RELIEF BY γ-IRRADIATION OF LYSINE INHIBITED DIAMINOPIMELATE SYNTHESIS IN AN *ESCHERICHIA COLI* AUXOTROPH

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ABSTRACT

Exposure of washed cells of a lysinehistidine *E. coli* double auxotroph (ATCC 13002) to ionizing radiations (34·7 k rad) reduces the inhibitory action of L-lysine on diaminopimelate synthesis from sucrose. A possible explanation of this phenomenon is presented.

INTRODUCTION

IT is well known that L-lysine inhibits the formation of diaminopimelic acid (DAP) in cultures of *E. coli* lysine auxotrophs[1,2]. This action may be attributed to repression of the synthesis and inhibition of the action of the allosteric aspartokinase III, an early enzyme in the biosynthetic pathway[3]. Lysine (250 μg/ml) is also strongly inhibitory to DAP synthesis by washed cells of *E. coli* ATCC 13002[4], a mutant which is auxotrophic for lysine and histidine. Since this mutant requires both of these amino acids for growth it has been possible to examine, in a histidine-free medium, the inhibitory action of lysine on DAP synthesis by cells in a situation in which they are unable to grow.

Knowledge that the regulatory site of certain allosteric enzymes[5,6] is considerably more sensitive to X-irradiation than the catalytic site prompted us to examine the possibility that irradiation of cells of *E. coli* ATCC 13002 might modify the inhibitory action of lysine on DAP synthesis in this organism.

MATERIALS AND METHODS

Washed cell suspensions: Late logarithmic cells from 20 h shaken cultures grown at 26° C in Sucrose-Salts medium[6] containing L-histidine (25 mg/l) and L-lysine (250 mg/l) were harvested by centrifugation at 1350 g for 10 min, washed twice by centrifugation with sterile salts medium and resuspended in salts medium.

Irradiation technique: Cell suspension were saturated with oxygen and exposed to radiations from a ^{60}Co source at a dose rate of 6·95 kR/min. Irradiated suspension were stored in ice prior to use.

Experiments with washed cells: Irradiated and unirradiated cells were suspended in sucrose-salts medium containing $CaCO_3$ (0·5% w/v) to buffer the medium; lysine was added as indicated, Incubation was at 37° C, in shaken flasks.

Analyses: Literature methods were used for the estimation of biomass, DAP[7] and the uptake of ^{14}C-lysine[8].

RESULTS AND DISCUSSION

The inhibitory effects of lysine (100 μg/ml) on the production of DAP by cells irradiated with 34·7 kR (cell viability reduced to *ca.* 1%) and by control cells were compared (Table I). The irradiated cells produced less extracellular DAP presumably owing to general radiation damage to enzyme systems. However, DAP synthesis was clearly less sensitive to lysine in these cells than in unirradiated non-growing control cells.

To gain an understanding of the nature of this effect the abilities of the two types of metabolizing cells to transport lysine were compared (Fig. 1, Table II). The results show that smaller amounts of lysine are taken up by irradiated cells which is reflected in a lower level of trichloracetic acid-soluble lysine in

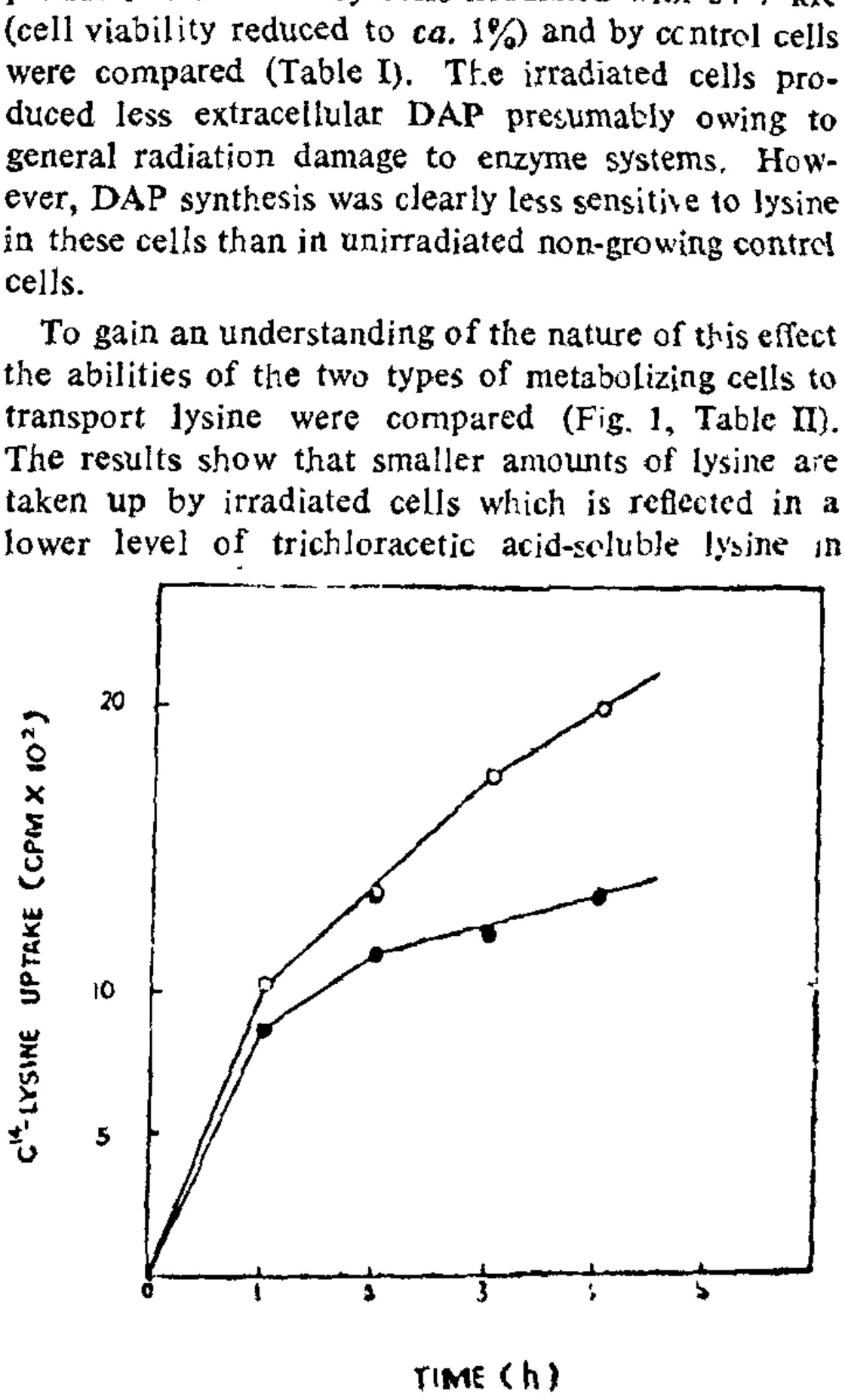

FIG. 1. Uptake of ^{14}C-lysine by irradiated and unirradiated metabolizing cells of *E. coli* ATCC 13002 Cells were irradiated with 20·8 kR (—●—●—) Unirradiated control cells (—○—○—).

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TABLE I

Inhibitory effect of L-lysine (100μg/ml) on the yield of extracellular DAP produced by irradiated and unirradiated cells of E. coli *ATCC* 13002

		2 h		5 h		20 h	
		DAP (μg/mg cell mass)	Inhibition %	DAP (μg/mg cell Mass)	Inhibition %	(μg/mg cell mass)	Inhibition %
Unirradiated Cells	Control	243		353		522	
	+Lysine	12	95·1	37	89·5	302	42·2
Irradiated Cells	Control	186		262		391	
	+Lysine	26	86·0	140	46·6	291	25·6

Cells are irradiated with 34·7 k R. (Results are averages of two independent determinations)

TABLE II

Distribution of ^{14}C *in cells and cell fractions of irradiated and unirradiated* E. coli *ATCC* 13002 *following uptake of* ^{14}C*-lysine*

Time of exposure to ^{14}C-lysine	Unirradiated cells			Irradiated cells		
	Total radio-activity in cells (cpm)	Radioactivity in trichloracetic acid (TCA)-insoluble fraction of cells (cpm)	Related lysine pool size (cpm)	Total radio activity in cells (cpm)	Radioactivity in TCA-insoluble fraction of cells	Related lysine pool size (cpm)
(*h*)	(*a*)	(*b*)	(*a*−*b*)	(*a*)	(*b*)	(*a*−*b*)
1	1499	370	1129	910	266	644
2	1991	513	1478	1220	354	866

Cells were irradiated with 34·7 k R and added to sterile sucrose-salts medium containing $CaCO_3$ (0·1% w/v) and L-lysine (100 ug/ml)

these cells. It thus seems likely that the reduced inhibitory effect of lysine on the production of DAP by irradiated cells is due, at least in part, to the less ability of these cells to concentrate lysine in the internal amino acid pool although the possibility of concomitant radiation damage to the regulatory site of the allosteric aspartokinase III cannot be excluded. It is perhaps worthy of note that x-irradiation of *Neurospora crassa* conidia has been shown to reduce the transportation of L-phenylalanine into these conidia[9].

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