

Deficiency of Coenzyme Q₁₀ in Gingival Tissue from Patients with Periodontal Disease

(nutrition/vitamin/dentistry/therapy/succinate dehydrogenase)

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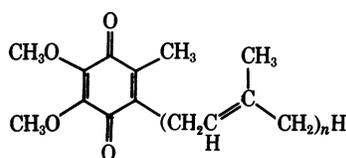
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ABSTRACT The specific activities of the succinate dehydrogenase-coenzyme Q₁₀ reductase in gingival tissue from patients with periodontal disease have been compared with the corresponding specific activities of normal human periodontal tissue. The gingival biopsies from patients having diseased periodontal tissue showed a deficiency of coenzyme Q₁₀, in contrast to those of the normal periodontal tissue which showed no deficiency. The presence or absence of a deficiency of coenzyme Q₁₀ in the succinate-coenzyme Q₁₀ enzyme system is appraised by determining the specific activity in the absence and again in the presence of exogenous coenzyme Q₃. An increase in specific activity of this mitochondrial enzyme system in the presence of exogenous coenzyme Q₃ reflects the mitochondrial deficiency of coenzyme Q₁₀. Such increases ranged from 38-120% and averaged 81% for the individuals with periodontal disease, and were highly significant statistically.

These data correlate with clinical studies in Japan that have indicated a therapeutic benefit of the administration of coenzyme Q₇ to many patients with severe and destructive periodontal disease and with the benefit of administration of hexahydrocoenzyme Q₄ to one such patient in the current work.

Coenzyme Q₁₀, [I](CoQ₁₀), exists in the mitochondria of all cells in the human body. Data on distribution and concentrations of CoQ₁₀ have been reported (1).



I, $n = 10$

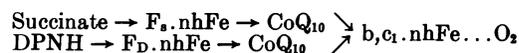
II, $n = 7$

III, $n = 3$

IV, $n = 4$ with terminal 3
units reduced

As a vitamin (2), CoQ₁₀ has an indispensable role in the electron transfer processes of respiration and coupled oxidative phosphorylation. While the presence of CoQ₁₀ in the respiratory chain is established, its position in relation to other components of the chain is not yet established. Nevertheless, the position of CoQ₁₀ has been localized and clarified by the

studies of several investigators, including Green (3), Ernster (4), and Chance (5) and their respective coworkers. In particular, it has been established that the molecular conformation of the enzyme sites of CoQ₁₀ for its coenzymatic role in succinoxidase and DPNH oxidase are different. Evidence for the existence of the two types of coenzymatic sites is based on many organic structure-coenzymatic activity relationships for members of the coenzyme Q Group (6) and derivatives according to Lenaz *et al.* (7), who depicted the two sites in the following abbreviated scheme.



A localization or enhancement of the deficiency of CoQ₁₀ at one or both of its enzymic sites in certain tissues could bear some correlation to disease of that specific tissue. An increasing deficiency of CoQ₁₀ would surely be correlated with an increasing severity of a disease associated with a specific tissue, regardless of its location in the human body.

The biosynthesis of coenzyme Q₁₀ in the human body is a nutritional process, since CoQ₁₀ is derived from dietary tyrosine by a largely known sequence of biosynthetic reactions (2). This sequence of reactions requires many of the known vitamins and minerals for the enzymic transformations. Any deficiency of one or more of the essential vitamins and minerals required for the biosynthesis of CoQ₁₀ could lead to a tissue deficiency of CoQ₁₀.

Deficiencies of CoQ₁₀ in mammals have been experimentally created in rabbits, which responded therapeutically to treatment with coenzyme Q (8). The vitamin activity of CoQ in other species that are placed on experimental diets indicates the existence of nutritional deficiencies of CoQ. These other species include rats (9), monkeys (10), chickens (11), and turkeys (11). The experimental diets that were fed to these diversified species bear certain nutritional similarities to the widespread dietary habits of man. Also, dietary deficiencies of vitamins, particularly folic acid, are more common for teenagers, adults, and the elderly than generally recognized. The report by Leery *et al.* (12) exemplified the existence of dietary deficiencies in the United States, which could readily lead to tissue deficiencies of CoQ₁₀.

The existence of CoQ₁₀ in the gingival tissue of man is apparent from the demonstration of the specific activities of the CoQ₁₀-enzyme system of mitochondrial origin described herein.

Abbreviation: CoQ, coenzyme Q.

This paper is No. CXL (140) in a series, Coenzyme Q.

Tanner (13) reported on a relationship between citric acid and periodontal lesions in rats. He observed that rats treated with citric acid exhibited severe porosity with atrophy and myelofibrosis accompanied by resorption of bone and hyperemia of the gingiva. Honjo *et al.* observed that aconitate hydratase (EC 4.2.1.3) and citrate hydro-lyase (EC 4.2.1.4) are stabilized by certain reducing agents, e.g., ascorbic acid. They tested coenzyme Q₇ [II] in scorbutic guinea pigs and found that prolonged administration normalized both the aconitate hydratase activity and the elevated citrate content of the alveolar bone. Matsumura *et al.* (15) administered CoQ₇ to rats receiving citric acid with effects on their periodontal lesions. Tsunemitsu *et al.* (16) described the protection afforded by CoQ₇ against the hypercitricemia resulting from alloxanization of rats and also observed that the hypercitricemia of some patients with periodontal disease was improved by the administration of CoQ₇. Akiyoshi *et al.* (17) described the effect of coenzyme Q₇ on pathologic changes of the periodontal tissues of guinea pigs maintained on a diet deficient for vitamin C.

Tsunemitsu and Matsumura (18) reported on the administration of CoQ₇ to patients who were 18–35 years of age and had severe and destructive periodontal disease and in whom the concentration of citrate in the blood was elevated. The hypercitricemia and clinical condition in 25 such patients was improved.

Nakamura *et al.* reported data, basic to studies on periodontal disease, concerning the phosphatase and transaminase activities of periodontal tissues in scurvy in guinea pigs (19, 20), and on the inhibition by citrate of alkaline phosphatase from alveolar bone (21).

It is the purpose of this paper to present a comparison of the specific activities of the succinate dehydrogenase-coenzyme Q₁₀ reductase of diseased and normal human periodontal tissue.

METHODS

Gingival Tissues. Gingival specimens were taken under local anesthesia from patients with chronic periodontitis and from patients without any clinical sign of inflammation in their gingival tissue. The tissues were removed with a scalpel, washed immediately with ice-cooled saline, and frozen until preparation of the mitochondrial enzymes. The specimens consisted of marginal gingiva that ranged from about 3–5 mm in width and from about 50–200 mg in weight.

Homogenization Procedures. Frozen tissue was placed on a dry ice-cooled Petri dish and was minced into very small pieces with a surgical scalpel. The minced tissue was added to 15 ml of 0.01 M Tris buffer—0.25 M sucrose (pH 7.8). This tissue suspension was homogenized for 3 min in a Potter-Elvehjem teflon-glass homogenizer at 200 rpm. The pestle clearance was 0.15 mm. All steps of this procedure and the subsequent isolation of mitochondria were conducted at 0°C.

Isolation of Mitochondria. The homogenate was centrifuged in 30 ml of the sucrose-Tris solution at 1000 × *g* for 15 min. The cellular pellet was discarded and the supernatant was centrifuged for 20 min at 25,000 × *g*. The resulting mitochondrial pellet was suspended in 0.25 M sucrose and analyzed immediately.

Enzyme Assay. Succinate dehydrogenase-CoQ reductase was assayed according to the method of Ziegler and Rieske

TABLE 1. Activities of the succinate dehydrogenase-coenzyme Q₁₀ reductase of periodontal tissue from diseased humans

Patient Sex	Age	Extent of the periodontal involvement	Specific activity	Specific	%
				activity with CoQ ₃	Increase with CoQ ₃ *
M	34	Minor	7.3	11.1	52
M	41	Moderate	13.4	26.2	95
F	32	Moderate	11.8	23.7	101
F	62	Moderate to severe	23.6	38.5	63
F	45	Moderate to severe	12.4	21.3	71
M	29	Moderate to severe	10.9	24.1	120
M	57	Severe	13.2	26.7	101
F	23	Severe	3.0	6.0	100
F	40	Severe	13.2	26.2	98
F	18	"Diabetic"	10.3	20.6	100
—	—	Diseased	11.9	16.4	38
—	—	Diseased	9.4	16.3	74
—	—	Diseased	16.3	27.5	68

Statistical analysis by Student's *t*-test: mean ± SD, 12.1 ± 4.76 (without CoQ₃); 21.9 ± 8.23 (with CoQ₃); *t* = 8.23 (*P* < 0.001).

* Average % increase, 81%.

(22), with a Hitachi Perkin-Elmer 139 spectrophotometer and microcuvettes having a 1-cm light path. The final reaction volume was 0.3 ml. Protein was determined by the method of Lowry *et al.* (23). The specific activity was expressed as nmol of dichlorophenol indophenol (DCIP) reduced per min per mg of protein. A mM extinction coefficient of 20 was used for the DCIP.

RESULTS AND DISCUSSION

In order to compare the specific activities of the succinate dehydrogenase-CoQ₁₀ reductase of normal human gingival tissue with that from patients having diseased tissue, gingival specimens were taken by the customary practice of the periodontist in hygienic treatment, and normal gingival tissue was obtained during the normal process of extraction of teeth. The patients in Tables 1 and 2 are typical and representative of the people who visit the periodontist or the dentist for treatment. These patients with periodontitis ranged in age from 18–62 years and were of both sexes. One patient had diabetes but, as usual, the nutritional and medical condition of the others who visited a periodontist or dentist was largely unknown. The normal individuals (controls) included teenagers, who may be presumed to be more "normal" than older

TABLE 2. Activities of the succinate dehydrogenase-coenzyme Q₁₀ reductase of periodontal tissue from normal humans

Patient Sex	Age	Specific activity	Specific	%
			activity with CoQ ₃	Increase with CoQ ₃ *
F	24	5.0	5.0	0
M	16	4.8	4.8	0
F	19	4.0	4.0	0
F	21	1.6	1.6	0
M	20	3.6	4.9	36

* Average % increase, 7%.

adults. The data in Table 1 are for 13 patients with diseased periodontal tissue and the data in Table 2 are from the tissue of five normal patients. Obviously, it is not often feasible to obtain gingival tissue from patients whose gingiva are normal.

All of the patients summarized in Table 1 had gingival tissue that were deficient in CoQ₁₀. This deficiency was revealed by determination of the specific activity of the succinate dehydrogenase-coenzyme Q₁₀ reductase isolated from the mitochondria. The specific activity of this CoQ₁₀-enzyme system from normal mammalian tissue, whether human or animal, is unchanged when the determination is repeated in the presence of exogenous CoQ. A repetition of the determination of the specific activity in the presence of exogenous coenzyme Q can reveal the existence of a deficiency of CoQ₁₀, and the magnitude of the increase in the specific activity directly corresponds to the magnitude of the deficiency. Additional background, theoretical considerations, and data in support of these statements have already been described in detail (25).

The periodontal tissue of only 1 of the 13 patients mentioned in Table 1 had an increase in specific activity with CoQ of less than 50%. Tissues of 7 of the 13 patients had increases of 95–120%. The average increase was 81%. The difference between the specific activities in the absence and presence of exogenous CoQ₃, were 12.1 and 21.9, respectively: $t = 8.23$; $P < 0.001$.

It is important to note that the extent of the periodontal involvement for 13 individuals ranged from minor to severe; in every case the mitochondrial CoQ₁₀-enzyme systems had a higher specific activity in the presence of exogenous CoQ₃, which showed the receptiveness of this enzyme to therapeutic improvement. One may presume that had coenzyme Q been administered therapeutically to these patients with periodontal disease, the condition and health of the tissue could have been substantially improved.

These data, which show a biochemical impairment of the essential energetic mechanisms for respiration and oxidative phosphorylation in gingival tissue, open up new ideas for research as well as the apparent therapy for periodontal disease that can accompany the traditional hygienic treatment. In 1969, Løe (25) made two statements of concept at the International Conference of Periodontal Research (a) "Bacterial plaque on teeth and gingiva is the only direct cause of marginal periodontal disease" and (b) "Provided plaque formation can be controlled, it is possible to maintain a qualitatively and quantitatively normal periodontium throughout old age". He also said: "—it seems justified to conclude that general malnutrition and lack of specific dietary compounds, do not cause periodontal disease, but may in a modest way influence the progress of already existing lesions". Although Løe did add that the above statements "may not be entirely correct", it is evident that his statements represent the current thinking of the majority of the members of the dental profession.

These studies on the relationship between CoQ and periodontal disease not only breach these statements of Løe, but raise the possibility that inadequate nutrition and a deficiency of coenzyme Q₁₀ may be primary rather than secondary to the problems of bacterial plaque.

The specific activities of the succinate dehydrogenase-coenzyme Q₁₀ reductase in tissues of humans with periodontal disease (Table 1) ranged from 3 to 24, with an average of 12. In comparison, the specific activity of this system for normal

human periodontal tissue averaged 3.8. The higher average specific activity for the diseased tissue has been confirmed (unpublished results).

This higher enzyme activity for diseased tissue, in contrast to normal tissue, has been observed by other criteria by other investigators. For example, Glickman *et al.* (26) have found, by histochemical techniques, a tendency toward increased oxygen consumption in gingival tissue with marked chronic inflammation and proliferation of connective tissue and epithelium. Burstone (27) found higher values for cytochrome in gingival tissue that were chronically inflamed, in comparison to the values for normal gingival tissue.

During these biomedical studies, a 43-year-old male who weighed 148 pounds and had periodontal disease came into our study. His gums were edematous and red and there was gingival bleeding. The right-lower-central incisor was loose. He was given 1 g of hexahydrocoenzyme Q₄ [IV], formulated in corn oil, on a daily basis. 1 Month later, the dosage was reduced to 500 mg of hexahydrocoenzyme Q₄, in the same formulation, for the duration of the study. At the time of dose reduction, the gums were less red and the patient reported that there was no more local itching, and that the abnormal taste was gone. 2 Months later, the incisor was not as loose and the abnormal taste was still absent, but there was occasional itching. X-ray examination revealed no evidence of special abscess or devitalized teeth. Alveolar recession was present in the lower anterior mandible. Bilaterally impacted third molars were present; on the left they were approaching a horizontal axis. 4 Months from the initiation of treatment, a report by the dentist stated that the gums were tighter and showed more pinkness. About 1 year later, another report by the dentist revealed a tremendous improvement in the gingival tissue, which was appropriately pink with normal stippling and no edema. The dental examination 8 months earlier had revealed edema with very red and bleeding tissue and with no stippling. It was concluded that "there has been a tremendous improvement". Other studies on the administration of coenzyme Q to patients with different degrees of periodontal disease are in progress.

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