Unbalanced Production of Reduced Nicotinamide-Adenine Dinucleotide Phosphate in Rheumatoid Synovial Lining Cells

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The abnormal permeability of lysosomal membranes in synovial lining cells of rheumatoid joints is related to the excessively reductive redox balance in these cells (Chayen, Bitensky, Butcher & Poulter, 1969a). The cause of this imbalance was not known.

Redox balance can be expressed by the NADPH/NADP⁺ concentration ratio. In the present study the maximal rates of production and of oxidation of NADPH were used to obtain an assessment of this ratio. The reactions were performed with neotetrazolium as the hydrogen acceptor, so that the activity of synovial lining cells alone can be measured by micro-densitometry.

To measure the rate of formation of NADPH from glucose-6-phosphate, phenazine methosulphate was used as intermediate hydrogen acceptor and neotetrazolium as final acceptor. The incubation media contained glucose-6-phosphate (1.5mg/ml), NADP⁺ (2.5mg/ml), phenazine methosulphate (0.2mg/ml), neotetrazolium chloride (3.0mg/ml) and polyvinyl alcohol (20%, w/v) in 50mM-glycylglycine buffer, pH 8.0. The maximum rate of oxidation of exogenous NADPH via the microsomal 'diaphorase' pathway was studied by adding NADPH (2.5mg/ml) instead of the glucose-6-phosphate and the NADP⁺; no phenazine methosulphate was present.

Sections of synovium from eight non-rheumatoid and seven rheumatoid joints were prepared by the method of Chayen, Bitensky, Butcher & Poulter (1969b) and incubated at 37°C in an atmosphere of N₂. The amount of reduced tetrazolium (coloured insoluble formazan) produced in each of 20 synovial lining cells from each section was measured by scanning and integrating micro-densitometry (Butcher, Poulter & Chayen, 1968; Chayen, Bitensky, Butcher, Poulter & Ubhi, 1970).

The rate of oxidation of exogenous NADPH was the same in rheumatoid and non-rheumatoid synovial lining cells ($E = 0.03\text{--}0.04/10\text{min}$ reaction). In the non-rheumatoid cells the rate of formation of NADPH was 2-3 times the rate of its oxidation ($E = 0.09\text{--}0.12/10\text{min}$). In contrast the rheumatoid cells produced 4 times as much NADPH in unit time ($E = 0.41\text{--}0.45/10\text{min}$) as did the non-rheumatoid cells even though their ability to oxidize NADPH remained unchanged. It follows therefore that the rheumatoid synovial lining cells have the potentiality of producing far more NADPH than can be oxidized, so that the NADPH/NADP⁺

concentration ratio could be displaced severely to the reductive side of equilibrium.

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The Coexistence of Two Metabolic Pathways in the *meta* Cleavage of Catechol by *Pseudomonas putida* N.C.I.B. 10105

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Two pathways for the metabolism of 2-hydroxymuconic semialdehyde in *Pseudomonas* strains have been described, one which involves a primary NAD⁺-dependent dehydrogenation to 4-oxalocrotonate (Nishizuka, Ichiyama, Nakamura & Hayaishi, 1962) and the other a primary hydrolysis to formate and 2-oxopent-4-enoate (Dagley & Gibson, 1965; Bayly & Dagley, 1969). The latter has been widely accepted as the general pathway following *meta* cleavage of catechol in *Pseudomonas*. We have recently shown the coexistence of both these catabolic steps in a naphthalene-grown pseudomonad (Catterall, Sala-Trepap & Williams, 1971).

We present here results showing that both pathways also coexist in phenol-grown cultures of the original strain of Dagley & Gibson (1965), *Pseudomonas* N.C.I.B. 10105 (*Pseudomonas* U, reported as *Pseudomonas putida* by Feist & Hegeman, 1969). The rate of metabolism of 2-hydroxymuconic semialdehyde by cell-free extracts was increased tenfold on addition of NAD⁺ (0.5 μmol). In the absence of added NAD⁺ 0.03 μmol of 4-oxalocrotonate was formed from 0.1 μmol of catechol or 2-hydroxymuconic semialdehyde. After treatment of the extract with a nicotinamide-adenine dinucleotidase preparation, the rate of disappearance of 2-hydroxymuconic semialdehyde in the absence of NAD⁺ was lowered by 30% and no 4-oxalocrotonate was formed. In NAD⁺-supplemented reaction mixtures approximately stoichiometric amounts of NADH and 4-oxalocrotonate were formed

from catechol or 2-hydroxymuconic semialdehyde. These results implicate both NAD^+ - and non- NAD^+ -dependent activities in the metabolism of 2-hydroxymuconic semialdehyde; they also show that the rate of disappearance of 2-hydroxymuconic semialdehyde in the absence of added NAD^+ cannot be taken as a direct measure of the hydrolase activity, as has been done (Feist & Hegeman, 1969). We have confirmed in this strain all the metabolic steps of the scheme reported by Sala-Trepát & Evans (1971) in *Azotobacter* (see page 19).

Unlike the pattern observed in *Azotobacter* species, the 2-hydroxymuconic semialdehyde hydrolase activity was greatly increased by growth on phenol in this strain. The enzymes functional in this scheme were inducible. The high inducible activities of 4-oxalocrotonate tautomerase and 4-oxalocrotonate decarboxylase substantiate the physiological importance of the NAD^+ -dependent dehydrogenase activity for the metabolism of 2-hydroxymuconic semialdehyde in this strain.

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The Physiological Significance of the Two Divergent Metabolic Steps in the *meta* Cleavage of Catechols by *Pseudomonas putida* N.C.I.B. 10105

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Cell-free extracts of *Pseudomonas putida* N.C.I.B. 10105 grown on phenol or any cresol (*o*-, *m*- or *p*-

cresol) showed an identical pattern of enzyme activities, independent of growth substrate. These extracts contained high activities of a *meta*-cleaving oxygenase that attacked catechol, 4-methylcatechol and 3-methylcatechol at constant relative rates of 30:100:48 respectively. The low specificity of the oxygenase extended to the enzymes metabolizing the ring-fission products. The NAD^+ -dependent aldehyde dehydrogenase present in all the extracts was able to metabolize 2-hydroxymuconic semialdehyde and the ring-cleavage product of 4-methylcatechol, namely 2-hydroxy-5-methylmuconic semialdehyde, at relative rates of 100:120 respectively, but did not attack the ring-cleavage product of 3-methylcatechol, namely 2-hydroxy-6-oxohepta-2,4-dienoate, in which the aldehyde group is absent. However, the non- NAD^+ -dependent hydrolase activity was very active on 2-hydroxy-6-oxohepta-2,4-dienoate but much less active on 2-hydroxymuconic semialdehyde and 2-hydroxy-5-methylmuconic semialdehyde (relative rates 100:5:2.5 respectively). All these activities were inducible. All extracts also showed high induced activities of 4-oxalocrotonate tautomerase and 4-oxalocrotonate decarboxylase, and the 4-hydroxy-2-oxovalerate aldolase activity was increased fivefold over its activity in succinate-grown cells.

Feist & Hegeman (1969) have shown that all the enzymes of the metabolic scheme of Dagley & Gibson (1965) in this strain are induced from the top by the primary substrate (phenol and cresols). Our results agree with this view, and in addition show that the enzymes of the 4-oxalocrotonate branch of the pathway are induced with the oxygenase and hydrolase; the whole set of enzymes therefore appears to be induced from the top. Our results suggest that the two branches of the pathway are responsible for the metabolism of different catechols. The NAD^+ -dependent aldehyde dehydrogenase and the other enzymes of the 4-oxalocrotonate branch are functional in the metabolism of catechol, 4-methylcatechol and their metabolic precursors, namely phenol and *p*-cresol,