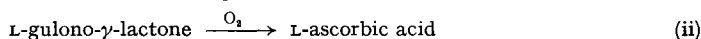
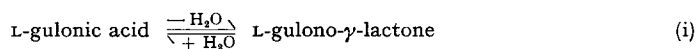


by comparing the stimulation of formation of L-ascorbate from L-gulonate in the presence of microsomes with the aldono-lactonase activity during a 60-fold purification of the factor; the activities were found to be parallel. The purified enzyme catalyzes the accumulation of L-gulonolactone from L-gulonate, measured by conversion to the hydroxamate following addition of alkaline hydroxylamine after the incubation period.

These results, which are consistent with isotopic experiments¹⁰ and with the independent work of YAMADA *et al.*¹¹, indicate the following reaction sequence:



These results thus demonstrate a role for the aldono-lactonase present in liver and kidney of many species^{9,11} and show that L-gulono- γ -lactone is the immediate precursor of L-ascorbate. On the other hand, L-gulonate rather than the lactone appears to be the direct precursor of L-xylulose^{2,12}.

*Department of Physiological Chemistry, The Johns
Hopkins School of Medicine, Baltimore, Md. (U.S.A.)*

CLARK BUBLITZ
ALBERT L. LEHNINGER

¹ M. UL HASSAN AND A. L. LEHNINGER, *J. Biol. Chem.*, 223 (1956) 123.

² C. BUBLITZ, A. P. GROLLMAN AND A. L. LEHNINGER, *Biochim. Biophys. Acta*, 27 (1958) 221.

³ Y. T. CHEN, F. A. ISHERWOOD AND L. W. MAPSON, *Biochem. J.*, 55 (1953) 821.

⁴ J. H. ROE AND C. A. KUETHER, *J. Biol. Chem.*, 147 (1943) 399.

⁵ J. J. BURNS, P. PEYSER AND A. MOLTZ, *Science*, 124 (1956) 1148.

⁶ L. W. MAPSON AND E. BRESLOW, *Biochem. J.*, 68 (1958) 395.

⁷ J. K. PALMER, *Conn. Agr. Exp. Sta. Bull.*, (1955) 589.

⁸ F. A. LOEWUS, R. JANG AND C. G. SEEGMILLER, *J. Biol. Chem.*, 222 (1956) 649.

⁹ J. WINKELMAN AND A. L. LEHNINGER, *J. Biol. Chem.*, 233 (1958) 794.

¹⁰ H. H. HOROWITZ AND C. G. KING, *J. Biol. Chem.*, 205 (1953) 815.

¹¹ K. YAMADA, S. ISHIKAWA AND N. SHIMAZONO, *Biochim. Biophys. Acta*, 32 (1959) 303.

¹² S. ISHIKAWA, *J. Biochem. (Tokyo)*, in the press.

¹³ W. C. SCHNEIDER AND G. H. HOGEBOM, *J. Biol. Chem.*, 183 (1950) 123.

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Transfer of radioactive sulfate from phosphoadenosine phosphosulfate to heparin

The description of a transplantable mouse mast-cell tumor by DUNN AND POTTER¹ suggested the possibility that this mastocytoma could be used for a study of heparin biosynthesis *in vitro*. Homogenates of this tumor were prepared, and incubated with inorganic ³⁵SO₄⁼. The heparin was extracted, and purified by paper chromatography, according to methods recently developed in this laboratory^{2,3,4}. It was observed that, under certain conditions, labeled inorganic sulfate was incorporated into heparin by the tumor homogenate. These results were recently reported in part³. In view of the work by LIPMANN and co-workers on the role played by PAPS in a number of different

Abbreviations: PAPS, 3'-phosphoadenosine-5'-phosphosulfate; DPN, diphosphopyridine nucleotide; ATP, adenosine triphosphate; UTP, uridine triphosphate.

sulfation reactions⁵, the possibility was considered that PAPS was involved in the sulfation of heparin. To determine whether this could be demonstrated in our system, [³⁵S]PAPS was incubated with homogenates of the Dunn-Potter mouse mast-cell tumor, and the heparin was extracted and its radioactivity determined.

Experimental conditions and results are shown in Table I. It is evident that the [³⁵S]PAPS served as a sulfate donor to the heparin. The radioactivity incorporated into heparin, expressed as % of total counts incubated, was about 10–15 times as great in the samples containing [³⁵S]PAPS as in those containing inorganic ³⁵SO₄⁼. These results suggest that PAPS is involved in the sulfation of heparin.

TABLE I
INCORPORATION OF ³⁵SO₄ FROM INORGANIC SULFATE
AND PAPS INTO HEPARIN IN MOUSE MAST-CELL TUMOR HOMOGENATES, *in vitro*

Each incubation sample contained, in a total vol. of 2.7 ml: 0.5 g tumor tissue (wet wt.), 120 μ moles Tris (hydroxymethyl)aminomethane buffer, adjusted to pH 7.4, 27 μ moles MgCl₂, 5.4 μ moles ATP, 2.7 μ moles L-glutamine, 0.27 μ mole UTP, 0.27 μ mole DPN, 4.8 μ moles NaHCO₃, adjusted to pH 7.4, 52 μ moles NaCl, 1.0 μ mole KCl, 0.70 μ mole CaCl₂, 2.2 μ moles D-glucose. In addition, each sample contained either carrier-free ³⁵SO₄⁼ (74·10⁶ counts/min), or 11.3 μ moles [³⁵S]PAPS (11.3·10⁴ counts/min). Incubated at 37° for 5 h.

Expt.	Nature of ³⁵ SO ₄ incubated	Mean radioactivity of heparin extracted from incubation samples			Incorporation %
		Experimental (counts/min)*	Heated control** (counts/min)*	Difference (counts/min)	
I	Inorganic PAPS	80,200 (3)	3,100 (6)	77,100	0.11
		1,980 (2)	130 (2)	1,850	1.6
II	Inorganic PAPS	73,800 (2)	3,100 (6)	70,700	0.10
		1,350 (2)	130 (2)	1,220	1.1

* Figures in parenthesis indicate number of samples incubated.

** The homogenate in control samples was heated to 65° for 20 min, cooled to room temperature, and then treated in the same manner as the homogenate in experimental samples.

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Department of Biochemistry and Nutrition, University of
Southern California, Los Angeles, Calif. (U.S.A.)

L. SPOLTER
W. MARX

¹ T. B. DUNN AND M. POTTER, *J. Natl. Cancer Inst.*, 18 (1957) 587.

² L. FREEMAN, R. POSTHUMA, L. GORDON AND W. MARX, *Arch. Biochem. Biophys.*, 70 (1957) 169.

³ L. SPOLTER AND W. MARX, *Federation Proc.*, 17 (1958) 314.

⁴ L. SPOLTER AND W. MARX, in preparation.

⁵ F. LIPMANN, *Science*, 128 (1958) 575.

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