REFERENCES

Haworth, W. N. & Hirst, E. L. (1933). *Chem. & Ind.* **52**, 645.
Harowitz, H. H., Doerschuk, A. P. & King, C. G. (1952). *J. biol. Chem.* **199**, 193.
Jackel, S. S., Mosbach, E. H., Burns, J. J. & King, C. G. (1950). *J. biol. Chem.* **186**, 569.
Mapson, L. W. (1953). *Proc. Nutr. Soc.* **12**, 339.
Ray, S. N. (1934). *Biochem. J* **28**, 996.

The Enzymic Conversion of L-Galactono-γ-Lactone to L-Ascorbic Acid by Plant Mitochondria

By L. W. MAPSON, *Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge and Department of Scientific and Industrial Research*

Belief that the synthesis of L-ascorbic acid in vivo does in fact proceed via the following routes:—

D-glucose—⩾ D-glucurono-γ-lactone—⩾ L-gulono-γ-lactone—⩾ L-ascorbic acid

D-galactose—⩾ D-galacturonic methyl ester—⩾ L-galactono-γ-lactone—⩾ L-ascorbic acid

would be greatly strengthened if it were shown that any of these steps could be carried out by enzyme preparations in vitro.

Our initial investigations were therefore planned to determine whether an enzyme extract could be obtained that would catalyse the last stage in this sequence of reactions, i.e. would convert either L-gulono-γ-lactone or L-galactono-γ-lactone to L-ascorbic acid. Germinating seedlings, in which L-ascorbic acid forms rapidly, were used as raw materials, extracts being prepared from them in the early stages of germination when those enzymes that can oxidize ascorbic acid are either absent or show lower activities than in the more fully developed plant.

Success was achieved with extracts in which the mitochondria (cytoplasmic particles) of the cell were preserved intact. Mitochondria prepared from partially germinated pea seedlings were able to convert L-galactono-γ-lactone rapidly to L-ascorbic acid, but could not effect the corresponding conversion of L-gulono-γ-lactone to L-ascorbic acid. The reaction proceeded smoothly until approximately 40% of the lactone had been transformed into L-ascorbic acid, and then ceased, but began again if an additional quantity of the lactone was added. The disappearance of the lactone, apart from that portion converted to L-ascorbic acid, was shown to be due to its conversion into the free galactonic acid, a reaction which proceeds simultaneously with the enzymically catalysed formation of L-ascorbic acid. Since galactonic acid is not itself converted to L-ascorbic acid, this reaction decreases the yield of L-ascorbic acid obtained from the γ-lactone.

D-galactono-γ-lactone, and D-manno-γ-lactone were not converted to L-ascorbic acid under these conditions, but D-altrono-γ-lactone was converted to D-arabo-ascorbic acid, findings which were in agreement with the previous results obtained from studies on the effect of these substances on the formation of ascorbic acid in vivo (Isherwood, Chen & Mapson, 1953); also 2-keto-L-gulonic acid, which has been suggested by some workers (Smythe & King, 1942; Smith, 1952) as a precursor of L-ascorbic acid, was not converted to L-ascorbic acid.

Since the change L-galactono-γ-lactone——⩾ L-ascorbic acid involves the transfer of hydrogen, the presence of a suitable hydrogen acceptor was essential and, of several tried, oxygen appeared to be the most efficient; in its absence there was no synthesis. The proportion of oxygen in the system could be reduced to 0·5% without any significant effect on the rate of the reaction, and not until it was reduced to 0·25% was there any marked retardation.

Further investigations showed that cytochrome oxidase was involved, the reaction being characteristically inhibited in the presence of cyanide and azide, and also by CO in the dark, this inhibition being reversed by light. Inhibitions with both azide and CO were more marked on the addition of either succinate or α-keto-glutarate and this was shown to be due to an increased competition for the available cytochrome. In the absence of inhibitors of cytochrome oxidase the cytochrome system in the mitochondria was more than sufficient to promote the maximum rate of oxidation of the lactone to L-ascorbic acid in addition to its normal role of promoting endogenous respiration. It follows therefore that the overall rate of the formation of L-ascorbic acid from the lactone is determined by the reactions involving the rate of hydrogen transfer from lactone to cytochrome.

No evidence was obtained for the participation of compounds containing high-energy phosphate bonds in the reaction. Arsenate could completely replace phosphate during the procedures used in isolating the mitochondria, and in the reaction mixture itself. ATP when added to the mitochondria in a concentration of 10^{-3}M reduced the rate of synthesis by 40%, an observation for which we have no explanation at present. 2 : 4-Dinitrophenol in concentrations which have been shown by Bonner & Millerd (1953) to suppress completely oxidative phosphorylation by plant mitochondria had no effect on the rate of formation of L-ascorbic acid from the lactone.

Most surprising was our failure during the course of this work to detect any formation of L-ascorbic acid from L-gulono-γ-lactone. It is true that in vivo the conversion of L-galactono-γ-lactone to L-ascorbic acid is about eight to ten times as great as the corresponding conversion of L-gulono-γ-lactone; nevertheless there is no doubt that L-gulono-γ-lactone when added to developing pea seeds does increase the formation of L-ascorbic acid in the tissues. It is an attractive hypothesis, as yet unproven, that synthesis in the plant normally proceeds by way of D-galacturonic acid (or derivatives)——⩾ L-galactono-γ-lactone——⩾ L-ascorbic acid, and that L-gulono-γ-lactone may be enzymically converted to L-galactono-γ-lactone and thence to L-ascorbic acid. Our failure to detect any formation of L-ascorbic acid from L-gulono-γ-lactone might be explained if, during extraction of the mito-

chondria, damage to such an enzyme had occurred. Other explanations are however possible and future work may reveal which is correct.

The work described in this paper was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

REFERENCES

Bonner, J. & Millerd, A. (1953). *Arch. Biochem. Biophys.* **42**, 125.
Isherwood, F. A., Chen, Y. T. & Mapson, L. W. (1953). *Nature, Lond.*, **171**, 348.
Smith, F. G. (1952). *Plant Physiol.* **27**, 736.
Smythe, C. V. & King, C. G. (1942). *J. biol. Chem.* **142**, 529.

Chairman's Summing-up

By LESLIE J. HARRIS, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*

On behalf of The Nutrition Society, I wish to thank very warmly those who have presented such an interesting and authoritative series of communications, and particularly the distinguished experts from overseas who have come specially to Britain in order to participate in the symposium.

High credit is due to the organizers of this most useful, and indeed historic meeting, namely to Dr C. P. Stewart and Dr A. P. Meiklejohn. Everyone present is grateful also to Drs Guthrie and Stewart for having undertaken the re-issue and editing of Lind's *Treatise of the Scurvy*; it has added greatly to the interest of the occasion.

Dr King in his paper has alluded in passing to the period, a good many years ago, when he was a young research worker at the Biochemical Laboratory in Cambridge, England; but he was altogether too generous in mentioning the episode which, I must confess, I had myself now almost completely forgotten, namely the first recorded occasion, apparently, when the suggestion had been put forward that vitamin C might prove to be identical with the then newly-isolated 'hexuronic acid'! I do recall however that, during the period he refers to, there were in that Institute several young American workers, some of whom have since become distinguished leaders in the fields of nutrition and biochemistry, including, for example, C. G. King and C. A. Elvehjem; they were regular attenders at the advanced lecture courses, including incidentally my own on vitamins and nutrition, and what I do distinctly remember is that one of the American guests had, on some occasion, expressed his own preference for the lectures given by Sir Frederick Hopkins: 'The other lecturers', he said, 'tell you all about the dope that is known, but "Hoppy" tells you all about the dope that isn't known!' I would like to-day to take my cue from that last remark, and, in opening the discussion, draw attention to what seem to me to be some of the principal gaps still remaining in our knowledge about vitamin C.