On the Concentration of Each Element, Zinc and Copper in Human Saliva Concerning the Taste Acuity

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SUMMARY

Measurments were made of zinc and copper levels in saliva by atomic absorption spectrophotometry. The zinc concentrations were 0.116 ± 0.034 ppm (mean ± S.D.) in resting mixed saliva and 0.117 ± 0.024 ppm in supernatant of the saliva.

The mean concentration of copper in supernatant is significantly higher ($p<0.01$) than in whole saliva.

There was no distinct correlationship between ninhydrin positive substances and zinc contents in whole saliva.

Ninhydrin-positive substances in diabetic saliva was significantly higher than normal subjects.

Paper electrophoresis was done using diabetic saliva, then we noticed four bands of ninhydrin positive substances. The largest amount of zinc was found at the nearest anode side band and no zinc was found in the band to the cathode.

INTRODUCTION

Saliva has increasingly been included in clinical laboratory specimens as reported by Okuda[1,2]. The zinc concentration in the blood has been known to have a relation with taste acuity. On the research of trace minerals, Mathur[3], and Wallenius[4] reported a relation between zinc contents in saliva and blood. Henkin[5,6] and Tomita *et al.*[7,8] measured amount of zinc in saliva, and discussed relation between zinc level in saliva and taste disorders.

Some papers discribed a relationship between salivary metals and nutrients in rats and humans[9-11]. Nilmer *et al.*[12] mentioned the amount of metal in saliva derived from dental metalic restoration and due to galvanic actions. The results of these measurements of zinc or copper, however, varied widely among investigators. In addition, a direct comparison of the metal concentrations does not make much contribution to the analysis of the concerns. It seems rather useful to analyze the data from the measurement of salivary metals in relation with the amount of glycoprotein (e.g. mucin) or sugar in saliva.

We measured the zinc and copper levels in the saliva of normal human subjects, and their relation with organic substances in the saliva was investigated to obtain pertinent information available for establishment of a reasonable method for analyzing the concentrations of metals in the saliva. To make up the limited number of the subjects with taste disorders, patients with diabetes were included in the present measurement because diabetes is accompanied with hypogeusia in some cases.

MATERIALS AND METHODS

1. Saliva collection

From 33 normal and 15 diabetic subjects, at least one hour after meal and rinsed out their mouths, then resting mixed saliva was collected in Zn- and Cu-free sample vials rinsed with deionized water. Unless measured immediately, the samples were frozen. At the time of measurement, they were thawed, diluted two times with deionized water, and used as the whole saliva samples. The supernatant of saliva were prepared by centrifugation at 1600 g for 15 minutes.

2. Saliva analysis

Zinc and copper levels were measured by atomic absorption spectrophotometory using Simazu Model AA-640-12. The sample was aspirated and atomized into acetylen flame. The analysis were done at 2139 Å for Zn and 3247 Å for Cu.

For measuring ninhydrin-positive substances, the sample and the ninhydrin reagent of amino-acid-autoanalysis grade were heated for 30 minutes in the boiling-water bath, and the absorption at 500 nm was determined and compared with the glycine standard. When the absorption was larger than 2.0, the sample was diluted and redetermined.

Sugar was colorimetrically determined at 490 nm by phenol-sulfuric acid method. Glucose was used as a standard.

For paper-electrophoresis, 12 × 30 cm paper strips (TOYO, No.51A) were rinsed with 5% formic acid-methanol and then with methanol, and dried. The supernatant of the saliva sample was concentrated to 1/10 volume at 40°C using rotary evaporator, and a 300 μl aliquot of the concentrate was applied in a length of 10 cm to the center of the paper strip. Electrophresis was performed in 1:16 mixture (pH 5.9) of 0.1 M sodium acetate and 0.1 M acetic acid by applying constant voltage of 10 V/cm paper for 60 min at room temperature. After the completion of electrophoresis, the paper strip was dried, and cut into segments 1.0 cm wide, from which zinc was extracted with 2 ml of 5% nitric acid, and was determined by atomic absorption spectrophotometry.

To locate ninhydrin-positive substances on the paper strip after electrophoresis, the above-mentioned ninhydrin reagent was sprayed, and the paper was heated to be specifically colored.

RESULTS AND DISCUSSION

Average levels of zinc and copper in the saliva are given in Table 1.

The zinc concentrations of the whole saliva samples and of the supernatant samples were 0.116 ± 0.034 and 0.117 ± 0.024 ppm, respectively. As these two did not significantly differ from each other, the supernatant fraction appeared to be alternative to whole saliva in measuring zinc.

The level of copper was 0.188 ± 0.084 ppm in the whole saliva, and 0.371 ± 0.123 ppm in the supernatant, the latter being twice as high as the former. This might be due to a considerable amount of sedimental components contained in whole saliva, which might lower the copper level in the fluid. When an aliquot of such sample taken by aspiration was analyzed, the level of copper in

the whole saliva may have been underestimated. Because of the small number of samples available, further studies are required for explaining this results.

Table 1. Zn and Cu concentration in saliva

	Whole saliva	Supernatant of Saliva
Zn	0.116 ± 0.034 ppm	0.117 ± 0.024 ppm (n = 11)
Cu	0.188 ± 0.084 ppm	0.371 ± 0.123 * ppm (n = 9)

* : $p < 0.01$

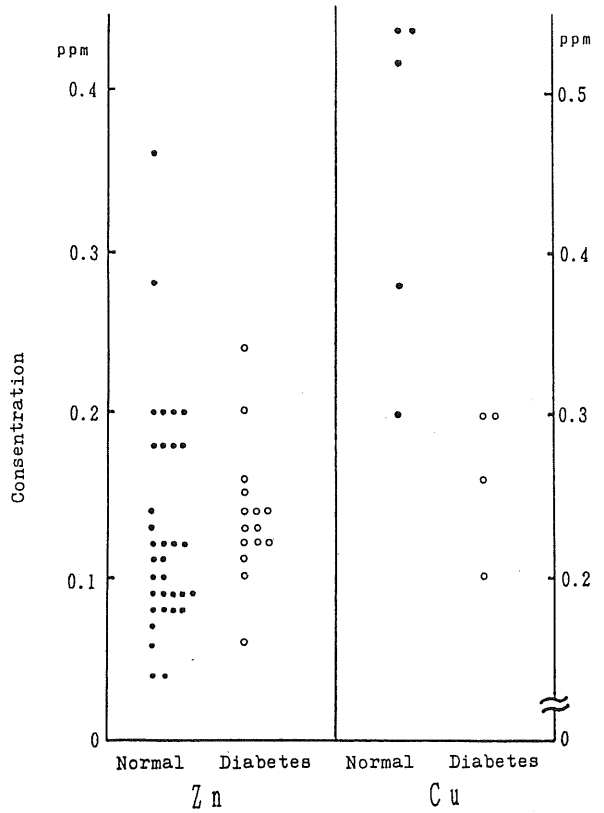

Fig. 1. Zinc and copper contentration in human saliva.

Fig. 1 shows the levels of zinc and copper in the saliva from the normal and diabetic subjects. The mean level of zinc was 0.131 ± 0.069 ppm in the normals, and 0.137 ± 0.040 ppm in the diabetics. There was no significant difference between the two. Four of the diabetics were dependent on insulin, but nothing remarkable was observed in their zinc levels. The standard deviation of the data was so large in all the cases, and so a direct comparison of the measured values of these metals did not seem to make much contribution to analyzing the concerns. Therefore, a reliable parameter to standardize these salivary metals was needed.

We picked up sugar and ninhydrin-positive substances, and determined their contents in the

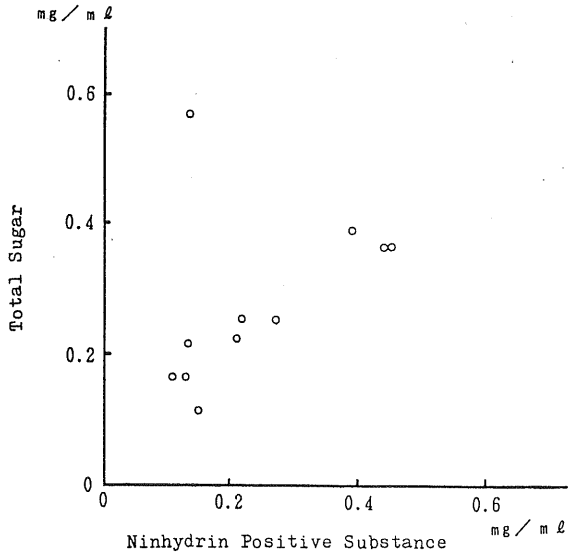

Fig. 2. The correlation between total sugar and ninhydrin positive substance in normal human saliva.

saliva. As shown in Fig.2, there was a positive correlationship between sugar and ninhydrin-positive substances except for one case. The correlation factor was $\gamma = 0.60$.

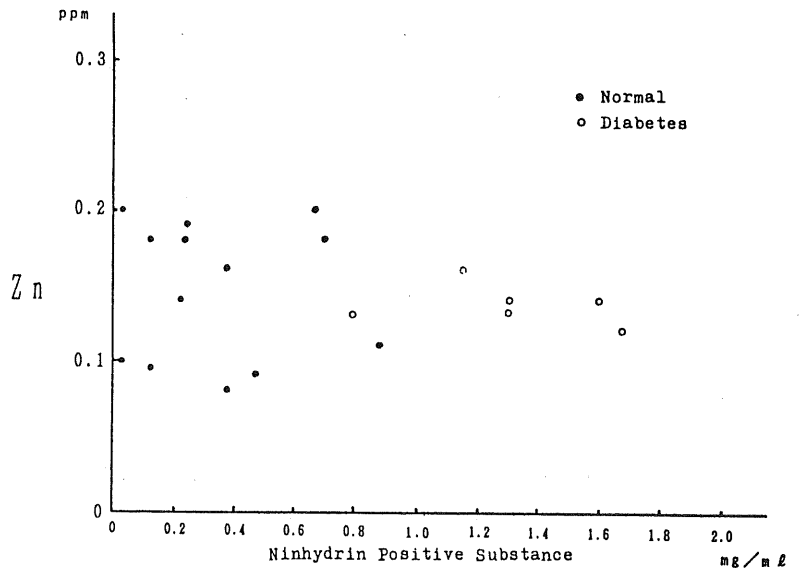

Fig. 3. The relation between zn and ninhydrin positive substance.

Fig. 3 shows the relation between the salivary levels of ninhydrinpositive substances and zinc. There was no correlationship between the two parameters. This figure, however, suggests that the level of ninhydrin-positive substances was higher in the diabetics than in the normal

subjects. Then, the mean levels was calculated separately for these two subject groups. As shown in Table 2, the mean in the diabetics (1.305 ± 0.293 mg/ml) was significantly ($p<0.01$) higher than in the normals (0.341 ± 0.261 mg/ml).

Table 2. Ninhydrin positive substances in human saliva

Normal Saliva	0.341 ± 0.261 mg/ml	(n = 13)
Diabetic Saliva	1.305 ± 0.293 * mg/ml	(n = 6)

* : $p < 0.01$

It suggests that the diabetics had a higher salivary glycoprotein (e.g. mucin) than the normal subjects, and that the salivary zinc was independent of the protein level.

The supernatant of saliva from the diabetics was concentrated to about 1/10 volume, and subjected to paper-electrophoresis to investigate in which protein fraction zinc localized. As seen from Fig.4 there were four bands of ninhydrin-positive substances. The largest in size was that remaining near the original point. Zinc was most concentrated in the fraction detected nearest to the anode. Since zinc in the body exists mostly in a protein-bound form, we had expected that zinc would be distributed in all the protein fractions. Actually, however, no zinc was found in the protein band lying nearest to the cathode. Free zinc may have some importance, but it was not analyzed in the present study.

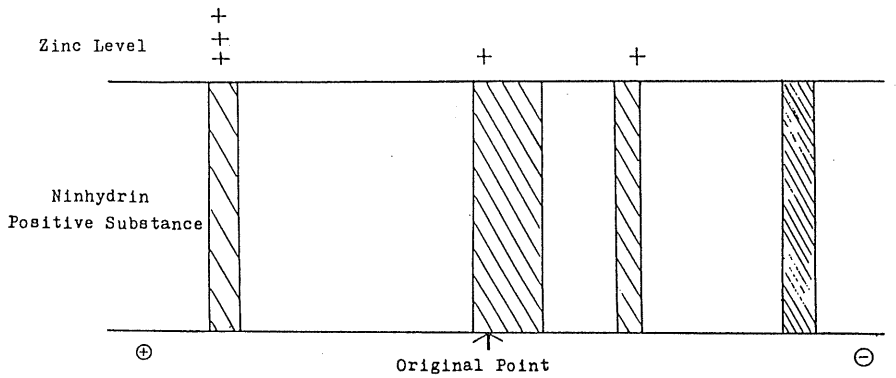

Fig.4.The pattern of paper electropholesis and zinc distribution of diabetic human saliva.

A considerable variation in the measured level of salivary zinc or copper is usually attributed to the technique of collecting saliva, circadian rhythm, sex difference, age, etc. In the present study, saliva was collected as resting mixed saliva, but no regulation was made for other factors. In further enlarging the subject number, those factors must be included in analysis. The copper level should be investigated in an increased number of saliva specimens.

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