Endogenous Respiration of Human Gingival Tissue. (24614)

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In the absence of marked anaerobic metabolism, it may be assumed that rate of oxygen consumption is a reasonable index of biochemical activity of a tissue. The value of 1.6 for endogenous QO_2 (dry weight) of human gingival tissue reported by Glickman *et al.*(1), using conventional Warburg Technic, appears to be quite low for a tissue which grows fast and heals rapidly. Laser(2) has shown that QO_2 of tissue in absence of added substrate decreases rapidly with time. A time-dependent decrease in respiration, together with delay in starting respiration trials, could well account for the low values previously obtained(1). To test such an explanation, we made use of a modified(3) Stern-Kirk microrespirometer and low incubation temperature (24°) to study time-dependence of endogenous respiration. Low temperature (under 8°) and speed were used in preparation of the slices(4).

Methods. Human gingival tissue, normal as judged by clinical and histologic standards, was obtained by surgical excision following block and/or local infiltration anaesthesia using Xylocaine HCl with adrenaline 1:100,000. The biopsy material was immediately placed into ice-embedded petri dish lined with filter paper, moistened with Ringer-phosphate solution(5). Slices 0.5 mm thick were prepared using Martin Slicer(6). To attain histologic uniformity, the biopsy was sliced in a direction perpendicular to mucosal surface. Slices were accumulated in cold Ringer-phosphate solution, and between 6 and 12 mg placed on lower shelf of platinum gold tissue rack. The rack was promptly lowered into the respiration chamber of respirometer which had been previously charged with 0.3 ml of Ringer-phosphate solution and equilibrated at 24°. A circle of filter paper to which was added 0.02 ml of 20% KOH was placed on upper shelf of rack. The lower shelf containing tissue slices was below surface of liquid, the upper shelf containing the CO_2 absorber was above the surface. The compensation chamber was charged in an identical manner except for tissue. The index droplet was centered, the instrument gassed with oxygen and needle valves closed. Readings were taken every 10 minutes for one hour. Time between excision of tissue and start of respiratory trials ranged from 15 to 30 minutes. Use of dental vibrator to shake microrespirometer, during previous trials established to our satisfaction that there was no rate-limiting factor in consumption of oxygen which could be removed by establishing turbulence in the bathing fluid. A portion from each biopsy sample, suitable for histologic examination, was placed immediately into 10% neutral formalin preparatory to processing and staining. Remainder of biopsy material (30-40 mg) was used to determine total nitrogen/unit wet weight of tissue. This value permitted calculation of QO_2 on the basis of wet weight of tissue(7). While wet wt. of larger pieces of tissue could be determined with reasonable accuracy, this was not so with small slices used in the respiratory trials. Instead, total nitrogen of contents of respiration chamber (tissue and fluid) was determined at end of trial, using the Kjeldahl-Nessler procedure outlined by Johnson(8) and the results multiplied by wet weight/total nitrogen obtained through analysis of larger piece of tissue.

Results. Studies were made on biopsy samples from 19 persons ranging in age 9-69 years. Values for total N/wet weight of 13 samples judged histologically, normal ranged from 0.0279 to 0.0355 with mean value 0.0316 ± .0036.*

Respiration trials at 24° were determined on 10 samples. QO_2 (wet) for individual sample averaged over 1 hour, varied from 0.265 to 0.446 with mean value 0.339 ± 0.068.* Mean QO_2 (N) was 10.67 ± 1.88.*

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* Stand. dev. $= \pm \sqrt{\frac{\Sigma (x-\bar{x})^2}{n-1}}$, where $\bar{x} = \frac{\Sigma x}{n}$

The value for each 10-minute period, in terms of μl O_2/min/mg N, for the average for 10 samples is shown in Fig. 1 as a bar graph. The marked dependence of value of endogenous respiration on time is evident. Average QO_2 (N) calculated on the basis of first 10 minutes would amount to 14.1, while calculated value for last 10 minutes would be 8.1. The value calculated for data for first 10 minutes may not be the maximum attainable value since, due to scarcity of normal gingival tissue at time these studies were underway, it was necessary to include biopsy samples which had been infiltrated with local anaesthetic. We estimated from comparison with samples obtained by block anaesthetic only, that infiltration of the anaesthetic lowers the value by 10-15%.

To permit a rough comparison with data of Glickman *et al.*, 3 sets of tissue were determined at 24° and 38°. Ratios of QO_2 in favor of higher temperature were 4.8, 3.9, and 3.2. Average of these values (4.0) is somewhat higher than the value obtained by Fuhrman and Field(4) for the same temperature range using liver slices.

Discussion. The marked time-dependence of rate of endogenous respiration of human gingival tissue points out the need, more so than with some other tissues, to control rigidly the conditions under which respiratory trials are made. This would be especially so in any comparison of normal and pathologic gingiva. Undoubtedly, reliable and reproducible values would be obtained if the determinations were carried out in presence of added substrate and in presence of CO_2(2). If our value obtained at 24° is multiplied by a conservative factor of 3 to convert to 37.5°, and the QO_2 (dry) obtained by Glickman *et al.*(1) is divided by 5 to convert to wet weight basis, our value at 37.5° would be 1.0 and would be roughly 3 times their value (0.3) at approximately the same temperature.

After completion of this investigation, Schrader and Schrader(8) reported on respiration of gingival tissue. They obtained a QO_2 (wet) of 0.475 at 37.5°, a value about one-

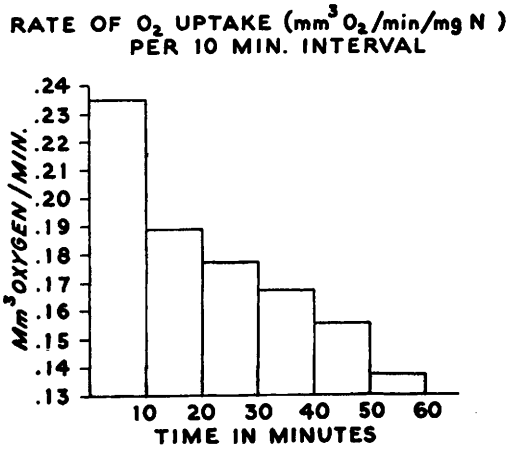

FIG. 1. Rate of endogenous oxygen uptake of normal gingiva for successive 10-min. intervals; composite of 10 studies at 24°.

half as large as our conservatively estimated value (1.0) at 37.5°. These workers used slices 0.3 mm thick with air as the gas phase. Unfortunately, from the point of view of data in Fig. 1, they incubated their slices at 37.5° for 20-30 minutes before starting an hour-long respiratory trial.

Summary. Rate of endogenous respiration of human gingival tissue is markedly dependent upon time. However, reasonably high values may be obtained for endogenous QO_2 if speed and low temperatures are used in preparation of the slices. Respiratory studies on gingiva should be carried out in the presence of added substrate.

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