

## Effect of inflammation upon human gingival oxidative metabolism

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Cytochrome oxidase and NADH cytochrome c reductase activities were analyzed biochemically in gingival biopsy specimens obtained from 22 male patients (age 23-72) undergoing periodontal treatment. Histologically, 13 specimens exhibited mild inflammation, while 9 showed more severe inflammatory responses. Cytochrome oxidase activity was significantly greater in the mildly inflamed than in markedly inflamed tissue samples. NADH cytochrome c reductase activity on the other hand was not significantly altered by the increasing degree of inflammation. The possible implication of the effect of inflammation upon oxidative enzymes is discussed in relation to degenerative and proliferative changes occurring in both types of tissue.

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### Introduction

Cytochrome oxidase (E. C. 1. 9. 3. 1.) and NADH cytochrome c reductase (E. C. 1. 6. 2. 1.) were determined in essentially normal human gingiva (Eichel & Sharhrik 1964), however these parameters have not been previously analyzed in relation to the effects imposed by gingival inflammation. Endogenous respiration studies revealed that the  $QO_2$  is stimulated in mild gingival inflammation, and depressed in highly inflamed gingiva (Glickman et al. 1949, Manhold & Volpe 1963). On the other hand recent polarographic studies (Zajack & Kindlova 1972) revealed that initial  $QO_2$  values remained constant in human gingiva and were not significantly altered by the degree of inflammation. In light of these divergent views, we wish to report on the effect of inflammation upon cytochrome oxidase and

NADH cytochrome c reductase activities in total homogenates of human gingiva.

### Methods and Materials

#### *Tissue Preparation*

Human gingival biopsy specimens were obtained from 22 male patients (age 23-72), undergoing periodontal treatment. Regional block anesthesia was administered to avoid infiltration of the tissue. The tissue was surgically removed and cut into two portions one of which was prepared for histologic study. The other was washed in cold 0.075 M phosphate buffer pH 7.0, to remove adhering blood and quickly frozen at  $-70^\circ\text{C}$  on dry ice. The frozen gingival samples were analyzed for their enzymatic activity within one day after their removal. Gingival homogenates were prepared by a modification of the methods (Hooper &

Bernstein 1966) utilized for the homogenization of rat skin. The frozen gingival tissue (25–50 mg wet wt.) was immersed in liquid  $N_2$  ( $-270^\circ C$ ) for 3 minutes, and pulverized to small granular fragments. The gingival fragments were homogenized in cold buffer in Ten Broeck ground glass homogenizers which were ground to fit with a clearance tolerance of 0.1–0.15 mm. The enzymatic activities were determined immediately after tissue homogenization.

#### Enzyme Analysis

Cytochrome oxidase (E. C. 1. 9. 3. 1.) (Wainio et al. 1951) was determined spectrophotometrically by following the oxidation of dithionite reduced cytochrome  $c^*$  at  $E_{550\text{ nm}}$  at  $25^\circ C$  in a Gilford Model 2400 spectrophotometer. In all experiments the auto-oxidation rate of ferrocytochrome  $c$  before the addition of the enzyme was negligible. The  $E_{550\text{ nm}}$  absorbance values for homogenate settling were for the most part negligible (0.0% in 16 samples), and where detected they ranged from 0.02–13.0% of the enzymatic value. The average settling value was  $2.5 \pm 0.9\%$  for the 22 samples which were analyzed. Settling values were determined by re-reducing the reaction media with excess dithionite, resuspending the homogenate in the reaction media, and following the change in optical absorbance at  $E_{550\text{ nm}}$  vs. time graphically. The settling values were subtracted from the total observed absorbance value obtained in the affected enzyme assays, and the specific activities were determined from the corrected values. The reaction mixture contained: 75 mM  $KH_2PO_4$ – $Na_2HPO_4$  buffer pH 6.75, 5.6  $\mu M$  cytochrome  $c$  and (25–150)  $\mu g$  protein (homogenate) in a final volume of 300  $\mu l$ . Cytochrome  $c$  concentration was determined from the millimolar extinction coefficient of cytochrome  $c$   $E_{550\text{ nm}} = 18.5\text{ mm}^{-1}\text{ cm}^{-1}$  for the reduced minus oxidized cytochrome  $c$ .

*NADH Cytochrome c Reductase.* NADH cytochrome  $c$  reductase (E. C. 1. 6. 2. 1.) (Jeng et al. 1968) was determined by following the reduction of cytochrome  $c$  at  $E_{550\text{ nm}}$  at  $25^\circ C$ . The millimolar extinction coefficient utilized for cytochrome  $c$  in the cytochrome oxidase assay was utilized for this assay. The assay system contained: 33 mM  $KH_2PO_4$ – $Na_2HPO_4$  pH 7.75 buffer, 8.2  $\mu M$  cytochrome  $c$ , 3.0mM KCN, 85  $\mu M$  NADH,\*\* and (25–150)  $\mu g$  protein (homogenate) in final volume of 300  $\mu l$ .

*Specific Activities.* Specific activities are expressed as nanomoles of cytochrome  $c$  oxidized (cytochrome oxidase) or reduced (NADH cytochrome  $c$  reductase) per mg protein per min.

*Protein Determination.* Tissue homogenates (10–20  $\mu l$ ) were digested in 0.05 N NaOH (final concentration) for one hour at  $25^\circ C$ , and analyzed for protein by the method of Lowry et al., (1951). Crystalline albumin\*\*\* was used as a standard.

#### Statistics

The mean, standard deviation and standard error were determined for each group. Values exceeding the limits  $\pm 1.96$  SD were discarded. The difference between each group was analyzed by the "student  $t$ " test and all values  $p < .05$  or less were considered to be significant.

#### Reagents

NADH\*\* (Grade 111), and cytochrome  $c^*$  (type 111 Horse Heart), were obtained from Sigma Chemical Corp., St. Louis, Mo. Crystalline albumin\*\*\* was obtained from Pentex Corp. Kankakee, Ill.

#### Histologic Evaluation

The biopsy specimens were cut at 7  $\mu$  and stained with hematoxylin and eosin. Each section was diagnosed as mild, moderate or

severely inflamed depending on the extent of inflammatory infiltrate found in the representative sections of the specimens.

### Results

#### *Histological and Clinical Evaluations*

Histologic evaluation of the biopsy specimens revealed that 13 gingival samples fell within the mildly inflamed category, and 9 samples were designated as severely inflamed. The specific activities of cytochrome oxidase, and NADH cytochrome c reductase activities were correlated with the observed degree of gingival inflammation and the data was statistically analyzed.

#### *Cytochrome Oxidase*

Cytochrome oxidase specific activity was found to decline from  $5.4 \pm 0.7$  in mildly inflamed to  $3.0 \pm 0.3$  in severely inflamed gingiva (Table I). The specific activity of the mildly inflamed was significantly greater ( $p < .001$ ) that that of the severely inflamed gingival samples.

#### *NADH Cytochrome c Reductase*

NADH cytochrome c reductase activity was not altered significantly by the degree of gingival inflammation. The specific activities were  $16.5 \pm 1.6$ , and  $15.3 \pm 2.1$  respectively in mildly and severely inflamed gingiva (Table I).

**Table I**  
Effect of inflammation upon oxidative metabolism of human gingiva

Degree of Inflammation	Enzyme Specific Activity, Cytochrome Oxidase	NADH Cytochrome c Reductase
Mild	$5.4 \pm 0.7$	$16.5 \pm 1.6$
Severe	$3.1 \pm 0.31$	$15.3 \pm 2.1$

Cytochrome oxidase and NADH cytochrome c reductase activity is expressed as nanomoles of cytochrome c oxidized, or reduced, per mg of protein per min.  $\pm$  standard error of the mean.

### Discussion

Although the consistency of inflamed gingiva may vary considerably from area to area, and thereby effect the degree of fragmentation, this does not appear to be the case in our preparations. The exposure of our gingival tissues to liquid  $N_2$ , pulverization of the tissue, thawing and subsequent homogenization appears to adequately disrupt the cells. Evidence for this interpretation lies in the observation that the distribution of NADH cytochrome c reductase in both mildly inflamed and markedly inflamed gingiva did not vary significantly. If the decline in cytochrome oxidase was influenced by our tissue preparation techniques, similar results should be observed in the NADH cytochrome c reductase distributions. The amount of settling in our assay system was found to be negligible for the most part and it could not account for the marked decline in cytochrome oxidase activity observed in markedly inflamed gingiva.

The elevated cytochrome oxidase activity observed in our study coincides with the observations made by Manhold and Volpe (1963) in their endogenous  $QO_2$  respiration studies of mildly inflamed and proliferating human gingiva. The increase in  $QO_2$  and cytochrome oxidase in gingiva may reflect increased mitochondrial high energy bond (ATP) synthesis, which is required to sustain the highly synthetic processes associated with DNA synthesis, mitosis, as well as cellular proliferation. Further evidence for the increased bioenergetic requirements in the pre-proliferating and proliferative stages of cell development is the observation that increased  $QO_2$  (endogenous respiration) (Glickman et al. 1949), and cytochrome oxidase (Fine 1970) reaches peaks in activity at a time when major increases in the mitotic index and proliferation (epithelialization) occur in regenerating gingival and skin

wounds. On the other hand the sharp decline in cytochrome oxidase and endogenous  $QO_2$  observed in markedly inflamed (Manhold & Volpe 1963, Glickman et al. 1949) gingiva appear to be reflections of changes occurring within the mitochondria as well as other subcellular components during significant gingival tissue destruction. We were unable to detect an increase in cytochrome oxidase in markedly inflamed and proliferating gingiva, although increases in the endogenous  $QO_2$  was detected by Glickman et al. (1949) in this type of gingival pathology. However, it is conceivable that as inflammation subsides, a pre-proliferative phase (S phase of mitosis) occurs within the migrating cells at the wound edge which requires increased ATP synthesis. Subsequently an increase in endogenous  $QO_2$  is also observed. Our cytochrome oxidase data, and the data reported for endogenous respiration  $QO_2$  do not coincide with the polarographic  $QO_2$  observations made by Zajicek and Kindlova (1972), who reported that the initial  $QO_2$  values remained constant in human gingiva, and were not significantly altered by the degree of inflammation.

NADH cytochrome c reductase activity was not markedly altered by the degree of inflammation in human gingiva. Our data for human gingiva parallel the observation made by Eichel and Shahrik (1964), for human non-inflamed gingiva. NADH cytochrome c reductase activity was found to be significantly greater than cytochrome oxidase in our studies which correlates with the observation made by Eichel and Shahrik (1964). On the other hand cytochrome oxidase activity (total homogenate) was found to be significantly greater than NADH cytochrome c reductase in normal rat gingiva (Fine 1970, Fine et al. 1973a) using identical assay methods. This suggests that NADH cytochrome c reductase is not primarily associated with gingival mitochondria. Re-

cent substantiating studies (Fine et al. 1973a, 1973b) showed the enzyme to be chiefly distributed within the soluble and microsomal subcellular fractions in rodent and human gingiva.

The variation in cytochrome oxidase activity with increasing inflammation provides information regarding the characteristics of terminal respiration in human gingiva in relation to the diseased state. The effect of inflammation upon NADH cytochrome c reductase distribution and specific activities at the subcellular level are currently under investigation.

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