LXVII. COENZYME-LINKED REACTIONS INVOLVING *l*(+)GLUTAMIC DEHYDROGENASE

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l(+)Glutamate in the presence of its dehydrogenase reduces coenzyme I [Dewan, 1938; Euler *et al.* 1938]. Reduced coenzyme in turn is oxidized enzymically by substrates such as aldehyde, acetoacetate, pyruvate and oxaloacetate [Green & Dewan, 1937; Euler *et al.* 1936; 1937]. The possibility of linkages between glutamate and these systems via coenzyme I would therefore seem likely. Adler *et al.* [1937] have linked the glutamate and alcohol dehydrogenase systems, and Dewan [1938] the glutamate and β-hydroxybutyrate systems.

This present communication describes coenzyme-linked reactions between the *l*(+)glutamic dehydrogenase system on the one hand and the malic and lactic systems on the other.

I. Preparation of the dehydrogenases

A very active preparation of glutamic, malic and lactic dehydrogenases may be obtained from pig heart muscle in the following manner. Two pig hearts are divested of fat and connective tissue, minced in a Latapie, washed 5 times with tap water and ground in a mortar with sand and 250 ml. *M*/25 phosphate buffer *p*H 7·2 for 1½ hr. The thick homogeneous paste is mixed with 350 ml. of the same buffer and centrifuged 5 min. to remove sand and cellular debris. The supernatant is brought to *p*H 4·6 (slightly yellow to chlorophenol red) with 10% acetic acid and centrifuged for 10 min. The precipitate is rubbed up with distilled water and again centrifuged 10 min. It is finally suspended in 70 ml. *M*/5 phosphate buffer *p*H 7·2. The preparation keeps its activity for about 5 days.

II. Methods of estimation

Since oxidation of *l*(+)glutamate results in the production of NH_3 and α-ketoglutarate [Euler *et al.* 1938; Dewan, 1938] the oxidation can be followed by estimation of NH_3 (Parnas method).

Lactic and malic acids were estimated by the manometric method described by Dewan & Green [1937].

III. Reaction between glutamate and pyruvate

The reactions were carried out anaerobically in Thunberg tubes at 38° for 1½ hr. Table I shows that glutamate is oxidized by pyruvate only in the presence of the enzymes and coenzyme. Lactic acid was also found only when the whole system was present. Table II shows that the lactic acid found was in close agreement with the theoretical amount calculated on the assumption that 1 mol. of lactic is formed for each mol. of NH_3 produced.

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Table I. *Controls for glutamate-pyruvate oxidoreduction*

Heart enzyme (ml.)	2·0	2·0	2·0	2·0	—
0·3% coenzyme I (ml.)	1·0	1·0	1·0	—	1·0
M/3 *l*(+)glutamate (ml.)	0·5	0·5	—	0·5	0·5
M pyruvate (ml.)	0·3	—	0·3	0·3	0·3
Water (ml.)	—	0·3	0·5	1·0	2·0
NH_3 (mg.)	0·15	0·01	0	0	0

Table II. *Ratio of observed to theoretical lactic acid calculated from NH_3 produced*

	Lactic acid in μl. O_2	Theory	Ratio
(1)	60	66	0·96
(2)	82	78	1·1
(3)	98	106	0·93

Table III. *Controls for the glutamate-oxaloacetate oxidoreduction*

Heart enzyme (ml.)	2·0	2·0	2·0	2·0	—
0·3% coenzyme I (ml.)	1·0	1·0	1·0	—	1·0
M/3 *l*(+)glutamate (ml.)	0·5	0·5	—	0·5	0·5
M/3 oxaloacetate (ml.)	0·3	—	0·3	0·3	0·3
Water (ml.)	—	0·3	0·5	1·0	2·0
NH_3 (mg.)	0·18	0·02	0	0	0

Table IV. *Ratio of observed to theoretical malic acid*

	Malic acid in μl. O_2	Theory	Ratio
(1)	122	114	1·1
(2)	86	72	1·1
(3)	88	102	0·9

IV. Reaction between glutamate and oxaloacetate

Oxaloacetate produces similar results when used as oxidizing agent (cf. Table III). Malic acid was also found only when the complete system was present. Table IV shows that the malic acid produced was approximately equal to the theoretical amount calculated on the assumption that 1 mol. is produced for each mol. of NH_3.

Oxaloacetic acid tends to decompose at 38° to pyruvic acid and CO_2. There was therefore the possibility of pyruvic acid acting as oxidizing agent. This was not so, however, since lactic acid was not formed under the conditions of the experiment.

Summary

The anaerobic oxidation of *l*(+)glutamate by pyruvate and oxaloacetate via coenzyme I has been described.

References

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