# TISSUE FLUID FLOWING INTO GINGIVAL POCKET

BY

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## ABSTRACT

The tissue fluid flowing into 129 human gingival pockets of upper anterior teeth in 15 minutes was collected on strips of filter paper. The tissue fluid from each pocket was weighed and the ninhydrin positive substance or free amino acids contained in it was estimated. These results were compared with clinical and histological findings. The weights of tissue fluid and the amounts of ninhydrin positive substance from all clinically healthy gingival pockets were found to be almost the same. There was a correlation between the tissue fluid weight and the amount of the ninhydrin positive substance in every type of periodontal disease. The amount of ninhydrin positive substance tended to increase with the progress of this disease. In many cases the amount of the ninhydrin positive substance increased where inflammatory cell infiltration and degeneration in subepithelial tissue and/or cell infiltration and roughness in pocket epithelium were recognized. The small amount of free amino acids per mg of tissue fluid was recognized in the case of gingivitis. The amount increased sharply in the case of slight periodontitis, but in the case of severe periodontitis, decreased slightly on the contrary of increase in the total ninhydrin positive substance.

## INTRODUCTION

Little is known how metabolic changes occur in periodontal tissues when periodontal disease is present[1]. Waerhaug[2,3] reported when he inserted indian ink or dye into the gingival pocket, they were eliminated in a short time. He supposed that this was a constant flow of tissue fluid into the pocket. McMillan, Burrill and Fosdick[4] found the collagen fiber in the exudate from the gingival pocket under the electron microscopic observation. Brill and others[5,6,7,8,9,10,11] reported that fluorescent sodium was found in a clinically healthy or inflamed gingival pocket shortly after it had been introduced into the subject, and that this amount of fluorescent sodium increased when inflammation and/or chemical stimulation was present.

If this is true, the fluid flowing into the gingival pocket may be most convenient window through which the metabolism of gingiva can be observed, and the study of this fluid may supply a new basic concept to peri-

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odontology. Brill[7,10] reported that the fluid from a clinically healthy gingival pocket contains a substance that has an antimicrobial effect, but this was denied by Collins and Gavin[12,13]. Gustfsson and Nilsson[14] reported that this fluid has a fibrinolytic system. The only chemical substances reported to be present in this fluid, however, were the ninhydrin positive substance reported by Brill[9], and the free amino acids and sugars reported by Konno[15].

Brill[9,11] was the only one who carried out a quantitative study of this fluid, but it seems that his work was not thoroughly enough from the quantitative standpoint because he measured the tissue fluid only planimetrically. The authors have therefore concentrated the study on the quantitative analysis of this fluid.

## Materials and Methods

This study was carried out on 129 gingival pockets of the upper anterior teeth of patients aged 17 to 37, who visited the hospital of the School of Dentistry, Tokyo Medical and Dental University. Each gingival pocket was classified according to the progress of the disease; clinically healthy gingiva, gingivitis, slight, mild and severe periodontitis.

The collection of tissue fluid was avoided for two hours after eating.

To remove the food debris and saliva, the gingival pockets were washed by spray and were dried with gauze and air. Vasline was put in the pockets of the upper premolors. Then the tissue fluid flowing into the gingival pocket was collected by filter paper strips sized $1 \times 10$ mm. The paper strips were inserted into each labial gingival pocket (8 strips at a central incisor, 6 strips at a lateral incisor and 9 strips at a canine) and were taken out 15 minutes later after drying.

A biopsy was performed for the labial marginal gingiva of canine, after the collection of fluid, to determine the histological condition. This biopsy material was fixed by Carnoy's solution, embedded in paraffin and stained by hematoxylin and eosin.

The weight of the tissue fluid was calculated from the difference of the weights of the strips of paper before and after insertion in the pockets. After this weighing, the amount of the ninhydrin positive substance in this fluid was determined by Yemm Cocking's method[16]. Glutamic acid was used as the standard.

In order to analyze quantitatively and qualitatively the ninhydrin positive substance, the tissue fluid in the strips was eluted in distilled water with heating. Then one dimensional paper chromatographic investigation was carried out. Butanol, acetic acid and water (4, 1, 2) were used as sol-

vents and 2% ninhydrin solution for coloring. Rf value was measured and the colored spots of amino acids were cut off. These cut pieces were eluted with 50% ethanol and the amount of amino acid was determined electrophotometrically at 570 mμ. Glutamic acid was used as the standard.

## Results

The fluid weights and the amount of ninhydrin positive substance of the various kinds of the clinically healthy teeth are shown in Table 1. Although the number of cases examined was few, there was not much

Table 1. Tissue fluid weight and ninhydrin positive substance weight in the case of clinically healthy gingiva

| | Tissue fluid weight | Ninhydrin positive substance weight |
|---|---|---|
| Central incisor | 0.6 mg | 15.3 γ |
| | 1.0 mg | 21.8 γ |
| Lateral incior | 0.6 mg | 12.1 γ |
| | 0.7 mg | 15.8 γ |
| Canine | 0.8 mg | 19.4 γ |
| | 1.3 mg | 23.4 γ |

Table 2. Relation between tissue fluid weight, ninhydrin positive substance weight and the progress of disease

| | No. of case | Tissue fluid weight | Ninhydrin positive substance weight | Ninhydrin positive substance weight/mg | regression line |
|---|---|---|---|---|---|
| Clinically healthy | 6 | 0.83±0.25 mg | 17.97± 3.90γ | 22.07± 6.12γ | |
| Gingivitis | 43 | 0.91±1.11 mg | 48.05±61.54γ | 52.80±63.12γ | $y=0.016x+0.14$ $r=0.87$ |
| Slight periodonititis | 47 | 1.38±1.30 mg | 82.33±45.48γ | 59.70±49.51γ | $y=0.014x+0.23$ $r=0.77$ |
| Mild periodontitis | 23 | 1.05±0.79 mg | 81.38±43.50γ | 77.47±49.70γ | $y=0.014x-0.09$ $r=0.79$ |
| Severe periodontitis | 10 | 1.03±0.72 mg | 232.63±75.85γ | 225.88±130.59γ | $y=0.011x-1.52$ $r=0.62$ |

difference among the various types of teeth.

The fluid weight and the amount of ninhydrin positive substance in various cases ranging from normal to severe periodontitis are shown in Table 2. The significant difference in fluid weight according to severity of periodontal disease could not be determined because of the large deviation

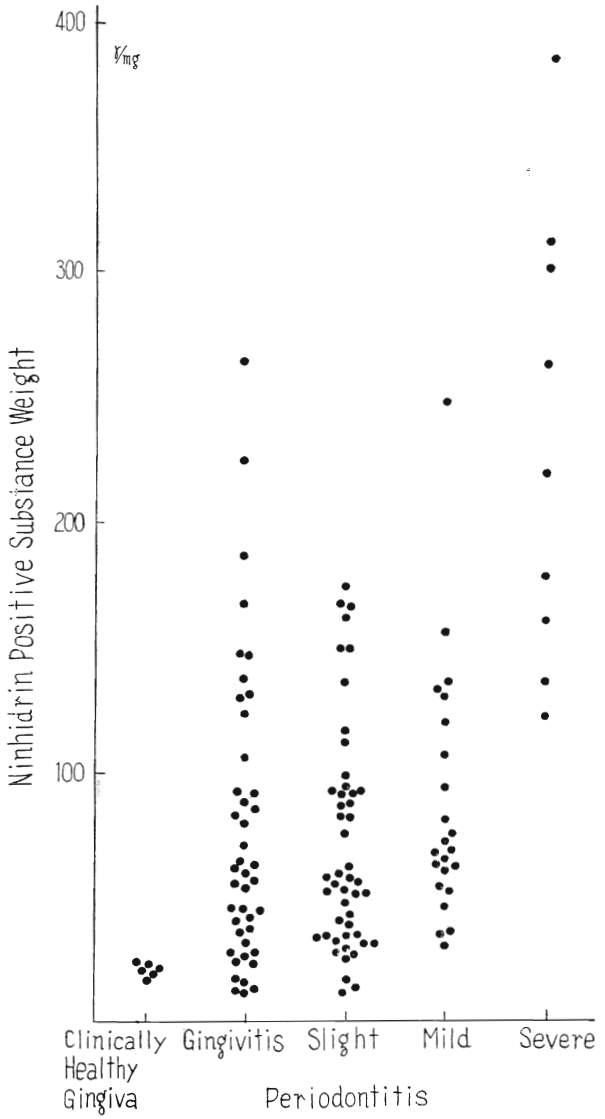


**Fig. 1.** The relation between ninhydrin positive substance weight per 1mg fluid weight and severity of the disease

in weight. But there was a distinct difference in the amount of ninhydrin positive substance between the case of severe periodontitis and the case of clinically healthy gingiva or the substance per 1 mg fluid weight increased with the progress of periodontal disease.

The relation between the fluid weight, and the amount of ninhydrin positive substance in the various disease is shown in Fig. 2. The straight lines in this figure are the regression lines. The equations for these lines are shown in Table 2. These results show that there is a correlation between fluid weight and ninhydrin positive substance weight in each case.

Table 3 and 4 show the relation between the histological changes and

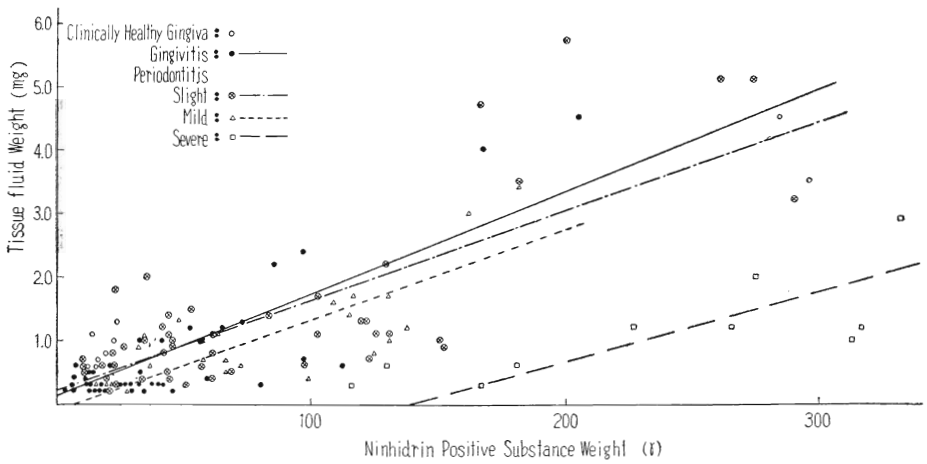


Fig. 2. The relation between fluid weight, ninhydrin positive substance weight and severity of the disease

Table 3. Relations between histological changes in subepitherial tissue and tissue fluid weight or ninhydrin positive substance weight

| | | Hypereamia | | Edem | | Inflammatory Cell infiltration | | Degnetation | |
|---|---|---|---|---|---|---|---|---|---|
| | | + | − | + | − | + | − | + | − |
| Tissue fluid weight | Above 1.6mg | 3 case | 6 | 4 | 5 | 8 | 1 | 6 | 3 |
| | Under 1.5mg | 13 | 17 | 15 | 15 | 28 | 2 | 18 | 12 |
| Ninhydrin positive substance weight | Above 30.1 γ | 13 | 15 | 15 | 13 | 27 | 1 | 20 | 8 |
| | Under 30.0 γ | 3 | 8 | 4 | 7 | 9 | 2 | 3 | 8 |

Table 4. Relations between histologic changes in pocket epithelium and tissue fluid weight or ninhydrin positive substance weight

| | | Break-down | | Roughness | | Inflammatory cell infiltration | |
|---|---|---|---|---|---|---|---|
| | | + | − | + | − | + | − |
| Tissue fluid weight | Above 1.6 mg | 5 | 4 | 8 | 1 | 7 | 2 |
| | Under 1.5 mg | 14 | 16 | 27 | 3 | 24 | 6 |
| Ninhydrin positive substance weight | Above 30.1 γ | 13 | 15 | 26 | 2 | 24 | 4 |
| | Under 30.0 γ | 4 | 7 | 8 | 3 | 7 | 4 |

the tissue fluid weights or the amount of ninhydrin positive substance. When the roughness and the cell infiltration in pocket epithelium and the cell infiltration and the degeneration in subepitherial tissue were recognized, the amount of ninhydrin positive substance tended to increase. But any significant correlation could not be found between the fluid weight and the histological change.

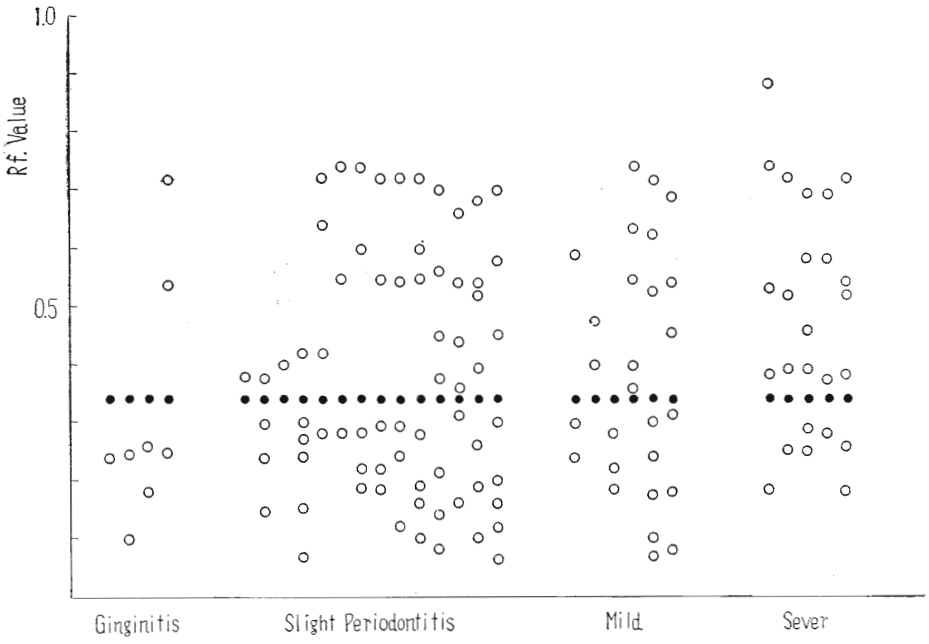


Fig. 3. Spots of free amino acids contained in tissue fluid
● : Glutamic acid

In paper chromatographic studies, glutamic acid appeared in every case, and several other spots were found, but these spots were not identified (Fig. 3).

Fig. 4 shows the free amino acid amount per 1 mg fluid weight and in various stages of periodontal disease. The amino acid amount increased sharply from gingivitis to slight periodontitis, but then decreased slightly in the case of severe periodontitis.

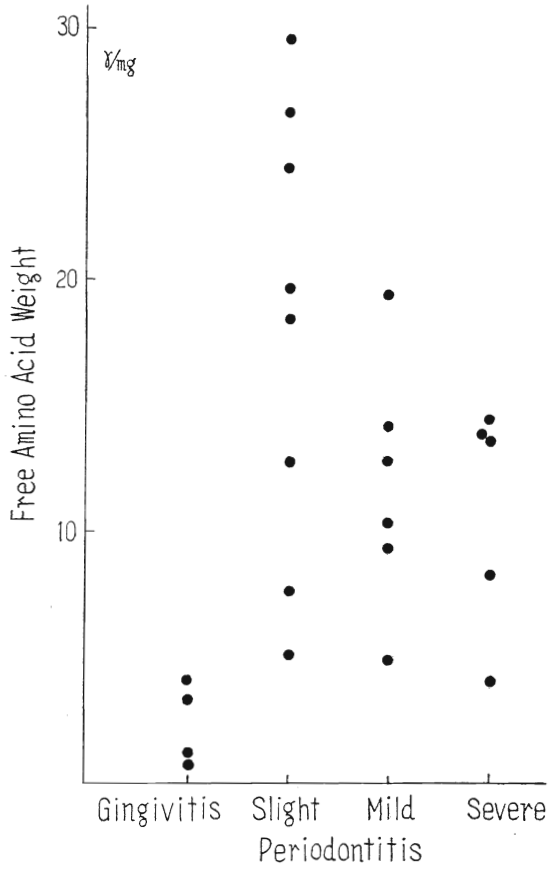


**Fig. 4. The relation between free amino acid weight per 1mg fluid weight and severity of the disease**

## Discussion

We authors believe the present method is more perfect than those which have been reported as far because the tissue fluid was collected with filter paper strips inserted in the gingival pocket for 15 minutes, and then

weighed and analyzed.

The numbers of filter paper strips inserted in the pocket, 8 to a central incisor, 6 to a lateral incisor and 9 to a canine were decided on the basis of the minimum acceptable numbers for each upper anterior tooth.

The tissue fluid weight and the amount of ninhydrin positive substance in clinically healthy gingiva, are shown in Table 1, and were negligible. So we concluded that the number of strips used did not have any effect on these weights.

The amount of free amino acid in saliva is reported to be 0.34–0.48 $\gamma$ per mg of saliva[17]. This value is lower than the amount of free amino acid in tissue fluid, so an apprehension to have dealt with saliva is excluded.

The amount of ninhydrin positive substance flowing into gingival pocket increased according to the clinical and histological change of periodontal tissue. Such a correlation, however, could not found in the fluid weight. Therefore, the amount of ninhydrin positive substance seems to serve more sensitively as an index to the progress of periodontal disease. On the other hand, Brill[11] reported that the gingival fluid of the dog fed with soft diet and having gingival inflammation was more than the healthy gingival fluid of the dog fed with hard diet. So he concluded that, when inflammation was present in the gingiva, the amount of tissue fluid flowing into the gingival pocket increased. This conclusion may be true. But this experiment seems to be not entirely quantitative on account of using planimetry and only two dogs were used.

In the one dimensional paper chromatographic study, the kinds of free amino acids that appeared were few in gingivitis, increased with the progress of periodontal disease up to a certain point, then decreased slightly as the disease became more severe. Glutamic acid appeared in all cases. This result was similar to Konno's data, but the kinds of amino acids observed in present study was less than that he detected, because he used a greater amount of tissue fluid.

Fig. 4 shows that free amino acid weight per 1 mg tissue fluid increased sharply from gingivitis to slight periodontitis, but then decreased slightly in the case of severe periodontitis. But the total amount of ninhydrin positive substance per 1 mg tissue fluid increased with the progress of the disease. These different tendencies between the ninhydrin positive substance weight and the free amino acid weight indicate that protein or high molcular peptide increases in the case of severe periodontitis. It is very interesting to note that the quality of ninhydrin positive substance changes in the various stages of the disease.

Studies of metabolic changes which occur with the progress of periodontal disease have been carried out by measuring gingival respiration and

dehydrogenase activities[18,19,20]. We believe that the inner relationship between these phenomena will be clarified with the accumulation of such studies.

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