ON THE EFFECT OF PYRIDOXAL ON THE TRANSPORT OF AMINO ACIDS IN EHRLICH MOUSE ASCITES TUMOR CELLS (EMAT)

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It was first reported by RIGGS et al. (1953) that pyridoxal stimulates the accumulation of glycine by EMAT cells during an incubation of one hour. This stimulation is maximal at 5 mM pyridoxal (CHRISTENSEN et al., 1954), whereas at higher concentrations pyridoxal inhibits the accumulation of glycine and considerably reduces the cellular level of K-ions. The influx of α-aminoisobutyrate (α-AIB) also increases in the presence of pyridoxal (CHRISTENSEN et al., 1958). So far no appreciable effect of pyridoxal on the influx of glycine has been demonstrated. We found instead that pyridoxal, used as its ethylacetal, while strongly stimulating the accumulation of glycine by these cells, distinctly reduces the efflux coefficient of this amino acid (1961). A depression of the α-AIB efflux by the phosphate derivative of pyridoxal has been reported by CHRISTENSEN (1960). Such observations suggest that pyridoxal acts merely by tightening the cellular membrane without necessarily influencing the active transport directly. The present studies, however, did not confirm this hypothesis and furthermore revealed a distinct but very rapid and transient effect of pyridoxal on the influx of glycine, which had not previously been observed.

Materials and Methods. Glycine and thiourea (Merck), L-methionine, α-AIB and pyridoxal-HCl (Fluka, Schweiz), 1-C^{14}-glycine,

C^{14}-H_3-L-methionine, 1-C^{14}-α-AIB (Amersham, England), and C^{14}-thiourea (Kernreaktor Karlsruhe), were used. The cells were harvested, prepared and incubated as previously described (BITTNER and HEINZ, 1963). The freeze-dried cells were extracted by 0.1 N nitric acid.

Results and Discussion. The effect of pyridoxal on the accumulation of glycine (L-methionine and α-AIB) was found to be highly dependent on the time of administration: The distribution ratio of label shows a distinct maximum after about 3-10 minutes and subsequently drops continuously below the control (Fig. 1,2). The height of this maximum seems to rise with the pyridoxal concentration (Fig. 3). The observed drop of accumulation after

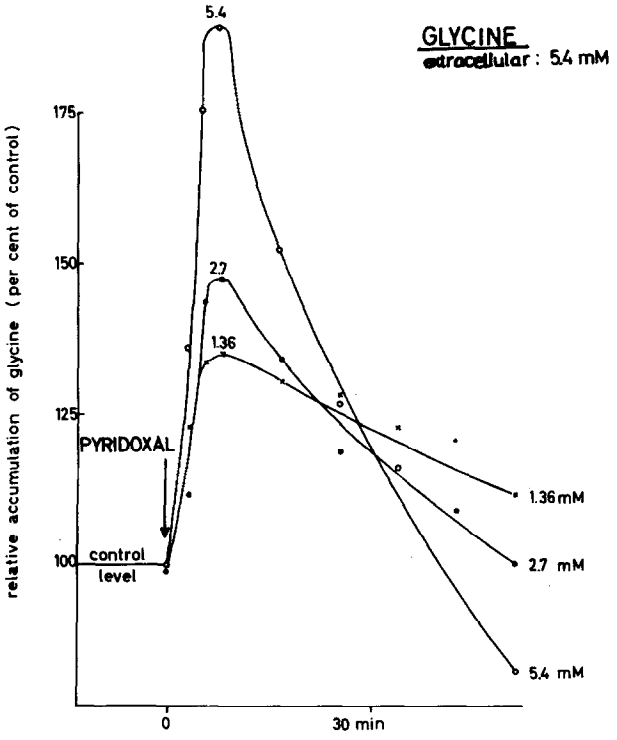


Fig.1: Time dependence of pyridoxal effect on amino acid accumulation in the steady state.

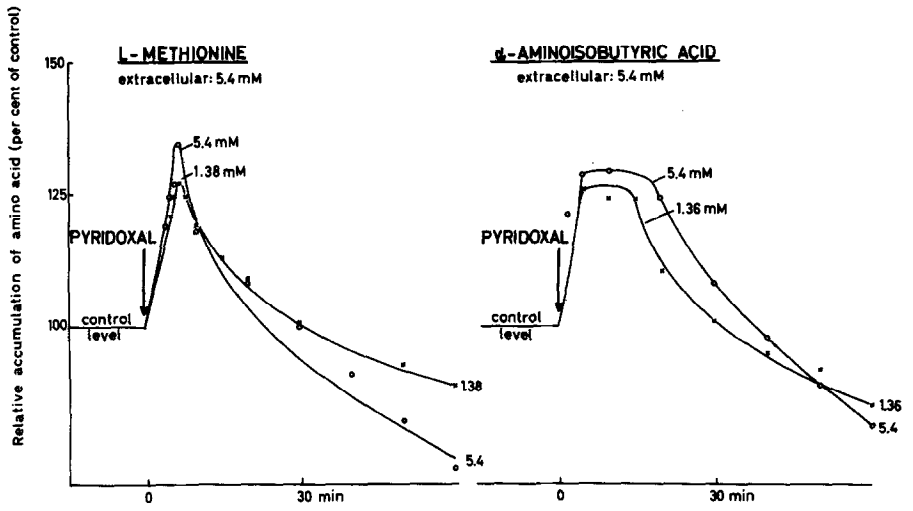
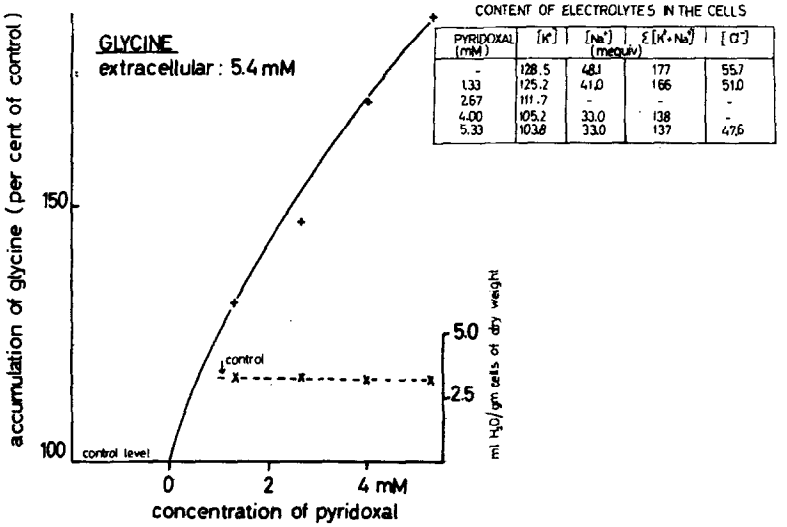


Fig.2: Time dependence of pyridoxal effect on amino acid accumulation in the steady state.



CONTENT OF ELECTROLYTES IN THE CELLS

PYRIDOXAL (mM)	[K⁺]	[Na⁺] (mequiv)	Σ[K⁺+Na⁺]	[Cl⁻]
-	128.5	48.1	177	55.7
1.33	125.2	41.0	166	51.0
2.67	111.7	-	-	-
4.00	105.2	33.0	138	-
5.33	103.8	33.0	137	47.6

Fig.3: The increase of glycine accumulation 10 minutes after addition of pyridoxal at different levels.-As a side effect pyridoxal also reduces the cellular electrolyte concentrations. So at 5.3 mM pyridoxal the sum of K and Na concentrations has dropped by about 23% and the Cl-concentration by about 15% of the control.

longer exposure to pyridoxal is hardly due to a damage of the transport system because a new peak appears upon readdition of pyridoxal (Fig. 4). Pyridoxal does not seem to alter the passive

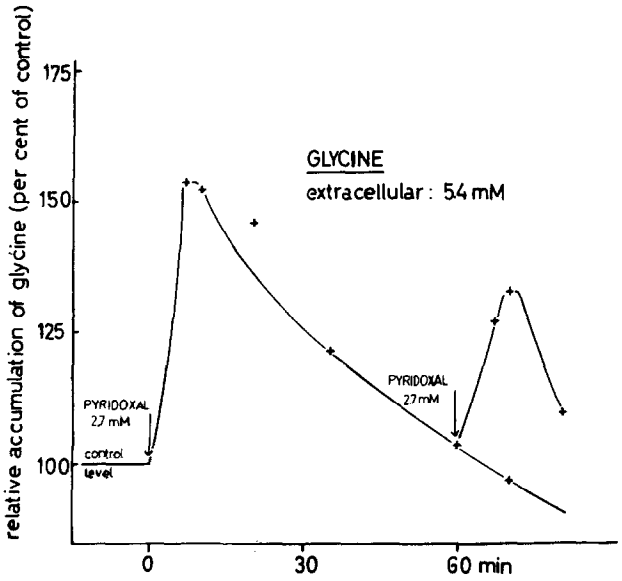


Fig.4: Time dependence of pyridoxal effect on amino acid accumulation in the steady state.

permeability of the cellular membrane, since it does not affect the uptake of thiourea which resumably penetrates the cellular membrane passively (Fig. 5). The initial peak of glycine uptake

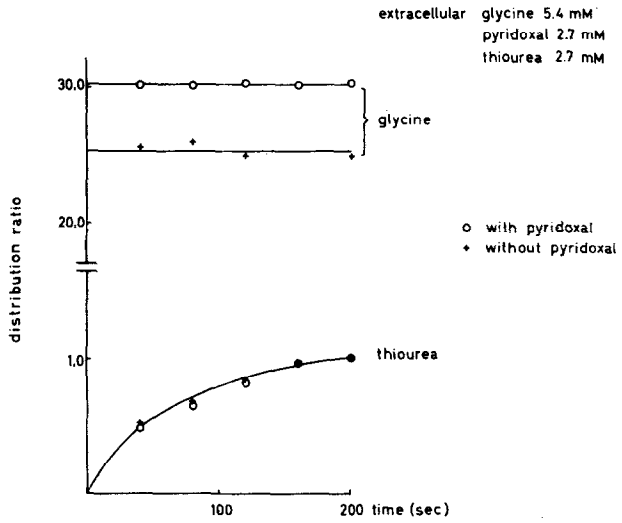


Fig.5: Uptake of C^{14}-thiourea with and without pyridoxal by 20^{o}, preincubation for 30 minutes at 37^{o} with glycine and pyridoxal.

upon pyridoxal administration was found to be mainly due to an acceleration of the influx which afterwards drops below the control (Fig. 6). Pyridoxin and pyridoxal phosphate were much less effective than pyridoxal, and desoxypyridoxin (5.4 mM) was ineffective.

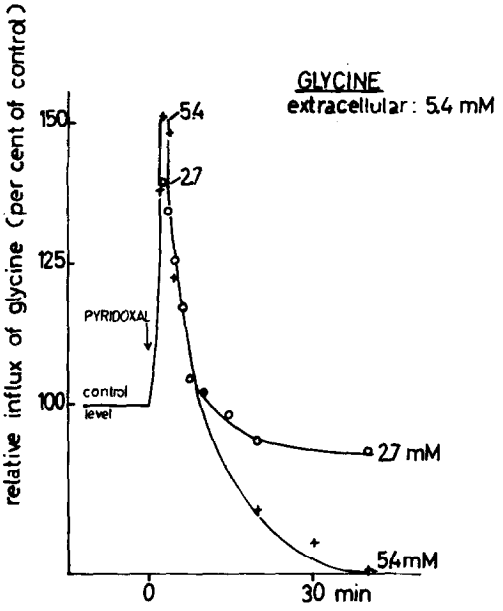


Fig.6: Effect of pyridoxal on the influx.

In agreement with CHRISTENSEN (1960) we found that agents blocking carbonyl groups do not alter the accumulation of amino acids in EMAT cells. In the presence of isonicotinic acid hydrazide (5.4 mM), however, the peak of glycine accumulation, caused by pyridoxal (1.35 mM) was reduced to about 50% of that with pyridoxal alone.

The above results cannot be fully explained at the present time. They suggest that pyridoxal, if it reacts with the transport mechanism, is rapidly destroyed or inactivated within the cell. Another possibility would be that pyridoxal acts as a carrier by-itself, but does so only as long as the gradient of

pyridoxal between the medium and the intracellular space is favorable. In this case, however, one would expect that each pyridoxal molecule entering the cell could at the most carry one amino acid molecule. The net movement of pyridoxal into the cell, however, seems to be too small to account for the total increase of glycine uptake.

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