

# Diabetes and Periodontal Disease

## Possible Role of Vitamin C Deficiency: An Hypothesis

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AN HYPOTHESIS is proposed relating the possible role of vitamin C deficiency as an etiologic factor contributing to periodontal disease in diabetes. The hypothesis is based upon the following: (1) transport of ascorbate across cell membranes may be impaired by glucose, but facilitated by insulin; (2) glucose utilization is significantly accelerated by sublethal concentrations of endotoxin; (3) endotoxin-induced histamine sensitivity of tissue is enhanced by ascorbic deficiency; and (4) ascorbic acid deficiency alters mucosal barrier function. The interrelationship of these factors is discussed.

Several studies have reported that the prevalence and severity of periodontal disease are significantly greater among people with diabetes than among those free of the disease.<sup>1-3</sup> The loss of alveolar bone<sup>4, 5</sup> also has been found to be greater among people with diabetes and to be more pronounced as the severity of the disease is increased.<sup>6</sup> A few studies, however, have failed to find an association between either bone loss, periodontal disease and diabetes.<sup>7, 8</sup>

The National Health Survey<sup>9</sup> attempted to correlate the association of periodontal disease and diabetes. Because the number of men and women with diabetes was small, the data from the study was not conclusive. The data also included conditions which were not directly associated with diabetes, but which many times are the sequelae of diabetes or often indirectly associated with the disease. If one views the data collectively, there is a strong suggestion that a relationship does indeed exist between diabetes and periodontal disease, but the association is difficult to prove conclusively.

### RATIONALE FOR THE DEVELOPMENT OF THE HYPOTHESIS

It is generally accepted that endotoxin is an etiologic agent in periodontal disease. This laboratory<sup>10</sup> has shown that the cementum of periodontally involved teeth exposed to the disease process contains endotoxin or endotoxin-like products; the remaining or uninvolved cementum is free of these materials. Also, it was established that these products are phenol-extractable and that they are toxic to cultured fibroblasts.

In another study<sup>11</sup> this laboratory demonstrated that human gingival fibroblasts did not attach to the portion of the root exposed to the disease process, *in vitro*,

whereas the remainder of the root allowed cells to attach, normally. Prior extraction of the roots with phenol-water or the mechanical removal of diseased cementum allowed the cells to attach normally. All things being equal, the extrapolation of these data to an *in vivo* situation dictates that a clinical success would depend upon complete removal of toxic materials from diseased cementum or the removal of the cementum itself.

A further study<sup>12</sup> in this laboratory found that concentrations of endotoxin which do not affect cell viability were cytotoxic to cellular organelles. All organelles studied displayed some degree of alteration which became more severe as the concentration of endotoxin was increased.

The most recent work in this laboratory<sup>13</sup> involved the study of macromolecular synthesis by endotoxin-treated fibroblasts. Because collagen synthesis was one of the macromolecules studied, ascorbic acid was added to the culture medium to insure maximum synthesis. During the course of these experiments, it was found that ascorbic acid had a consistent protective effect against the toxicity of the endotoxin added to the model system; further studies<sup>14</sup> confirmed these observations.

### ASCORBIC ACID IN CONNECTIVE TISSUE METABOLISM

The biochemical lesions of scurvy and the known actions of ascorbic acid in biologic systems have been summarized in several reviews.<sup>15-22</sup> Probably the best known and most intensively studied effect of scurvy is the lesion in wound healing and connective tissue formation; wounds which are allowed to heal in a scorbutic animal lack tensile strength and reopen easily. These lesions can be attributed to the breakdown of mesenchymal connective tissue, probably as a result of the failure of new collagen synthesis to replace losses resulting from normal turnover.<sup>23-24</sup> Without adequate Vitamin C, connective tissue cells fail to achieve optimum hydroxylation

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of proline and lysine in nascent collagen, and produce a molecule that is deficient in hydroxyproline and hydroxylysine. This abnormal molecule is denatured at body temperature, and may either accumulate within the cells or be secreted in a form which cannot form fibers.<sup>25-27</sup> The molecular lesion is related to a cofactor-like role of ascorbic acid in the action of prolyl and lysyl hydroxylases.<sup>28, 29</sup>

#### DEVELOPMENT OF THE HYPOTHESIS

##### The Effect of Glucose on Ascorbic Acid Transport

Gore et al.<sup>30</sup> have shown with electron microscopy that the vascular lesion of scurvy involves collagenous structures in the basement membranes, and this is also the site of lesions in diabetic microangiopathy.

These data propose that the intracellular availability of ascorbic acid would be impaired in certain tissues by either hyperglycemia or lack of insulin. The suggestion is that diabetic microangiopathy, the main complication of human diabetes, may be a consequence of local ascorbate deficiency.<sup>31</sup>

Mann and Newton<sup>32</sup> have shown that glucose will impair the transport of ascorbic acid into human red blood cells, a tissue not dependent on insulin. Other sugars that inhibit transport show a hierarchy, D-glucose = D-mannose > L-arabinose = D-xylose > D-fructose = L-sorbose suggesting that these sugars share a common transport mechanism with ascorbate. Species that require dietary ascorbate, such as man, must also have an appropriate mechanism for transporting this into cells. The available data then, support the hypothesis that hyperglycemia, no matter what the cause, will restrict the intracellular supply of ascorbate.

##### The Effect of Endotoxin on Glucose Utilization

Hinslaw et al.<sup>33</sup> explored the influences modifying glucose uptake in canine blood administered LD<sub>100</sub> *E. coli* endotoxin. Assaying the role of the white blood cell in glucose utilization, they found a significant increase in glucose uptake and lactic acid production. They also found that blood from dogs pretreated with sublethal doses of endotoxin, *in vivo*, utilized glucose at an accelerated rate when subjected to endotoxin, *in vitro*. Further studies<sup>34</sup> also indicated that glucose utilization is significantly accelerated by sublethal and lethal concentrations of either *E. coli* endotoxin or live *E. coli* organisms incubated with canine blood under controlled *in vitro* conditions.

Berry et al.<sup>35</sup> also confirmed the fact that endotoxins, in the form of crude vaccines or as purified cell wall lipopolysaccharides, deplete animals of their carbohydrate reserves, resulting in early hyperglycemia and eventual hypoglycemia. These same investigators pointed out that endotoxin stimulates cellular glycolysis in a fashion similar to that obtained with insulin. They also observed that endotoxin increased the consumption

of glucose by leukocytes and macrophages. This work would suggest that endotoxin possesses an insulin-like action.

Wolfe et al.<sup>36</sup> studied the effects of lethal doses of *E. coli* endotoxin in glucose kinetics and cardiovascular responses of conscious dogs. The plasma glucose level fell steadily after endotoxin administration to severely hypoglycemic levels preterminally. The fall in plasma glucose appeared to be due primarily to an increased uptake in the early phase, while decreased hepatic glucose output also contributed to the later, preterminal phases.

##### The Effect of Insulin on Ascorbate Transport

Ralli and Sherry<sup>37, 38</sup> observed a diminution of the level of plasma ascorbate in dogs after the administration of insulin. Since neither the urinary ascorbate nor metabolic products were increased by this treatment with insulin the effects were presumed to be a result of accelerated tissue uptake. The implications of these findings are that the uncontrolled diabetic, may experience tissue deficiency of ascorbate. Pauling's<sup>39</sup> contention that the human requirement for ascorbate may be much larger than the officially defined requirement may be important in the diabetic state, whereas prolonged periods of marginal deficiency may result in changes in both the diabetic and nondiabetic.

##### The Alteration of the Mucosal Barrier by Ascorbate Deficiency

Ascorbic acid deficiency has been quantitatively related to an altered mucosal barrier function,<sup>40, 41</sup> decreased resistance to infectious disease,<sup>42</sup> increased incidence of periodontal disease,<sup>43</sup> and altered synthesis of basement membrane collagen.<sup>44, 45</sup>

Alfano et al.<sup>46</sup> showed that oral mucosal tissues from scorbutic animals demonstrated significantly greater permeability to [<sup>3</sup>H]endotoxin than did tissues from normal healthy animals and that ascorbate deficiency on mucosal barrier function is not rapidly reversible. They suggest that ascorbate may not be stored to appropriate levels in mucosal epithelium thereby compromising the barrier function of the basement membrane. Their findings support the contention that acute episodes of malnutrition may potentially compromise host defenses for prolonged periods of time and may thus predispose to diseases such as periodontal disease.

##### Ascorbate Deficiency, Endotoxin and Histamine Release

It has been reported that endotoxin from various Gram-negative organisms is capable of sensitizing mice to histamine.<sup>47-51</sup> However, the histamine sensitizing agent is digested by trypsin and inactivated by heat at 100°C thereby indicating a protein component. Heating and trypsinization, however, does not affect either the direct toxicity of endotoxin or the lethal rate of histamine

shock; indirect endotoxin effects have been proposed. Endotoxin, apparently induces synthesis of histamine resulting from endotoxin-mediated activation of the enzyme histidine decarboxylase.<sup>47</sup>

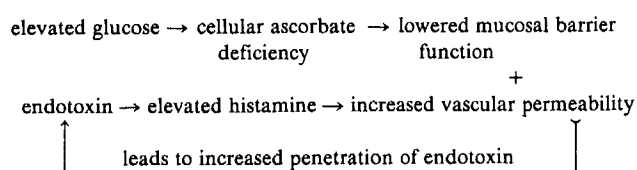
Lewis and Nicholls<sup>52</sup> studied the effect of ascorbic acid deficiency in relation to the sensitivity of the ileum to histamine and the release and catabolism of histamine in guinea pigs. They observed that, although there is no change in the metabolism of histamine in scurvy, there is a definite increase in the sensitivity of tissue to histamine.

If rats are administered ascorbic acid along with drugs which increase histamine levels, there results decreased urinary histamine levels indicating detoxification of histamine. In the guinea pig, histamine producing or histamine releasing drugs result in a decreased urinary ascorbic acid level indicating greater utilization of the ascorbate.<sup>53</sup> Furthermore, in guinea pigs fed an ascorbate-free diet, as the blood ascorbate content began to fall, the blood level of histamine started to rise steadily, reaching a maximum long before the onset of scurvy.<sup>54</sup>

*Based Upon the Above Information, the Following Hypotheses are Proposed:*

1. Transport of ascorbate across cell membranes may be impaired by glucose, but facilitated by insulin.
2. Glucose utilization is significantly accelerated by sublethal concentrations of endotoxin.
3. Endotoxin-induced histamine sensitivity of tissue is enhanced by ascorbic acid deficiency.
4. Ascorbic acid deficiency alters mucosal barrier function.

If these hypotheses are valid, those species requiring exogenous ascorbate, such as man, would be in greater difficulty if they were also hyperglycemic, as in uncontrolled diabetes. That is to say that the intracellular availability of dehydroascorbate (DHA), the transportable form of Vitamin C, would be impaired in certain tissues by either hyperglycemia or lack of insulin. Furthermore, if tissues in the uncontrolled diabetic are challenged with endotoxin (as in periodontal disease) they would be in triple jeopardy since (1) endotoxin accelerates tissue utilization of glucose, (2) accelerated glucose transport compromises the intracellular supply of ascorbate, and (3) endotoxin-induced histamine sensitivity of tissues is enhanced by ascorbate deficiency, directly. The resulting high activity of histamine has a response in the reaction on blood vessels (increased permeability) which allows more endotoxin to enter the circulation. Thus, a vicious cycle is produced:



#### Significance of the Proposed Hypothesis

There is little doubt that endotoxin is involved in periodontal disease. Certainly its role in the chronicity of the disease is not disputed, but its role in initiation is more complex. Since this laboratory first discovered the presence of cementum-bound endotoxin in the cementum of periodontally-involved teeth, much research and effort have been directed towards either chemically modifying the toxic principle of periodontally-involved roots or mechanically removing the offending agent(s). The original discovery, in effect, has markedly influenced the therapeutic management of the disease.

The recent finding by this laboratory that ascorbic acid under specific conditions is able to modify the toxicity of endotoxin, opens a new approach to the potential management of cementum-bound endotoxin in periodontal disease. The use of ascorbic acid therapy in periodontal disease therapy has a long history with very little conclusive evidence that it is of value. This paper proposes that ascorbic acid's lack of efficacy is related primarily to its transport across cell membranes. In the case of diabetes where the transport of ascorbic acid across cell membranes is influenced by hyperglycemia, the problem of intracellular ascorbic acid deficiency becomes more apparent and may describe to some extent the nature of periodontal disease in diabetes.

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