Some Factors Influencing Enzymic Activities of *Corynebacterium creatinovorans.* (18041)

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Introduction. Dubos and Miller(1) isolated a soil bacterium, later named *Corynebacterium creatinovorans* (Strain "NC"), which could be adapted to grow on creatinine as its sole source of carbon and nitrogen. Krebs and Eggleston(2) found that the enzymes in this organism responsible for the oxidation of creatinine, lactose and malic acid were totally adaptive (according to Karström) (3), whereas those responsible for the oxidation of uric acid, amino acids and lactic acid were partly adaptive and the enzymes responsible for the oxidation of glucose or glycerol were constitutive. As a result of work with this organism on the oxidation of acetate (4) and the adaptive nature of its acetate oxidation system(5), we became interested in some of its other metabolic activities, some results of which are presented here.

Methods. Corynebacterium creatinovorans was grown in media consisting of a carbon source (either glucose, glycerol or acetate), 0.8%; $(NH_4)_2SO_4$ or NH_4Cl, 0.2%; NaCl, 0.4-0.5%; yeast extract, 0.1%; phosphate buffer, pH 7.0, 0.005 M, plus minerals in the following amounts per liter of medium: 3% $MgSO_4$, 1.5 ml; 1% $CaCl_2$, 5 ml; 1% Na_2CO_3, 4 ml; 1% $NaHCO_3$, 2 ml; 0.1% $MnSO_4$, 1 ml; 0.01% HNO_3, 1 ml; 0.1% $FeCl_3$, 0.3 ml. The medium was aerated, and after 24 hours' growth at approximately 28°C, the cells were harvested, washed twice and finally suspended in distilled water. The yield of cells was generally about 4 g, wet weight, per 2 liters of medium. Dry weight determinations were obtained on all cell suspensions. Standard Warburg technic was used, all reaction vessels being buffered with 0.01 M phosphate buffer at pH 7.0, with KOH in the alkali well. The results are expressed as Q_{O_2}, or μl O_2/mg dry weight of cells/hour. Blank Q_{O_2} values (usually 8-16) have been subtracted.

Results. Following the recommendations of Karström(3) and Gale(6) that an adaptive enzyme is one whose production is markedly increased (at least 5-fold), and a constitutive enzyme is one whose production is not significantly increased (less than doubled) by the presence of the *specific substrate* during growth, it appears from the data in Table I that the acetate oxidation enzyme system is adaptive, the glycerol-oxidizing enzyme is semi-adaptive and the glucose-oxidation enzyme system is truly constitutive.

The maximum activity of a culture is

TABLE I.
Activity of Cells Grown in Different Media.

Substrate	Cells grown on Glucose Q_{O_2}	Glycerol Q_{O_2}	Acetate Q_{O_2}	Nutrient agar Q_{O_2}
Glucose	60	33.9	33.3	41.8
Glycerol	62.8	218.8	66.5	61.5
Pyruvate	12.9	12.1	88.8	28.8
Lactate	18.3		31.0	39.6
Butyrate	6.9	5.2	21.8	
Acetate	53.2	37.2	203.3	
Glycolate			5.5	
Glyoxalate	4.5	3.0	24.0	
Oxalate			0	
Glycine	15.8	22.3	67.3	65.2
Citrate			6.8	
α-ketoglutarate	20.6	18.1	8.0	
Succinate			2.5	
Fumarate	17.1	6.4	11.5	12.1
Malate	10.2	6.9	27.8	16.2
Mg (dry wt) of cells per vessel	4.04	4.04	4.00	4.07

1. Dubos, R., and Miller, B. F., *J. Biol. Chem.*, 1937, v121, 429.

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3. Karström, H., *Ergeb. Enzymforsch.*, 1938, v7, 350.

4. Kalnitsky, G., and Barron, E. S. G., *J. Biol. Chem.*, 1947, v170, 83.

5. Singer, T. P., and Barron, E. S. G., unpublished.

6. Gale, E. F., *Bact. Rev.*, 1943, v7, 139.

TABLE II.
Activity of Cells Grown for 1-3 Days in Acetate Medium.

Substrate	Cells harvested after 1 day Q_{O_2}	2 days Q_{O_2}	2.5 days Q_{O_2}	3 days Q_{O_2}
Glucose	33.3	38.6	40.9	15.4
Glycerol	66.5	101.5	116.1	64.7
Pyruvate	88.8	38.2	23.5	13.6
Lactate	31.0	19.8	21.4	13.1
Butyrate	21.8	15.6	9.6	8.6
Acetate	203.3	100.3	74.6	30.4
Glycolate	5.5	13.4		2.5
Glyoxalate	24.0	23.1		13.4
Oxalate	0	6.2		0
Glycine	67.3			
Citrate	6.8	8.9		4.0
α-ketoglutarate	8.0	5.9		14.6
Succinate	2.5	5.0		4.0
Fumarate	11.5	1.0		1.0
Malate	27.8	27.8	30.5	14.6
Mg (dry wt) cells per vessel	4.00	4.04	3.84	3.96

usually shown after 18-24 hours growth(6). With this organism, however, the activity with some substrates was greater after 48-60 hours growth (Table II).

TABLE III.
Lag in Acetate Oxidation by Cells Harvested After 2 Days.

Time, hr	Activity of cells harvested after 2 days Q_{O_2}	2.5 days Q_{O_2}	3 days Q_{O_2}
1st	100.3	74.7	30.4
2nd	120.7	139.8	49.8
3rd		147.0	54.2

The activity on different substrates depends not only on the growth medium and the age of the culture, but also on the duration of the test (Table III).

These results would indicate the need for similar studies with other organisms and raises the question as to what might be found during the logarithmic phase of growth.

Summary. The variations in metabolic activity of the soil organism, *Corynebacterium creatinovorans* (Strain "NC"), grown in media containing different carbon sources is described.

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An Antichylomicronemic Substance Produced by Heparin Injection.* (18042)

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Heparin *in vivo* abolishes alimentary lipemia. This effect is not obtained when heparin is added to blood *in vitro*(1-3). Perfusion of heparinized lipemic blood through different parts of the body results in the disappearance of the lipemia suggesting that no specific organ is involved(2). The effect observed is due to a change in the physical state of the fat since the total plasma lipid level is not altered during short term experiments. Heparin injection has also been found to increase the rate of fat uptake from the intestine(3). In the present work the possibility that a surface active substance is produced by heparin administration has been explored.

The chemical composition of heparin would not suggest surface activity(4), and surface tension measurements on 8 heparin preparations in aqueous solution (1 mg/ml) showed none was present. Preliminary experiments

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† Atomic Energy Fellow.

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