TABLE 2. CHROMATOGRAPHY OF 5-AI3AA, 5-AT AND URINARY METABOLITE

Material	Rf		
	System 1	System 2	System 3
Normal mouse urine	no spot	no spot	no spot
5-AI3AA	0·86	0·86	0·71
Urine containing the metabolite	0·80		
5-AI3AA in normal urine	0·80		
Urinary metabolite doubly chromatographed from urine containing the metabolite	0·86	0·86	0·71
5-AI3AA doubly chromatographed from 5-AI3AA in normal urine	0·86	0·86	0·71
5-AI3AA and urinary metabolite doubly chromatographed	0·86*	0·86*	0·71*
5-AT in normal mouse urine	0·60		
5-AT	0·60	0·29	0·36

* Only one spot obtained.

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Elimination and catabolism of ^{35}S-heparin by neoplastic mast cells in culture

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NEOPLASTIC mast cells, both in culture and in the mouse, synthesize histamine, 5-hydroxytryptamine,[1, 2] and heparin.[3] The turnover rate of ^{35}S-sulfate and ^{14}C-glucosamine in heparin was shown to be the same;[4] the results suggest that the turnover of ^{35}S-sulfate in heparin is a measure of the turnover of heparin, but no attempt was made to learn the fate in the cells of heparin. It has been shown that both histamine and 5-hydroxytryptamine are eliminated unchanged by these cells.[5]

X-1 cells, derived from the Dunn-Potter P-815 mastocytoma, were grown in a medium containing 2 mc of ^{35}S-sulfate.[4] The cells and all methods were essentially identical with those used to study the turnover of heparin.[4] The cells were collected by centrifugation and divided into two portions. One aliquot was washed three times with ice-cold 0·9% NaCl and extracted for ^{35}S-heparin;[4] the other was placed in isotope-free medium and incubated. After 24 hr both the medium and cells were collected; the cells were divided into two portions, one of which was washed and extracted for ^{35}S-heparin and the other incubated in isotope-free medium. At the end of the 24-hr period of incubation the heparin of the cells was extracted; in each of the fractions of medium the ^{35}S-heparin also was determined.

Table 1 shows that in the first 24 hr, 268,000 counts/min of ^{35}S-heparin were lost from the cells, and during the second day, 120,000 counts/min of ^{35}S-heparin. Concomitantly, only 31,300 and 16,400 counts/min of ^{35}S-heparin, respectively, were recovered in the medium. The remainder of the radioactive material, comprising 88·3% and 86·4% of the ^{35}S-heparin that was lost from the cell, was dialyzable and may be regarded as degraded heparin.

TABLE 1. LOSS OF ^{35}S-HEPARIN FROM P-815-X-1 MAST CELLS TO MEDIUM

Day	Loss of ^{35}S-heparin from cells	Increase of ^{35}S-heparin in medium
	(counts/min)	
1	268,000	31,300
2	120,000	16,400

The ^{35}S-heparin from both the cells and the medium was chromatographed on Whatmann 3MM paper in 0·04 M ammonium formate: isopropanol (65:35 v/v). After development the paper was cut into 1-cm strips, and radioactivity was measured in a liquid scintillation counter.[4] As shown previously,[4] the ^{35}S-heparin obtained from the cells was resolved into several components, one of which was immobile—i.e. Rf 0·00 (Fig. 1). The ^{35}S-heparin obtained from the medium contained only the immobile component.

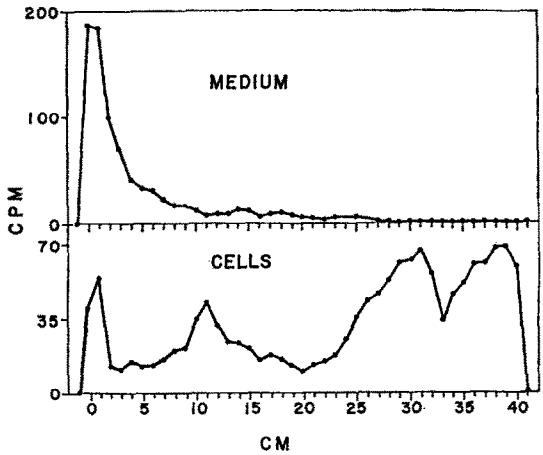

FIG. 1. Paper chromatograms of the ^{35}S-heparin found in the medium and in the cells.

Thus the major portion of the ^{35}S-heparin is catabolized by the cells to dialyzable material and, in addition, a small portion, representing only one of the components of intracellular heparin, is eliminated into the medium. This latter component, which is nitrogen-rich,[6] may be heparin in association with the cytoplasmic granules, which appear to be extruded from normal mast cells.[7]

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The influence of oximes on the acetylthiocholine hydrolysis rate

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IN STUDIES of the reactivation of alkylphosphate-inhibited cholinesterase, using the method of Ellmann *et al.*,[1] we noticed that the presence of oximes in concentrations of 10^{-3}M or more accelerated the non-enzymic hydrolysis of acetylthiocholine. This observation was further established by using the Warburg technique. The simultaneous decomposition of acetylthiocholine by cholinesterase and oxime in reactivated samples therefore might give results showing reactivation exceeding 100 per cent measured by the technique of Ellmann *et al.*

Modification of the original procedure by replacement of enzyme with oxime resulted in a linear relationship between the rate of acetylthiocholine hydrolysis and the oxime concentration. This is demonstrated in Fig. 1. which shows the change in absorbance per minute with different concentrations of pyridine-2-aldoxime-N-methiodide.

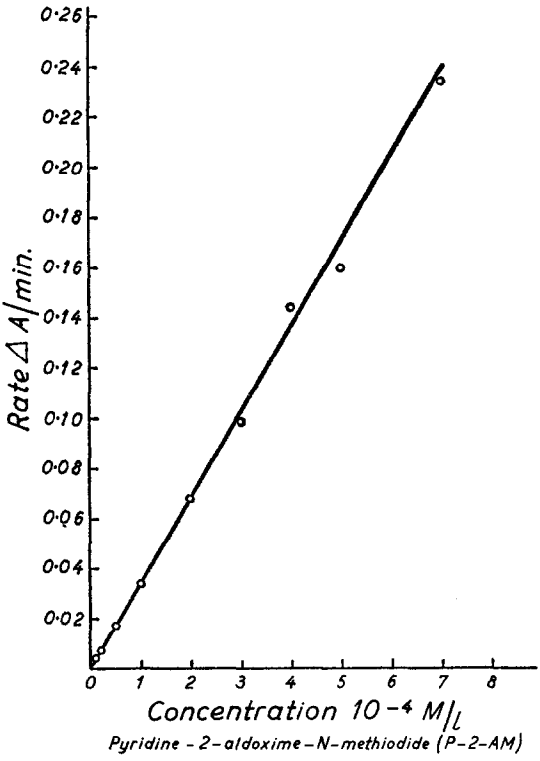

FIG. 1.