

0.3 mm and an experimental anion exchange resin kindly put at our disposal by Pharmacia Fine Chemicals, Uppsala, Sweden. This resin had been prepared from cross-linked dextran (Sephadex) by introduction of quaternary ammonium groups. The particle size was about 35 μm and the exchange capacity 2.7 mequiv./g. The cation exchange resins were the same as those used previously.⁴

Aqueous solutions of carbon disulfide were prepared by passing nitrogen saturated with carbon disulfide into a flask with water. Wet anion exchanger (20 g) obtained by careful suction was stirred with 100 ml of the solution at 20°. After various lengths of time the solution was freed from resin and analyzed by passing nitrogen through the solution with subsequent absorption of the carbon disulfide in M sodium ethoxide solution in ethanol. The carbon disulfide was determined spectrophotometrically as ethyl xanthate at 301 nm.⁷ The variations in the water contents of various ionic forms of the same resin⁸ have only a slight influence upon the results and no corrections have, therefore, been applied.

The cation exchange resins were dried before the experiments in which 12 g of dry resin were added.

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Transport of B-Vitamins in Microorganisms

VI. The Non-specificity of the Effect of Exogenous ATP on the Uptake of Labelled Thiamine by Non-proliferating Thiamine Deficient Cells of *L. fermenti*. A Reappraisal

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It was observed earlier¹ that exogenous ATP stimulated, in certain cases, the uptake of ³⁵S-thiamine by thiamine deficient non-proliferating cells of *L. fermenti*, but was without effect on normal cells. The stimulating effect was observed only with severely thiamine deficient cells (thiamine content in the growth medium 1 $\mu\text{g}/\text{ml}$) employing in the incubation mixture 20 mM ATP, 0.01–0.02 M potassium phosphate pH 5.5, 10^8 – 10^{10} cells/ml and certain other components.

Results of comparative studies on the permeability of thiamine depleted and normal cells towards a number of compounds other than thiamine did not indicate any generally increased permeability of the deficient cells.² It did not seem probable, therefore, that ATP had easier access to the thiamine depleted cells than to the normal ones.

It was found, during subsequent studies on thiamine transport in *L. fermenti*, that the effect of ATP did not always take place when the conditions of the incubation with labelled thiamine were different from those employed in the originally reported experiments.¹ For example, changing the pH and/or buffer from the originally employed potassium phosphate pH 5.5 to pH 6.8 or to Tris-HCl pH 6.8 gave variable results with respect to the ATP effect. Experiments were therefore undertaken to investigate whether or not the stimulation by ATP of thiamine uptake was a true energy effect. In these experiments the effect of ATP was compared with that of AMP at equimolar concentrations. Certain representative results are shown in Table I. It can be seen that the effect of ATP varied with the buffer and the pH employed.

Table 1. The effect of ATP and AMP on the ^{14}C -thiamine uptake by non-proliferating thiamine deficient cells of *L. fermenti*. Incubation mixture: sodium chloride 0.15 M, ascorbic acid 0.03 M, MgCl_2 0.02 M, variable buffer 0.02 M, ATP or AMP 0.02 M, 10^8 cells/ml, ^{14}C -thiamine 2×10^{-6} M, total volume 5 ml. Incubation for 20 min at 37°C . Radioactivity counted by the liquid scintillation method.³

Buffer	Relative uptake of radioactivity			
	In the absence of exogeneous energy	In the presence of ATP ^a (Sigma)	ATP ^a (Fluka)	AMP ^b (Sigma)
K-phosphate pH 5.5	100	250	190	220
K-phosphate pH 6.8	100	40	120	140
Tris-HCl pH 6.8	100	115	127	110
Tris-HCl pH 6.8, without MgCl_2	100	270	276	220

^a disodium salt

^b sodium salt

In certain cases a depression of the thiamine uptake was observed instead of a stimulation. There were also certain differences between the preparations of ATP obtained from Sigma and from Fluka. The effect of AMP was, in most cases, essentially similar to the effect of ATP, except when ATP exerted a depressing effect on the uptake. It must therefore be concluded that the stimulating effect of ATP, observed under certain conditions, does not depend on this compound as an energy carrier. Rather, it seems that the stimulating effect may depend on some contaminant, which is presumably present both in the ATP and in the AMP preparations. It is interesting to note in this connection a report by Lorand *et al.*⁴ according to which different commercial preparations of crystalline ATP disodium salt were hydrolyzed to widely varying degrees by myofibrillar ATPase. The authors suggest that the observed variations may depend on the varying Ca^{2+} content of the respective preparations.

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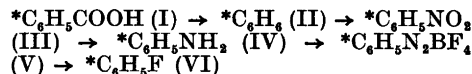
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Preparation of a Mixture of Mono- ^{13}C -fluorobenzenes

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The preparation of the statistical mixture of 1-, 2-, 3-, and 4- ^{13}C -fluorobenzenes has been performed according to the following scheme:



$^{13}\text{C}-\text{C}_6\text{H}_6$: Starting from 1.969 g (15 mmoles) of (I) (55 % enriched in ^{13}C) 1.11 g (14.3 mmoles) of (II) was obtained using the method of Ref. 1.

$^{13}\text{C}-\text{C}_6\text{H}_5\text{NO}_2$: 1.11 g (14.5 mmoles) of (II) was nitrated by the method of Ref. 3 using a mixture of conc. HNO_3 (1.35 ml) and conc. H_2SO_4 (1.35 ml) at 50° , (optimal temp.). After washing with 3×300 ml NaOH (1 M) and 3×300 ml H_2O and drying over 0.2 g anhydrous CaCl_2 , the product was distilled at 22°C using vacuum-line technique. The fraction in the vapour pressure range from 1.5–0.6 mm Hg consisted of 1.47 g (12 mmoles) of (III).

$^{13}\text{C}-\text{C}_6\text{H}_5\text{NH}_2$: 0.86 g (7.0 mmoles) of (III) was dissolved in 10 ml methanol. 150 mg 10 % Pd on C was added. While continuously stirred this mixture absorbed 470 (calc. 500) ml H_2 in 3 h. 0.5 g molecular sieves 'A5' was now added to remove water. After the removal of methanol *in vacuo* the residue was distilled on the vacuum-line. The fraction in the vapour pressure range from 2.0–0.9 mm Hg at