by comparing the stimulation of formation of L-ascorbate from L-gulonate in the presence of microsomes with the aldonolactonase 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 experiments[10] and with the independent work of YAMADA *et al.*[11], indicate the following reaction sequence:

$$\text{L-gulonic acid} \underset{+\,H_2O}{\overset{-H_2O}{\rightleftharpoons}} \text{L-gulono-}\gamma\text{-lactone} \qquad \text{(i)}$$

$$\text{L-gulono-}\gamma\text{-lactone} \xrightarrow{O_2} \text{L-ascorbic acid} \qquad \text{(ii)}$$

These results thus demonstrate a role for the aldonolactonase 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].

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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 POTTER[1] 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 $^{35}SO_4^=$. 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 part[3]. 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 reactions[5], the possibility was considered that PAPS was involved in the sulfation of heparin. To determine whether this could be demonstrated in our system, [^{35}S]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 [^{35}S]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 [^{35}S]PAPS as in those containing inorganic $^{35}SO_4^=$. These results suggest that PAPS is involved in the sulfation of heparin.

TABLE I

INCORPORATION OF $^{35}SO_4$ 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 μmoles Tris (hydroxymethyl)aminomethane buffer, adjusted to pH 7.4, 27 μmoles $MgCl_2$, 5.4 μmoles ATP, 2.7 μmoles L-glutamine, 0.27 μmole UTP, 0.27 μmole DPN, 4.8 μmoles $NaHCO_3$, adjusted to pH 7.4, 52 μmoles NaCl, 1.0 μmole KCl, 0.70 μmole $CaCl_2$, 2.2 μmoles D-glucose. In addition, each sample contained either carrier-free $^{35}SO_4^=$ ($74 \cdot 10^6$ counts/min), or 11.3 mμmoles [^{35}S]PAPS ($11.3 \cdot 10^4$ 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	80,200 (3)	3,100 (6)	77,100	0.11
	PAPS	1,980 (2)	130 (2)	1,850	1.6
II	Inorganic	73,800 (2)	3,100 (6)	70,700	0.10
	PAPS	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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