RATE LIMITING EFFECTS OF PYRIDINE NUCLEOTIDES ON CARBOHYDRATE CATABOLIC PATHWAYS OF MICROORGANISMS

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When glucose dissimilation of a marine pseudomonad, Pseudomonas natriegens, was studied, enzymes of both the Embden-Meyerhof pathway and of the hexose monophosphate pathway were detected in extracts of glucose-grown cells. Data from radiorespirometric experiments indicated that approximately 92 and 8% of glucose actually catabolized were routed via the Embden-Meyerhof and the hexose monophosphate pathways respectively (Eagon and Wang, 1962). No pyridine nucleotide transhydrogenase or reduced triphosphopyridine nucleotide (TPNH) oxidase could be detected in cell-free extracts of P. natriegens. Thus, triphosphopyridine nucleotide (TPN) supply appeared to be the rate limiting step in the operation of the hexose monophosphate pathway (Eagon, 1962).

The hexose monophosphate pathway has been shown to be rate limited primarily by the rate of TPN supply in a variety of mammalian tissues, including thyroid tissue (Dumont, 1961), corneal epithelium (Kinoshita, 1957), liver (Cahill et al., 1958), ascite tumor cells (Wenner et al., 1958), erythrocytes (Brin and Yonemoto, 1958) and mammary tissue (McLean, 1960). This hypothesis has not been

Table 1. Pathways participation in various microorganisms.

Microorganism	Pathways (% Participation) EMP*	ED*	HMP*	Reference
Bacillus subtilis	65	0	35	(Wang and Krockov, 1962)
	80	0	20	(Wang et al., 1958b)
Escherichia coli	72	0	28	(Wang et al., 1958b)
Penicillium chrysogenum	77	0	23	(Wang et al., 1958b)
Pseudomonas natriegens	92	0	8	(Eagon and Wang, 1962)
Saccharomyces cerevisiae	88	0	12	(Wang et al., 1958b)
Streptomyces griseus	97	0	3	(Wang et al., 1958b; Wang et al., 1958a)
Acetobacter suboxydans	0	0	100	(Kitos et al., 1958)
Pseudomonas aeruginosa	0	71	29	(Stern et al., 1960)
Pseudomonas fluorescens	<17	>33	>50	(Lewis et al., 1955)
Pseudomonas (Hydrogenomonas) saccharophila	0	100	0	(Wang et al., 1958b; Stern et al., 1960)

*EMP=Embden-Meyerhof pathway; ED=Entner-Doudoroff pathway; HMP= hexose monophosphate pathway.

advanced for microorganisms, however.

Experiments described in this communication were performed to provide answers to two questions: (a) Is the hexose monophosphate pathway of microorganisms as a group rate limited by the rate of TPN supply in a fashion similar to mammalian tissue? and, (b) How do microorganisms that predominantly or exclusively utilize a hexose monophosphate pathway overcome the rate limiting effects of TPN supply?

The microorganisms utilized for these experiments are listed in Table 1. Participation of the Embden-Meyerhof, hexose monophos-

phate and Entner-Doudoroff pathways in each microorganism is indicated as per cent simultaneous participation during glucose catabolism. The number of species represented in Table 1 was limited by those whose pathways participation have been well documented by means of C^{14}-tracer techniques. At the same time, few microorganisms have been shown to utilize predominantly hexose monophosphate or Entner-Doudoroff pathways. Those that do so are largely species of pseudomonads.

Table 2 records the specific activity of reduced diphosphopyridine

Table 2. DPNH-oxidase, TPNH-oxidase and pyridine nucleotide transhydrogenase in extracts of various microorganisms.

Microorganism	E_{340}/min/mg Protein X 10^3		
	DPNH	TPNH	TPNH DPN
Bacillus subtilis	230	27	33
Escherichia coli	765	11	21
Penicillium chrysogenum	163	9	10
Pseudomonas natriegens	275	<1	<1
Saccharomyces cerevisiae	122	3	5
Streptomyces griseus	310	2	5
Acetobacter suboxydans	289	0	7
Pseudomonas aeruginosa	335	53	238
Pseudomonas fluorescens	357	132	303
Pseudomonas (Hydrogenomonas) saccharophila	25	15	17

Protocol: Beckman spectrophotometer, 30 C; 100 µmoles of tris-HCl buffer (pH 7.65); 0.5 µmoles each of DPN, DPNH and TPNH; 0.1 ml of extract; total volume, 3.0 ml.

nucleotide (DPNH) oxidase, TPNH-oxidase and pyridine nucleotide transhydrogenase observed in cell-free extracts of those microorganisms indicated in Table 1. Comparison of these results with data in Table 1 shows a strong correlation between the simultaneous presence of readily demonstrable TPNH-oxidase and pyridine nucleotide transhydrogenase and those microorganisms predominantly utilizing hexose monophosphate and Entner-Doudoroff pathways. DPNH-oxidase, on the other hand, was present in all extracts. Little or no TPNH-oxidase or pyridine nucleotide transhydrogenase activity was detected in extracts from those microorganisms predominantly utilizing the Embden-Meyerhof pathway. The low level of activity detected could be attributed to the non-specific activity of DPNH-oxidase or the presence of small amounts of intermediary products serving as hydrogen acceptors for TPNH. The low level of activity for Pseudomonas saccharophila may be due to the destruction of the cofactors, DPN and TPN, as previously reported (Doudoroff et al., 1958). Comparison of the rates of TPNH oxidation to DPNH oxidation, however, clearly suggests the presence of TPNH-oxidase.

Whereas Acetobacter suboxydans appears not to be in line with the rule that organisms utilizing the hexose monophosphate pathway possess TPNH-oxidase and pyridine nucleotide transhydrogenase, such is not the case. Cheldelin (1961) has shown that A. suboxydans can use either DPN or TPN as cofactors in the hexose monophosphate pathway. Furthermore, A. suboxydans was reported to lack a Krebs tricarboxylic acid cycle and the hexose monophosphate-pentose cycle functioned as the terminal oxidation route. In all systems studied to date, it is DPNH and not TPNH that is involved in energy generation by oxidative phosphorylation through the terminal oxidase (i.e. cytochrome) system. While Cheldelin (1961) did not

indicate whether the DPN-system was the predominant one, these results indicate that A. suboxydans uses DPN as cofactor for energy generation and TPNH is oxidized by coupling to reductive biosynthetic reactions. Similarly, other microorganisms lacking TPNH-oxidase or pyridine nucleotide transhydrogenase whose hexose monophosphate pathway is rate limited by TPN supply may oxidize TPNH by coupling to reductive biosynthetic reactions. This hypothesis has been advanced previously for P. natriegens (Eagon, 1962).

These data indicate, then, that the hexose monophosphate pathway in microorganisms is rate limited by the rate of TPN supply in a manner similar to mammalian tissue. Microorganisms that utilize exclusively or predominantly hexose monophosphate or Entner-Doudoroff pathways have acquired additional or modified enzyme systems which overcome the rate limiting effects of TPN supply. Two such systems are clearly understood: (1) As indicated in this report, the acquisition of TPNH-oxidase and pyridine nucleotide transhydrogenase; and, (2) Glucose-6-phosphate and 6-phosphogluconate dehydrogenases that utilize either DPN or TPN as cofactors, such as in the case of A. suboxydans (Cheldelin, 1961) or Leuconostoc mesenteroides (DeMoss, Gunsalus and Bard, 1953).

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