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Study of CoQ_{10}-Enzymes in Gingiva from Patients with Periodontal Disease and Evidence for a Deficiency of Coenzyme Q_{10}*

(succinate dehydrogenase-coenzyme Q_{10} reductase/DPNH-cytochrome *c* reductase)

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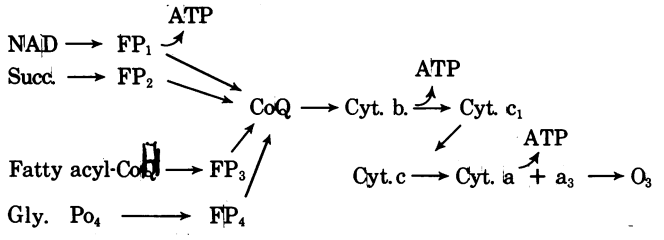
ABSTRACT **The specific activities of both the succinate dehydrogenase-coenzyme Q_{10} reductase and the DPNH-cytochrome *c* reductase [NADH:(acceptor)oxidoreductase, EC 1.6.99.3] were determined in mitochondria from 40 diseased gingival biopsies from patients with periodontal disease and from 24 control biopsies from nondiseased areas (clinically evaluated) of gingival tissues from the same mouths of the patients from whom the diseased gingival tissues were taken. The control tissue was taken during normal surgical procedures, such as for gingival recontouring and tuberosity removal.**

The diseased gingival biopsies showed a mean specific activity for the succinate dehydrogenase-coenzyme Q_{10} reductase which was higher ($P < 0.02$) than that of the control biopsies, and which increased ($P < 0.01$) when the assays utilized exogenous coenzyme Q_3, and corresponded to an average deficiency of coenzyme Q_{10}-enzyme activity of 35%. About 60% of the 40 diseased gingival tissues showed a deficiency of coenzyme Q_{10} at its site in this succinate-coenzyme Q_{10} enzyme. Of the 24 control tissues, 20% showed deficiencies of coenzyme Q_{10}. As a group, the control tissues showed no deficiency of coenzyme Q_{10}.

No deficiency of coenzyme Q_{10} at its site in DPNH-cytochrome *c* reductase was observed for either the control or diseased gingival tissues, as groups or individually.

The basic biochemistry of coenzyme Q has been studied for about 15 years. Coenzyme Q_{10} is intrinsic to human tissue, including the gingiva, and is a vitamin according to the basic science of nutrition. The dominant biochemical interest in coenzyme Q has been in its fundamental and indispensable role in the electron transfer reactions of respiration and coupled oxidative phosphorylation in mitochondria. The following depiction of the respiratory chain, including coenzyme Q, is from Lehninger's *Biochemistry* (1) and may be considered representative of the efforts of many biochemists to determine the exact position of coenzyme Q in the supramolecular chain of reactions of the respiratory process.



In 1967, Tsunemitsu and Matsumura first described the clinical administration of a form of coenzyme Q (CoQ_7) to patients with severe destructive periodontal disease and hypercitricemia (2). They reported that the hypercitricemia was normalized by the oral administration of coenzyme Q_7. This result was supported by subsequent evidence that coenzyme Q_7 significantly normalized the decreased levels of aconitate hydratase in scorbutic alveolar bone and kidney (3).

Although these and other (4, 5) significant reports were from clinical studies of coenzyme Q and periodontal disease, the mechanism of the role of coenzyme Q in periodontal disease had not been known until Littarru *et al.* (6) and Nakamura *et al.* (7) reported a deficiency of coenzyme Q_{10} in patients with periodontal disease. It was first found by Littarru *et al.* (6) that gingival biopsies from patients with diseased periodontal tissue showed a deficiency of coenzyme Q_{10}, in contrast to patients with normal periodontal tissue who showed no deficiency; that report provided an account of the first treatment with coenzyme Q of a patient in the United States with periodontal disease. The clinical result was beneficial and encouraged continuing clinical studies.

Nakamura *et al.* (7) studied the enzyme activities of the succinate dehydrogenase-coenzyme Q_{10} (CoQ_{10}) reductase in the absence and in the presence of exogenous coenzyme Q_3. There was no meaningful increase in the enzyme activities of this mitochondrial CoQ_{10}-enzyme from normal gingival tissue, which indicated that there was no deficiency of CoQ_{10} at its succinate enzyme site in these normal gingival tissues. However, a study of this CoQ_{10}-enzyme in gingival mitochondria from patients with obvious periodontal disease indicated a deficiency of CoQ_{10} at its succinate site.

Matsumura *et al.* (8) cooperatively conducted a double-blind trial of coenzyme Q in patients with destructive periodontal disease who had not benefited adequately from oral hygiene. Clinical examinations and statistical analyses revealed therapeutic improvement from the administration of coenzyme Q. It was concluded that for certain patients oral hygiene combined with therapy with coenzyme Q could provide improved treatment and longer benefits.

We now describe data and their significance for succinate dehydrogenase-coenzyme Q_{10} reductase and DPNH-cytochrome *c* reductase [NADH:(acceptor) oxidoreductase, EC 1.6.99.3], for control gingival nondiseased tissues and diseased tissues from the same mouths of a large group of patients having periodontal disease. It was not feasible to obtain truly normal (nutritionally healthy) gingival biopsies which would constitute the desirable control because of the reluctance of the test group to be subjected to additional or experimental surgery. However, comparison of pairs of tissue biopsies (from

Abbreviations: S.A., specific activity; DCIP, 2,6-chlorophenol indophenol.

* This is publication No. 171 in a series on coenzyme Q.

TABLE 1. *Data on CoQ_{10}-enzymes of control human periodontal tissue*

			Succinate dehydrogenase-CoQ_{10} reductase				DPNH-cytochrome *c* reductase			
No.	Age	Sex	S.A.*	+10 nm CoQ_3	A.C.†	% Def‡	S.A.*	+10 nm CoQ_3	A.C.†	% Def.‡
1	53	F	4.0	3.5	—	—	3.2	3.2	—	—
2	34	F	1.3	1.3	—	—	3.5	2.8	—	—
3	37	F	3.7	4.6	25	20	16.2	18.2	12	11
4	36	F	5.0	4.0	—	—	13.6	8.6	—	—
5	36	F	2.3	2.3	—	—	10.0	10.0	—	—
.6	54	M	5.2	5.2	—	—	19.5	19.5	—	—
7	29	F	1.8	3.1	72	42	22.2	22.2	—	—
8	51	M	4.4	4.5	2	2	12.7	13.6	7	7
9	49	M	4.1	8.1	98	49	30.7	31.2	2	2
10	24	M	2.7	3.2	19	16	39.1	35.3	—	—
11	38	F	2.5	1.9	—	—	9.3	7.5	—	—
12	78	M	2.0	2.0	—	—	13.7	15.9	16	14
13	52	M	1.9	2.0	5	5	36.4	34.4	—	—
14	32	M	2.4	2.7	13	11	38.6	39.2	2	2
15	46	M	5.0	4.7	—	—	78.3	84.0	7	7
16	13	F	7.3	7.3	—	—	47.7	45.8	—	—
17	52	F	3.9	3.1	—	—	32.4	31.0	—	—
18	31	F	3.6	6.4	77	44	30.7	23.3	—	—
19	48	F	2.7	3.2	19	16	21.2	19.3	—	—
20	37	F	2.7	2.9	7	7	6.1	6.1	—	—
21	20	M	5.1	5.6	10	9	48.9	43.8	—	—
22	54	M	4.2	6.5	55	35	33.0	31.1	—	—
23	55	M	1.9	2.9	51	34	43.1	45.9	6	6
24	52	F	2.7	3.0	11	10	20.6	20.6	—	—

* Specific activity (S.A.) is expressed as nmoles of DCIP or cytochrome *c* reduced per min/mg of protein.
† Activation coefficient (A.C.).
‡ % Deficiency of CoQ_{10}-enzyme activity.

the same mouth) clinically evaluated as periodontally diseased and periodontally healthy is of extreme interest because the overall nutritional status of the patient would not be a factor, especially since some inflammatory cells are found in all "clinically healthy tissue" and the nutritional status of other control patients would have to be determined.

MATERIALS AND METHODS

Gingival Biopsies. The method (7) of obtaining the biopsies was as follows. In general, gingival tissues were obtained by use of a surgical scalpel and a local anesthesia. The specimens consisted mainly of interdental papilla and/or marginal gingiva and measured 3–5 mm in diameter and weighed about 100–200 mg. The tissues were immediately washed in cool saline and frozen until used for the preparation of mitochondria. The patients selected were aged 17–78 years, were of both sexes, and had clinical signs of severe destructive periodontal disease.

Preparation of Mitochondria. The biopsies were cut into small pieces and added to 15 ml of 0.01 M Tris·HCl–0.25 M sucrose buffer, pH 7.8. The mixture was homogenized for 2 min in an ice-cooled homogenizer fitted with a Teflon pestle of the Potter-Elvehjem type, and then the mixture was centrifuged at 1000 × *g* for 15 min. The supernatant was transferred into another tube and centrifuged again at 18,000 × *g* for 20 min. The resulting pellet was washed three times by suspending it in the same buffer and centrifuging at 18,000 × *g*. The final pellet was suspended in 0.25 M sucrose solution for the enzyme determination.

Enzyme Assay. The protein content in the mitochondrial preparation was measured by the method of Lowry *et al.* (9). The determination of the specific activity (S.A.) of succinate dehydrogenase-coenzyme Q_{10} reductase was performed essentially by the method of Ziegler and Rieske (10) and is based on the rate of reduction of coenzyme Q by succinate, which is determined indirectly by measuring the rate of reduction of 2,6-chlorophenol indophenol (DCIP) at 600 nm. The indophenol is rapidly reduced by the hydroquinone of either coenzyme Q_3 or Q_{10} and is not reduced in the absence of coenzyme Q.

The specific activity of DPNH-cytochrome *c* reductase was determined essentially by the method of Hatefi and Rieske (11). The reduction of ferrocytochrome *c* by DPNH was measured spectrophotometrically at 550 nm.

The specific activities of the enzymes are expressed in terms of nanomoles of reduced cytochrome *c* or DCIP per min/mg of protein of the enzyme solution.

The activation coefficient and percent deficiency of the coenzyme Q_{10} enzyme activity were calculated as follows:

$$\text{Activation Coefficient} = \frac{\text{S.A. with } CoQ_3 - \text{S.A.}}{\text{S.A.}} \times 100$$

% Deficiency of the CoQ_{10} Enzyme Activity

$$= \frac{\text{S.A. with } CoQ_3 - \text{S.A.}}{\text{S.A. with } CoQ_3} \times 100$$

TABLE 2. *Data on CoQ_{10}-enzymes of diseased human periodontal tissue*

			Succinate dehydrogenase-CoQ_{10} reductase				DPNH-cytochrome *c* reductase			
No.	Age	Sex	S.A.*	+10 nm CoQ_3	A.C.†	% Def.‡	S.A.*	+10 nm CoQ_3	A.C.†	% Def.‡
1	34	F	3.7	7.6	106	51	21.0	32.4	54	35
2	37	F	7.5	7.5	—	—	12.2	12.2	—	—
3	36	F	5.6	7.3	31	23	28.7	28.7	—	—
4	36	F	4.7	6.7	43	30	21.6	25.9	20	17
5	54	M	7.3	8.3	—	12	25.5	30.4	19	16
6	45	F	2.3	5.0	122	54	27.0	27.0	—	—
7	45	F	4.2	9.2	122	54	26.8	26.8	—	—
8	52	F	2.9	2.9	—	—	15.8	15.0	—	—
9	32	F	2.9	5.8	100	50	31.3	31.3	—	—
10	42	M	10.7	15.0	40	49	53.3	46.3	—	—
11	53	M	3.0	3.9	30	23	15.7	15.7	—	—
12	29	F	3.2	6.9	115	54	30.8	30.8	—	—
13	51	M	13.3	26.6	100	50	71.6	73.3	2	2
14	51	M	5.8	12.7	118	54	47.4	50.2	6	6
15	49	M	8.2	17.2	110	52	44.5	37.1	—	—
16	44	F	1.6	3.7	131	57	41.3	40.3	—	—
17	51	M	1.6	2.4	50	33	27.5	30.8	12	10
18	44	M	1.5	3.3	120	55	35.5	40.7	15	13
19	51	M	1.1	1.5	36	27	23.7	19.1	—	—
20	51	M	1.2	2.1	75	43	9.5	7.6	—	—
21	50	M	1.8	1.9	6	5	11.2	10.4	—	—
22	42	M	2.6	2.2	—	—	9.8	11.1	13	12
23	38	F	4.5	7.9	76	43	33.5	29.8	—	—
24	78	M	7.4	12.8	73	42	34.5	46.0	33	25
25	36	M	1.4	1.5	7	7	31.6	33.4	6	6
26	52	M	2.9	4.4	52	34	57.2	59.1	3	3
27	49	M	1.4	1.4	—	—	32.4	30.0	—	—
28	32	M	2.1	3.5	67	40	54.1	46.5	—	—
29	45	M	3.8	6.0	59	26	24.5	22.1	—	—
30	46	M	4.0	6.3	58	37	37.3	33.1	—	—
31	13	F	10.0	12.6	26	21	92.2	79.0	—	—
32	52	F	9.6	11.8	23	19	60.1	45.0	—	—
33	31	F	5.8	10.5	81	45	66.9	46.9	—	—
34	48	F	9.4	14.8	59	36	50.9	41.3	—	—
35	37	F	2.5	2.7	8	7	27.3	26.5	—	—
36	20	M	5.5	6.8	25	19	30.2	34.9	16	13
37	45	M	3.1	3.1	—	—	26.3	28.0	6	6
38	44	M	2.7	2.9	7	7	64.0	52.4	—	—
39	55	M	5.4	8.7	61	38	43.1	45.9	—	—
40	52	F	2.7	2.9	7	7	16.3	17.3	6	6

* Specific activity (S.A.) is expressed as nmoles of DCIP or cytochrome *c* reduced per min/mg of protein.
† Activation coefficient (A.C.).
‡ % Deficiency of CoQ_{10}-enzyme activity.

RESULTS

The specific activities of both succinate dehydrogenase-CoQ_{10} reductase and DPNH-cytochrome *c* reductase were determined in the same preparations of mitochondria so that data for activities, increases, and deficiencies of the enzyme systems could be compared.

The specific activities of both enzymes in the mitochondria which were obtained from the control tissues are shown in Table 1. (The significance of this control tissue is discussed in the *Introduction*.) This control tissue is not truly normal, healthy gingival tissue because it is clinically nondiseased gingival tissue from the mouths of patients having diseased areas of gingival tissue. It is difficult to obtain truly normal gingival tissue from an individual with good nutrition and no dental problem. However, the control tissue showed no clinically distinct signs of disease upon visual inspection. The data on the specific activities of the two CoQ_{10}-enzymes for these particular control tissues are reasonably consistent with the control data previously reported (6) for individuals having no periodontal disease.

Table 2 shows the specific activities of the succinate dehydrogenase CoQ_{10}-reductase and the DPNH-cytochrome *c* reductase of the severely diseased gingival biopsies. The succinate-CoQ_{10}-enzyme in mitochondria from these diseased biopsies showed a mean specific activity of 4.52 ± 1.99. The difference between the specific activities of the succinate-CoQ_{10}-enzyme for the diseased and control gingiva is significant ($P < 0.02$), and it is notable that the level of the mean specific activity of the diseased gingiva is higher than that for the control gingiva (Table 3). [The higher level of enzyme

TABLE 3. *Summary of data on succinate-CoQ10 reductase*

Gingiva	Specific activity: No addition	Specific activity: With CoQ_3	Average activation coefficient	Average % deficiency of CoQ_{10} enzyme activity
Control (24)	3.43 ± 1.44	3.91 ± 1.81	15.8	12.3
Diseased (40)	4.52 ± 1.99*	7.00 ± 5.28	52.8†	35.3

* Significantly different by $t = 2.42$ ($P < 0.02$).
† Significantly increased with CoQ_3 by $t = 2.78$ ($P < 0.01$).

activity for a diseased tissue was previously discussed with citations to the literature (6).] The mean specific activity of the diseased gingiva, when the assay was carried out with exogenous coenzyme Q_3, increased to 7.00 ± 5.28, which is significant ($P < 0.01$) and this increase in activity corresponded to an average of 35% deficiency of CoQ_{10}-enzyme activity. In contrast, there was no significant increase in the mean specific activity of the enzyme from control gingiva when the assays were carried out in the presence of exogenous coenzyme Q_3; the 12.3% increases of activity are not significant. Levels of deficiency of CoQ_{10}-enzyme activity of over 25%, and particularly over 50%, for specific individuals, are considered significant.

The data in Table 4 show that the mean specific activity of the DPNH-cytochrome *c* reductase from diseased gingiva was 35.4 ± 21.0; the corresponding level of activity for the control tissue was 26.3 ± 17.7. The difference in the mean specific activity of the DPNH-cytochrome *c* reductase of the diseased gingiva in comparison to that of the control tissue has borderline "significance" ($0.05 < P < 0.1$). The mean levels of specific activities of the enzyme from both the diseased and control gingiva showed no change when the assays were repeated in the presence of exogenous coenzyme Q_3. Consequently, no deficiencies of coenzyme Q_{10} for the activity of DPNH-cytochrome *c* reductase was observed for either the diseased or control tissue.

DISCUSSION

Comparison of these data on the two CoQ_{10}-enzymes of the gingiva showed deficiencies of coenzyme Q_{10} only in the succinate dehydrogenase-CoQ_{10} reductase. Consequently, it can be concluded that there was a deficiency of coenzyme Q_{10} in the diseased gingiva.

Although there is biochemical and statistical value to interpreting the mean specific activities with and without exogenous coenzyme Q_3 for the groups of control and diseased gingival tissues, it is also important to inspect the data for the activities of both enzymes for individual patients, which is possible from the data in Tables 1 and 2. For example, 18 of the 24 control gingival tissues (Table 1), or 72%, showed no significant increase in specific activity when the assay for the succinate-CoQ_{10}-enzyme was carried out in the presence of exogenous coenzyme Q_3, which is as expected for reasonably normal tissue. One of the 24 specimens showed an increase with exogenous coenzyme Q_3 of CoQ_{10}-enzyme activity of only 20%, which is not considered significant. The remaining

TABLE 4. *Summary of data on the DPNH-cytochrome c reductase*

Gingiva	Specific activity: No addition	Specific activity: With CoQ_3
Control (24)	26.3 ± 17.7	25.5 ± 18.3
Diseased (40)	35.4 ± 21.0*	34.0 ± 15.9

* $t = 1.78$ ($0.05 < P < 0.1$).

5 of the 24 patients, or 20%, showed deficiencies of CoQ_{10}-enzyme activity with exogenous coenzyme Q_3 of 34, 35, 42, 44 and 49%. These increases of about 35–50% very likely indicate that these five subjects were not truly normal with respect to the nutritional levels of coenzyme Q_{10} in their gingival tissue and perhaps elsewhere in the tissues of their bodies. This observation that 20% of 24 patients biochemically show deficiencies of coenzyme Q_{10} when they are otherwise clinically considered "normal" on the basis of periodontal inspection, seems reasonable on the basis of nutritional science.

Similarly, inspection of the specific activities of the DPNH-cytochrome *c* reductase of each of the 24 patients (Table 1) shows that not a single patient in this control group showed a significant increase in CoQ_{10}-enzyme activity when the assay was conducted in the presence of exogenous coenzyme Q_3.

The data in Table 2 for the individual patients with severe periodontal disease show that about 63% of the group of 40 patients showed significant deficiencies of the succinate-CoQ_{10}-enzyme activity in contrast to about 20% for the control group. The difference between the specific activities of the succinate-CoQ_{10}-enzyme for the diseased and control gingiva is significant ($P < 0.02$). In the diseased gingival tissues, there was a significant increase in the activity of the succinate-CoQ_{10}-enzyme, showing a deficiency of coenzyme Q_{10}, but there was no comparable increase in the activity of the DPNH-CoQ_{10}-enzyme. In other words, the succinate-CoQ_{10}-enzyme seemed to be affected preferentially over the DPNH-CoQ_{10}-enzyme in some specific manner by whatever cause a nutritional deficiency of coenzyme Q_{10} develops in the individual.

We have previously reviewed (7) a nutritional basis for the development of a deficiency of coenzyme Q_{10} at its site in the succinate enzyme of diseased gingival tissue. A significant deficiency of coenzyme Q_{10} at this one site is understandable on the basis of a difference between the affinities of coenzyme Q_{10} at its sites in the two apoenzymes, according to the data presented. Presumably, the diseased gingival tissue could require more energy—achieved through the bioenergetics of respiration, which requires coenzyme Q_{10}—to generate new tissue. The apoenzyme for both succinate dehydrogenase-CoQ_{10} reductase and DPNH-cytochrome *c* reductase of the newly forming tissue may not acquire the needed coenzyme Q_{10} because the biosynthesis of coenzyme Q_{10} in the tissue is not increased. Coenzyme Q_{10} might be transferred from the succinate enzyme to the DPNH enzyme because of the higher affinity between coenzyme Q_{10} and the DPNH enzyme. Alternatively, the DPNH enzyme may preferentially receive newly biosynthesized coenzyme Q_{10} because of the higher affinity.

A deficiency of coenzyme Q_{10} at the succinate site of diseased gingival tissue could be the result of the disease process and/or just a classical nutritional deficiency in the body. That both causes of such a deficiency may take place is evidenced by the data in the control tissues.

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