INCORPORATION OF RADIOACTIVE GLUCOSE IN THE EXTRACELLULAR PENICILLINASE OF *STAPHYLOCOCCUS AUREUS*

POLLOCK AND KRAMER[1] showed that amino acids are the main precursors in the biosynthesis of extracellular penicillinase by *Bacillus cereus* and that the exopenicillinase was not derived from the endopenicillinase. The possibility that glucose may serve in the synthesis of the extracellular penicillinase by *Staphylococcus aureus* No. 72, a strain known to produce exopenicillinase as inducible enzyme[2], was examined and the results are reported in this communication.

Staphylococcus aureus No. 72 incubated in 100 ml peptone water (peptone, 1% and sodium chloride, 0·5% and adjusted to pH 7·4) in 250 ml Erlenmeyer flasks was incubated on a wrist-action shaker maintained at 37° C for 24 hr. To this were added 1 ml of uniformly labelled ^{14}C-glucose (carrier-free) with a specific activity of 0·05 mc/ml (22 mc/mM of glucose) and benzyl penicillin ('Crystopen' Glaxo) of 3,000 I. U/ml and again incubated for 3 hr.

The culture was centrifuged at 10,000 rpm at 4° C for 20 min and the cell-free supernatant was decanted. The presence of the enzyme was tested by a modified procedure of Foley and Perret[3]. A drop of the enzyme preparation was placed on a strip of Whatman No. 1 filter-paper previously soaked in 2% starch solution and was air-dried. Over this drop, another drop of benzyl penicillin was added and the paper strip incubated at 37° C for 20 min. Subsequently, the paper strip was dipped in Gram's iodine solution. Appearance of a colourless zone, where the drops were placed, surrounded by a blue background indicated the presence of the enzyme penicillinase in the enzyme preparation.

The enzyme was purified and concentrated by adsorption on cellulose phosphate powder following the method of Richmond[2]. To 100 ml of the cell-free supernatant, 0·5 g cellulose phosphate (Sigma) was added and stirred for 1 hr at room temperature. The cellulose phosphate was spun down at 10,000 rpm at 4° C for 20 min and the sediment washed twice with distilled water. The enzyme adsorbed on cellulose phosphate was eluted with 30 ml of 2 M *Tris*-HCl buffer (PH 7·5) and by washing the adsorbant twice with 10 ml portions of the buffer each time and the eluates pooled. The enzyme activity, as well as the radioactivity incorporated in the enzyme preparation were determined at various stages of the experiment. A gas-flow Proportional Counting System (Electronics Corporation of India) was used in determining the radioactivity. The results are presented in Table I.

TABLE I

Incorporation of ^{14}C-labelled glucose in the exopenicillinase of Staphylococcus aureus *No. 72*

Sample	Enzyme activity	Radioactivity	
		CPM/ml	S.D.
Culture liquid (with cells)	+	19,227	± 118
Culture–filtrate (cell–free)	+	7,580	± 58·2
Enzyme preparation (purified)	+	105	± 12·8

+: Presence of activity.
CPM: Counts per minute.
S.D.: Standard deviation.

There was clear indication of the labelled ^{14}C-glucose, supplied in the medium, getting incorporated in the cells and in the extracellular penicillinase, although only a fraction of the activity supplied in the medium was recovered in the enzyme itself. Presumably glucose supplied in the medium may act as a precursor in the biosynthesis of extracellular penicillinase by *S. aureus*, after its conversion into intracellular amino acids, though not directly.

Radio-tracer Lab.,
University of Agri.
Sciences,
Bangalore-24, *April* 13, 1973.

S. KRISHNASWAMY.
B. V. GANTOTTI.
A. BALASUBRAMANIAN.

1. Pollock, M. R. and Kramer, M., *Biochem. J.*, 1958, 70, 665.
2. Richmond, M. H., *Ibid.*, 1963, 88, 452.
3. Foley, J. M. and Perret, C. J., *Nature, London*, 1962, 195, 287.