pairs of animals were killed at 24hr., 48hr. and 1 week. At the end of the experimental period the following enzymes were estimated in the heart, liver, kidney and skeletal muscle: cytochrome oxidase, NADH-cytochrome *c* reductase, succinate dehydrogenase, malate dehydrogenase, α -glycerol phosphate dehydrogenase, lactate dehydrogenase, glucose 6-phosphate dehydrogenase, pyruvate kinase, glutamate-oxaloacetate transaminase and glucose 6-phosphatase. The enzymes of the heart showed no progressive change, but NADH-cytochrome *c* reductase, succinate dehydrogenase, pyruvate kinase and lactate dehydrogenase were all significantly decreased at some stage of the exposure to hypoxia. The enzyme pattern of the liver became less oxidative, but there was no corresponding increase in the activity of the glycolytic enzymes. Glucose 6-phosphatase had only one-tenth of the activity of the controls. It is suggested that the loss of mitochondrial and microsomal enzymes reflects a diminished phospholipid synthesis for membrane formation. In the kidneys there was no loss of oxidative capacity but a slight increase in glucose 6-phosphatase and lactate dehydrogenase activity. There was a marked increase in the glycolytic enzymes of the muscle after 48hr. of hypoxia.

Hurtado, A. (1964). In *Handbook of Physiology, Section 4: Adaptation to the Environment*, chapter 54. Washington, D.C.: American Physiological Society.

Hurtado, A., Velásquez, T., Reynafarje, C., Lozano, R., Chavez, R., Aste-Salazar, H., Reynafarje, B., Sanchez, C. & Muñoz, J. (1956). Rep. 56-1, School of Aviation Medicine, U.S.A.F., Randolph Field, Texas.

Reynafarje, B. (1962). *J. appl. Physiol.* **17**, 301.

Tappan, D. V. & Reynafarje, B. (1957). *Amer. J. Physiol.* **190**, 99.

Tappan, D. V., Reynafarje, B., Potter, V. R. & Hurtado, A. (1957). *Amer. J. Physiol.* **190**, 93.

Valdivia, E. (1956). Rep. 55-100, School of Aviation Medicine, U.S.A.F., Randolph Field, Texas.