Mitochondrial oxidative activity in human rheumatoid synovial lining cells

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SUMMARY The activities of two mitochondrial enzymes, succinate dehydrogenase and cytochrome oxidase, have been measured by quantitative cytochemistry and microdensitometry in the synovio-cytes of rheumatoid and non-rheumatoid synovial lining cells. Although both tended to be higher in the former, there was no statistically significant difference in the activities of either enzyme in these tissues. However, when cytochrome oxidase activity was measured without exogenous cytochrome c, the activity in the rheumatoid synoviocytes was highly significantly elevated. It is suggested that these findings may indicate only that the cytochrome c-cytochrome oxidase complex in the rheumatoid cells is more stable, possibly because of the increased availability of phospholipids in these cells.

Such studies as have been done on oxidative carbohydrate metabolism of human synoviocytes from rheumatoid synovial tissue have concentrated rather on the glycolytic, Embden-Meyerhof pathway. Using manometric methods on specimens of synovial tissue, both Dingle and Page Thomas (1956) and Roberts *et al.* (1967) found increased activity of this pathway in rheumatoid tissues. In such studies the increased cellularity of rheumatoid synovial tissue may account for the increased glycolytic activity. However, by means of quantitative cytochemistry, which allows measurement of the activity in the synoviocytes free from contributions from infiltrating cells, Henderson (1977) showed increases in the activity of some of the enzymes of the glycolytic pathway. Thus it seems likely that glycolytic activity is enhanced in rheumatoid synoviocytes and this raised the question of whether mitochondrial activity was similarly increased to meet the increased glycolytic flux. Very little work has been done on this question. It was therefore decided to examine the activity of two key mitochondrial oxidative enzymes: succinate dehydrogenase, which is the most active of the Krebs' cycle enzymes, and cytochrome oxidase, which is the terminal acceptor of the respiratory chain. Previous work by Butcher (1970) had validated the cytochemical measurement of these enzymes.

Materials and methods

Non-rheumatoid specimens were taken from the knee during routine arthrotomy for internal derangements. They came either from quiescent joints or from otherwise normal joints following recent mechanical trauma. The rheumatoid specimens were taken mainly from the knee during the course of synovectomy; all the patients from whom the rheumatoid synovium was taken had 'definite' or 'classical' disease according to the diagnostic criteria of the American Rheumatism Association (Ropes *et al.* 1959). All specimens were taken in a bloodless field but within 10 min of the application of the tourniquet. Pieces of such tissues approximately 4 mm³ were chilled by precipitate immersion in *n*-hexane (BDH 'free from aromatic hydrocarbons' grade, boiling range 67-70°C). After no longer than 1 min the specimens were removed from the hexane and stored at -70°C in corked dry glass tubes. They were then sectioned at 10 µm in a Bright's cryostat maintained at a cabinet temperature of -25 to -30°C with the knife cooled to -70°C by having solid carbon dioxide packed around its haft. The sections were flash-dried on to slides (Chayen *et al.* 1973). For each specimen several sections were stained with toluidine blue to establish the precise histology.

Succinate dehydrogenase activity (E.C. 1.3.99.1) was measured by the procedure of Butcher (1970). The reaction involves the oxidation of succinate in the presence of the intermediate hydrogen-acceptor, phenazine methosulphate (PMS) which

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accepts reducing equivalents from the flavoprotein of the dehydrogenase (Slater, 1961) which are then transferred quantitatively to neotetrazolium chloride (obtained from Merck GmbH, and purified by extraction with chloroform before use). The reduced neotetrazolium salt is a strongly coloured formazan which was measured specifically in synoviocytes at 585 nm by the use of a Vickers M85 scanning and integrating microdensitometer. The stoichiometry of the production of the formazan has been determined by Butcher and Altman (1973).

Cytochrome oxidase (E.C. 1.9.3.1) activity was measured by the method of Butcher *et al.* (1964). The activity of this enzyme in this reaction depends on the endogenous concentration of oxidised cytochrome c. Normally the reaction is done without exogenous cytochrome c; to demonstrate the maximal activity, sections were reacted in the normal medium reinforced with 10^{-4} mol/l cytochrome c (Sigma). The reaction product is an indophenol produced by the reaction between N-phenyl-*p*-phenylene diamine, 1-hydroxy-2-naphthoic acid and oxidised cytochrome c; the cytochrome is then re-oxidised by cytochrome oxidase. This forms an insoluble reaction-product which was measured in synovial lining cells at 550 nm. As controls, serial sections were reacted in the same reaction medium containing potassium cyanide (10^{-3} mol/l).

For each reaction, 15–20 fields, each containing 2–3 synovial lining cells, were measured in each of duplicate or triplicate sections ($\times 40$ objective; scanning spot of area $0.5 \mu\text{m}$ diameter). The integrated relative absorption per unit field, recorded by the microdensitometer, was converted to units of absolute integrated extinction (IE) per unit field by suitable calibration. The significance of the results was evaluated by Student's *t* test.

Results

CYTOCHROME OXIDASE ACTIVITY IN SYNOVIOCYTES

The direct activity of cytochrome oxidase in these cells, that is without the addition of exogenous cytochrome c, was measured in the synovial lining cells of 16 specimens of non-rheumatoid and 15 specimens of rheumatoid synovial tissue. The small residual 'activity' found in the presence of cyanide was subtracted from the activity recorded without KCN. The activity in the non-rheumatoid specimens was 2 ± 0.61 (mean $\pm$ standard error) whereas that in the rheumatoid cells was 18 ± 5.71 . The difference was highly significant ($P < 0.001$).

The maximal cytochrome oxidase activity was measured in the synoviocytes in 7 samples of non-rheumatoid and 8 of rheumatoid synovial tissue

Table 1 The effect of exogenous cytochrome c (cyt c) on cytochrome oxidase activity in synovial lining cells (after subtraction of KCN insensitive activity).

Tissue	Case No.	Activity (IE $\times 10^3/10$ min)	
		No cyt c	Plus cyt c
Non-rheumatoid	1665	1	26
	1824	1	8.5
	1877	0.5	6
	1916	5	5.5
	1935	3	21
	1982	2	7.5
	1989	3	6.5
	Mean (and standard error)	2 (0.61)	11.5 (3.26)
Rheumatoid	1815	16.5	26
	1819	16	40
	1854	3.5	9
	1911	8.5	20.5
	1928	48	37
	1941	13	17
	1946	18	17.5
	1980	—	21
	Mean (and standard error)	18 (5.71)	24 (3.96)

No significant difference in activity in presence of exogenous cytochrome c ($0.5 > P > 0.25$).

(Table 1). Although the difference in activity without added cytochrome c remained significant, the activities in the presence of added cytochrome c were not significantly different ($0.5 > P > 0.25$). At best, the rheumatoid specimens showed twice the maximal activity shown by the non-rheumatoid with considerable overlap of activities.

SUCCINATE DEHYDROGENASE ACTIVITY

The activity of this enzyme has been measured in the synoviocytes of 9 specimens of non-rheumatoid and 11 specimens of rheumatoid synovial tissue. As with the total cytochrome oxidase activity, the succinate dehydrogenase activity in the rheumatoid specimens tended to be higher and the overall mean activity was nearly twice that in the non-rheumatoid specimens. However, there was considerable overlap of activities (Table 2) and the results were not statistically significant.

Discussion

Previous biochemical studies (Dingle and Page Thomas, 1956; Roberts *et al.*, 1967) and cytochemical investigations (Henderson, 1977) indicated that rheumatoid synoviocytes had elevated glycolytic activity. The activity of the hexose monophosphate pathway has also been shown to be considerably increased in rheumatoid synovial lining cells (Butcher *et al.*, 1973; Henderson, 1977). Thus it seems likely that there is an increased rate of carbohydrate metabolism in rheumatoid synoviocytes. The question was whether or not this was matched by increased mitochondrial oxidative activity.

Table 2 Succinate dehydrogenase activity ($IE \times 10^3/10 \text{ min}$) in synovial lining cells

Non-rheumatoid		Rheumatoid	
Case No.	Activity	Case No.	Activity
1652	36	1677	23
1655	33	1681	20
1665	73	1692	82
1712	16	1696	74
1717	6	1709	33
1877	25	1711	98
1883	7	1722	26
1916	2	1815	81
1935	51	1819	26
		1911	37
		1928	44
Mean (and standard error)			
28 (8.13)		49 (8.36)	

Difference in activity between non-rheumatoid and rheumatoid lining cells not significant.

The present investigation concentrated on two mitochondrial enzymes. The first, cytochrome oxidase, is the terminal enzyme of the mitochondrial electron-transport chain that is responsible for the production of ATP by mitochondrial oxidative phosphorylation. Lemberg and Barret (1973) showed that there is close agreement between the cytochrome oxidase content of a tissue and its oxygen utilisation. Thus the measurement of the maximal cytochrome oxidase activity (assayed in the presence of added cytochrome c) should give an indication of the oxygen consumption of the lining cells. The second enzyme studied was succinate dehydrogenase which is the most active of the tricarboxylic acid cycle (Lehninger, 1975). Mitochondrial membranes are said to be freely permeable to its substrate (Chappell, 1968). Thus the activity recorded in this study should represent the maximal activity of the tricarboxylic acid cycle in the synoviocytes.

Although there was some suggestion of elevated levels of succinate dehydrogenase and maximal cytochrome oxidase in the rheumatoid synoviocytes, there was such overlap of values that the results were not statistically significantly different. This contrasts with the marked changes in glucose 6-phosphate dehydrogenase activity recorded by Butcher *et al.* (1973) and by Henderson (1977) who obtained values of 70 ± 48 (mean integrated extinction $\times 10^3/\text{unit field}/10 \text{ min}$) in non-rheumatoid as against 499 ± 217 in rheumatoid synoviocytes. Henderson (1977) also found a marked increase in lactate dehydrogenase activity in rheumatoid synovial lining cells (652 ± 316 as against 156 ± 77 in non-rheumatoid cells).

Although there was no significant difference in the maximal cytochrome oxidase activity between rheumatoid and non-rheumatoid synoviocytes, the

level of activity found in the absence of added cytochrome c was markedly and significantly different. Cytochrome c is a soluble molecule which is easily removed from mitochondria (Lemberg and Barret, 1973). It is also readily lost from unfixed cryostat sections such as those used in this study (Roels, 1974). It is known that cytochrome c binds to cytochrome oxidase, the degree of binding being dependent on phospholipid concentration, so that binding is increased parallel to an increase in phospholipid concentration (Tzagoloff and MacLennan, 1965). Recent studies have revealed an increase in the available (unbound or unmasked) phospholipids in human rheumatoid synoviocytes (Henderson *et al.*, 1978). It is therefore possible that the increased activity of cytochrome oxidase (tested without added cytochrome c) in rheumatoid synoviocytes is due to the greater binding of cytochrome c to cytochrome oxidase as a result of the increased concentration of unbound phospholipids. Such phospholipids could interact with the cytochrome c-cytochrome oxidase complex to increase the binding of cytochrome c and prevent solubilisation of the molecule. Conversely, in the non-rheumatoid cells the cytochrome c would be less tightly bound and more easily solubilised into the reaction-medium.

Thus these results imply that the increased availability of phospholipids in rheumatoid synoviocytes (Henderson *et al.*, 1978) may assist the binding of cytochrome c and the stability of the cytochrome c-cytochrome oxidase complex, so enhancing the capability of rheumatoid mitochondria. They show that, despite some enhanced glycolysis, and considerable elevation of the hexose monophosphate pathway, there may be no great difference in the potential mitochondrial oxidative activity associated with the rheumatoid condition.

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