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EFFECT OF 2:5-DICARBETHOXY-3:4-DIHYDROXY-THIOPHENE (DICETOL) ON GLUCOSE METABOLISM OF HOMO- AND HETEROFERMENTATIVE BACTERIA

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Abstract—Effects of 2:5-dicarbethoxy-3:4-dihydroxy-thiophene (Dicetol) have been studied on *Leuconostoc mesenteroides* and *Lactobacillus arabinosus*, with reference to glucose metabolism.

Dicetol has been shown to inhibit the pentose phosphate system as well as the Embden-Meyerhof pathway of glucose dissimilation. The possible sites of action have been discussed and anaerobic bacteria have been used to determine the specificity of inhibitors in the pathways of glucose metabolism.

AN APPROACH to chemotherapy of cancer has been proposed assuming predominance of the pentose phosphate pathway of glucose metabolism in tumour tissue.[1] Some compounds implicated to act as anti-metabolites of the pentose phosphate pathway intermediates, have been synthesised and tested for their antitumour properties.[2–6] However, the mode of action of these compounds is not well defined. In animal systems having more than one pathway of glucose utilisation, it becomes difficult to assess if these compounds preferentially inhibit the pentose phosphate pathway. Differential conversion of the specifically labelled glucose to carbon dioxide[7] has been used as a measure of effectiveness of these compounds.[6] However, limitations of this procedure are known.[8] In the heterofermentative *Leuconostoc mesenteroides* glucose dissimilation proceeds exclusively via a pentose phosphate system,[9–13] unlike that in the homofermentative bacteria which produce lactic acid through the Embden-Meyerhof system.[13,14] Each of these types of bacteria would, therefore, provide an isolated system with respect to glucose metabolism. This paper reports the effect of 2:5-dicarbethoxy-3:4-dihydroxy-thiophene referred to as 'Dicetol'[2,3] on glucose metabolism, based on inhibitory responses on *Leuconostoc mesenteroides* and *Lactobacillus arabinosus*.

METHODS

Cultures of *Leuconostoc mesenteroides P-60* and *Lactobacillus arabinosus 17-5*,* were maintained on nutrient agar stabs throughout the course of these studies. The composition of the growth medium was as described by DeMoss *et al.*,[9] and consisted of bactotryptone, 10 g/l of the medium; yeast extract, 10 g; glucose, 10 g; K_2HPO_4,

* Obtained through the courtesy of the Department of Chemical Technology, University of Bombay.

5 g; and salts solution, 20 ml (containing per 100 ml, $MgSO_4 \cdot 7H_2O$, 0·8 g, $MnCl_2 \cdot 4H_2O$, 0·19 g, NaCl, 0·04 g, $FeSO_4 \cdot 7H_2O$, 0·04 g, and 12 N HCl, 2 ml). To ensure uniform growth in all these experiments preinoculated medium was added to the sterile tubes (9 ml/tube) containing Dicetol* and other additions (1 ml/tube as aqueous solutions). Turbidimetric readings were taken after 20–24 hr of incubation at 37°, on a Klett–Summerson colorimeter at 660 mμ (red filter). Growth has been expressed as per cent of normal growth in the nutrient medium without addition of Dicetol and other compounds.

Dicetol-induced growth inhibition was reversed by the addition of some metabolites such as glutathione, cysteine ,cystine, etc. Concentration of the added substances was chosen arbitrarily as 1·0 mg per tube, except nicotinamide-adenine-dinucleotide (NAD) which was 0·2 mg per tube. Dicetol and other compounds as solutions in water were Seitz filtered and asceptically added to the sterile tubes.

RESULTS

Addition of Dicetol to the growth medium inhibits growth of *Leuconostoc mesenteroides* as well as that of *Lactobacillus arabinosus* at the same dose level of 0·3 mg per tube (Table 1). Since these organisms represent two different pathways of glucose

TABLE 1. EFFECT OF DICETOL ON THE GROWTH OF *Leuconostoc mesenteroides* AND *Lactobacillus arabinosus*

Dicetol added (mg/10 ml medium/tube)	Growth per cent	
	L. mesenteroides	*L. arabinosus*
0·0	100	100
0·1	66	77
0·2	33	44
0·3	0	6
0·4	0	0
0·5	0	0

metabolism, it would appear that the inhibition might be at a site common to both.

Table 2 gives the extent of reversal of growth inhibition caused by Dicetol on addition of some metabolites. Since the concentration of the added substances has been arbitrarily chosen the results represent only qualitatively this reversal effect. Dicetol added per tube was 0·3 mg as this is the minimal dose where both these organisms stop growth. The results show that cysteine and glutathione restore growth, while such an effect is not shown by cystine, indicating that Dicetol in some way interferes with the free –SH groups.

Similar reversals have been obtained with NAD pyruvate, alanine, and serine; however, lactate addition does not reverse the growth inhibition. It would also be observed that pyruvate, cysteine, alanine and serine stimulate growth in *Leuconostoc mesenteroides* but not in *Lactobacillus arabinosus*. The significance of this is discussed below.

* Sample of Dicetol was kindly supplied by Dr. M. B. Sahasrabudhe.

DISCUSSION

The experiments reported above bring out that Dicetol affects growth of micro-organisms utilising glucose by way of either the Embden–Meyerhof pathway or the pentose phosphate system. It appears, therefore, that this compound has no specific effect on the pentose phosphate pathway as reported.[2, 3]

TABLE 2. REVERSAL OF GROWTH INHIBITION CAUSED BY DICETOL BY ADDING DIFFERENT METABOLITES TO THE GROWTH MEDIUM OF *Leuconostoc mesenteroides* AND *Lactobacillus arabinosus*

Addition	Growth per cent	
	L. mesenteroides	*L. arabinosus*
No addition	100	100
Dicetol (0·3 mg/tube)	0	0
Glutathione	100	100
Dicetol + glutathione	66	33
Cysteine	150	100
Dicetol + cysteine	90	85
Cysteine	100	100
Dicetol + cystine	0	0
NAD	100	100
Dicetol + NAD	66	44
Pyruvate	150	100
Dicetol + pyruvate	90	90
Lactate	100	100
Dicetol + lactate	0	0
Alanine	150	100
Dicetol + alanine	80	60
Serine	120	100
Dicetol + serine	80	60

The suitability of anaerobic bacteria for screening purine, pyrimidine, and folic acid analogues has been suggested by some authors.[15–17] Dependence of the tumour cell on glucose for energy purposes is more than that of the normal cell. High lactic acid production of tumour cells resembles that of anaerobic bacteria which are incapable of utilising oxygen for energy and therefore obtain energy by fermenting glucose to lactic acid.[18] The bacteria employed in these studies are facultatively anaerobic in which electron transport chain, di-and tri-carboxylic acid cycles are absent and glucose is the exclusive energy source. Impairment in tumour growth by Dicetol[3] could be due to interference of this compound with the energy metabolism in general.

The principal system for the regeneration of NAD in anaerobic bacteria is the lactic dehydrogenase system,[9, 10, 13] which uses $NADH_2$ formed during the initial reactions. In *L. mesenteroides* both NAD and/or NADP carry out reactions of the pentose phosphate pathway in contrast to animal systems which are NADP specific.[12] The need of this organism for NAD is, therefore, more than that of *L. arabinosus*. This could be easily observed with respect to the stimulation of growth on addition of pyruvate and amino acids giving rise to pyruvate. The product of alanine transminaase, cysteine desulphurase and serine deaminase (dehydrase) is pyruvate. When a hydrogen acceptor like pyruvate, acetate etc. was added to the growth medium similar stimulation was reported.[9, 13] It may also be expected that neither growth stimulation nor reversal of growth inhibition caused by Dicetol, should be observed with lactate

addition. Addition of NAD alone also reversed growth inhibition. It would mean that availability of NAD could be the limiting factor for the growth of both the bacteria. This could be affected as a result of inefficient functioning of NAD regenerating system or NAD synthesising system. It is interesting to note that NAD and free –SH compounds restore growth when added separately (Table 2). If Dicetol could have any specific effect it could be at a site where both DPN and free –SH groups have a function.

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