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