38. GLYOXALASE OF *BACTERIUM COLI*

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NEUBERG & GORR [1925] observed that *Bact. coli*, both in culture and when dried, caused the conversion of methylglyoxal into lactic acid. Using yeast and *B. pasteur*, Lohmann [1932] investigated the properties of glyoxalase, the enzyme concerned, and determined the nature of its coenzyme or prosthetic group.

Cell-free extracts of *Bact. coli* prepared with the wet-crushing mill of Booth & Green [1938] contained an active glyoxalase. The following results were obtained with such extracts.

I. *Preparation.* *Bacterium coli commune* Escherich (National Collection of Type Cultures No. 86) was the source of the enzyme. Extracts were prepared as previously described [Still, 1941]. The extracts were used without heating or fractional precipitation, and retained their activity for 10 days without appreciable loss if kept at 0°.

II. *Methods.* The activity of the enzyme was measured in Warburg manometers by following the liberation of CO₂ from NaHCO₃-CO₂ buffer at pH 7.2. The methylglyoxal was prepared from 'Oxantin' by distillation in the presence of H₂SO₄ and estimated by the bisulphite fixation method of Clift & Cook [1932].

III. *Coenzyme.* The extract as prepared above showed very little activity, and this could be almost completely eliminated by dialysis against running water for 12 hr. (Table 1).

Table 1. *Effect of dialysis on glyoxalase in extract*

Manometers containing 1.0 ml. extract, 0.4 ml. M/10 NaHCO₃, 0.4 ml. 0.23 % methylglyoxal. Total volume 3.2 ml.

	μl. CO ₂ /31 min.
Extract undialysed, no substrate	1.5
Extract undialysed, + substrate	25.0
Extract dialysed, no substrate	4.5
Extract dialysed, + substrate	2.0
No enzyme, + substrate	2.0

Addition of reduced glutathione more than restored the activity present before dialysis (Table 2).

Table 2. *Effect of GSH on activity of extract*

Manometers containing 1.0 ml. extract, 0.4 ml. M/10 NaHCO₃, 0.4 ml. 0.47 % methylglyoxal and 3.0 mg. GSH. Total volume 3.2 ml.

	μl. CO ₂ /20 min.
Extract undialysed, no substrate	3.0
Extract undialysed, + substrate	190.0
Extract dialysed, no substrate	3.5
Extract dialysed, + substrate	143.5
Extract dialysed, + substrate, no GSH	6.0

Oxidized glutathione in equivalent amounts did not replace GSH as coenzyme. However, an equivalent amount of cysteine brought about a considerable

increase in the glyoxalase activity of the dialysed extract (Tables 3 and 4). Lohmann [1932] showed that with his yeast preparations no other SH-compound could replace GSH as coenzyme for glyoxalase. No explanation is offered for the action of cysteine in this instance.

Table 3. *Effect of concentration of GSH*

Manometers containing 1.0 ml. dialysed extract, 0.4 ml. $M/10$ NaHCO_3 , 1.7 mg. phenylglyoxal. Total volume 3.2 ml.

GSH mg.	$\mu\text{l. CO}_2/17$ min.
—	26.0
0.25	60.5
0.5	70.5
1.0	85.0
2.0	96.0
3.0	88.0

Table 4. *Effect of GSH, GSSG and cysteine*

Enzyme	Additions to manometers (ml.)				
	0.8	0.8	0.8	0.8	0.8
$M/10$ NaHCO_3	0.4	0.4	0.4	0.4	0.4
Phenylglyoxal	0.4	0.4	0.4	—	0.4
GSH 2 mg./ml.	1.4	—	—	—	—
GSSG 4 mg./ml.	—	1.4	—	—	—
Cysteine-HCl 1 mg./ml.	—	—	1.0	1.0	—
Water	0.2	0.2	0.6	1.0	1.6
CO_2 output, $\mu\text{l.}/15$ min.	202	9.5	127.5	3.0	33

IV. *Substrates.* Glyoxalase attacks several keto-aldehydes. The bacterial enzyme decomposed both methyl- and phenyl-glyoxals. Hayashi [1929] reported that *Bact. coli* attacked phenylglyoxal much less rapidly than methylglyoxal. This did not appear to be the case with the extracted enzyme.

Under suitable conditions the reaction proceeds to completion and the acid can be estimated. In one such experiment 540 $\mu\text{l. CO}_2$ were evolved, representing 92.5 % of theory. Lactic acid was estimated by the method of Friedemann & Graesser [1933]; 2.08 mg. were found, 2.17 mg. being the amount expected.

SUMMARY

Cell-free extracts of *Bact. coli* contain the enzyme glyoxalase, with properties essentially the same as those of the enzyme obtained from *B. pasteur* and yeast.

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