CARBOHYDRATE IN PUS AND EXUDATE FROM GINGIVAL POCKETS

—including investigation with regard to relationship of blood sugar level to glucose concentration in gingival pocket fluid of periodontitis associated with diabetes mellitus—

BY

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ABSTRACT

First of all, the free sugars in pus from the gingival pockets of periodontitis, and pus of alveolar and gingival abscess were observed by paperchromatography.

Secondly, the relation between total weight, glucose content in pus and exudate from gingival pockets and the severity of periodontitis, the pocket depth, sex and age was quantitatively analyzed.

Furthermore, the effect of blood sugar level upon the glucose concentration in gingival pocket fluid of periodontitis associated with diabetes mellitus was examined.

As the results, glucose spot was detected in all cases of periodontitis, alveolar and gingival abscess. Besides glucose, ribose and desoxyribose spots also were found in some cases of involvement mentioned above.

The amount of pus was proportional to the severity of periodontitis and an increase of pocket depth. In addition, the weight of exudate was more increased in periodontitis than in clinically healthy gingiva.

A characteristic finding was not obtained from sex and age differences.

In glucose concentration per 1 mg of gingival pocket fluid it was found that in clinically healthy gingiva it was approximately equal to the blood sugar level of healthy person (70–110 mg%) and it was increased slightly in periodontitis than in clinically healthy gingiva. And in periodontitis the tendency to decrease gradually proportionally to the severity of inflammation was shown.

In the cases of periodontitis involving diabetes with hyperglycemia glucose content in gingival pocket fluid was increased, as the blood sugar level was increased.

It is pointed out that an increase of glucose consumption in gingival tissue may be related to an increased severity of diabetes.

Finally, there was a close correlation between the glucose concentration per 1 mg of pus and that of exudate in the same patient of periodontitis. This finding is regarded as evidence that tissue fluid may always exude through the gingival pocket epithelial layer into the gingival pocket.

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Introduction

The nature and biological roles of the tissue fluid flowing into the gingival pocket have been studied extensively by many researchers since Brill et al.[1] found instant appearance of a fluorescent substance, injected intraveneously, into the tissue fluid. And amino acids and the related substances in the pus of the gingival pocket were investigated by Konno[2] and also by Sueda[3].

These results were suggestive of that the chemical constituents in the fluid of the gingival pocket may reflect partially the metabolic processes or effects performed in the periodontal tissue.

In order to clarify the carbohydrate metabolism in the periodontal tissue, the authors attempted to analyze qualitatively and quantitatively carbohydrates in the pus accumulated in the gingival pocket of patients involved periodontal disease, especially in the cases associated with diabetes mellitus which is currently recognized to have a close relationship to the etiology of the periodontal disease.

Carbohydrates in the tissue fluid flowing into the pocket of clinically healthy gingiva were also determined for the control experiment.

Experimental Materials and Methods

I. Experimental materials.

The subjects of this investigation were comprised 77 male and female patients, aged from 15 to 65 who had visited to the dental and medical hospitals. They were divided into 33 persons involving periodontal disease and 35 persons who had suffered from periodontal disease associated with diabetes mellitus as the experimental groups, besides 9 persons with clinically healthy gingiva used for comparison group.

The diagnosis of periodontal disease was made by the clinical examination and X-ray photographic finding. The presence of diabetes mellitus was determined from the blood glucose level which was above 140 mg% either at 2 and 3 hours after a meal or at the fasting time, measured by the Hagedorn-Jensens method.

Before the collection of samples was taken, the food debris, the dental plaque and the saliva adhere to the gingiva and the tooth surface were wiped with cotton gauze and then they were removed by spraying with physiological saline solution.

In order to avoid contamination by the saliva, the area to be operated on was isolated with cotton gauze rolls and was dried with compressed air.

The collection of samples was taken by means of filter paper strips;

the previously weighed 20 filter paper strips (approximately 1.0 mm wide and 20 mm long) were inserted simultaneously into the labial gingival pockets of the maxillary central incisors, lateral incisors, and canines in the proportion of 8, 6 and 6 sheets respectively, until resistance to minimal pressure was encountered.

Collection was continued for five or fifteen minutes to absorb any fluid present in the gingival pockets and after removal for examination they were weighed again.

II. Fraction of samples.

The extraction and fractionation scheme was as follows.

Samples taken by the filter paper strips

↓

extracted with 1.0 ml of Pyridine for 24 hours in refrigerator and was then centrifuged (4.000 r.p.m., 15 min)

↓

The supernatant was dried by evaporation at reduced pressure (40°C)

↓

The dried residue was again extracted with 2.5 ml of 70% ethanol and was then centrifuged (4.000 r.p.m., 5 min)	The dried residue was dissolved in 5.0 ml of distilled water and was then treated with ion-exchange resins
↓	↓
The supernatant was dried by evaporation at reduced pressure (40°C)	The filtrates was dried by evaporation at reduced pressure (40°C)
↓	↓
The dried residue was dissolved in 0.02 ml of distilled water	The dried residue was extracted with 2.5 ml of 70% ethanol and was then centrifuged (4.000 r.p.m., 5 min)
	↓
	The supernatant was dried by evaporation at reduced pressure (40°C)
	↓
	The dried residue was dissolved in 0.02 ml of distilled water
(Fraction 1)	(Fraction 11)

III. Qualitative analysis of carbohydrate in the samples.

The samples fractionated by the above described method were spotted onto the filter paper (20 mm wide and 400 mm long) and in the ascending system one dimensional paper chromatography was carried out for 15 hours at room temperature (22–25°C), with phenol/ammonia water (4/1) or with n-buthanol/pyridine/water (5/3/2) as a solvent.

After development, sugar spots were indicated with aniline hydrogen phthalate or with silver nitrate reagents.

IV. Quantitative analysis of carbohydrate in the samples.

After the fractionation of sample was also made, finally each sample was dissolved in 2.0 ml of distilled water.

The glucose concentration in it was examined by the Somogyi-Nelsons method.

V. Relation of blood sugar level to glucose content in gingival pocket fluid of periodontitis associated with diabetes mellitus.

Twenty-six patients of periodontitis associated with diabetes mellitus were selected. They comprised 9 slight and 4 severe cases of periodontitis according to the group of 100 mg% in blood sugar value (slight diabetes) and 6 slight and 7 severe cases of periodontitis due to the group of 200 mg% in blood sugar value (severe diabetes).

The patients were examined by the following method:

1) All subjects were given a glucose tolerance test, in which each patient's blood was taken at two hours after a meal and at the same time the gingival pocket fluid was collected by the procedure already stated above.

2) After taking of blood and gingival pocket fluid, the blood sugar level and the glucose content in the fluid were determined quantitatively by the above described method.

Results

Qualitative analysis of carbohydrates in the samples:

In periodontitis 2 sugar spots appeared as illustrated in Table I. In these spots, the one spot which showed range of Rf value from 0.40 to 0.64, revealed in all cases. While the other one spot was detected in 2 cases of sample number 2, 4 and it was ranged according to Rf value from 0.61 to 0.63.

As compared with the authentic sugar developed simultaneously, the former was comparable to spot of glucose and latter was corresponding to Rf value of ribose. Furthermore, it was observed in the case of sample number 4 that when the paperchromatography was carried out after mixing glucose and ribose into the sample, 2 sugar spots were located at the same positions of authentic glucose and ribose. It was also discovered in case of sample number 6 that one sugar spot run by n•buthanol/pyridine/water (5/3/2) solvent showed Rf value from 0.63 to 0.64 and it was comparable to authentic glucose.

Secondly, one sugar spot appeared in all cases of alveolar abscess (sample number 8–14) as shown in Table 1. Since it was ranged at Rf value

Table I. Rf value of free sugars contained in pus

		I		II		III		authentic sugars developed simultaneously*					Fraction of samples
		A	B	A	B	A	B	GL	GC	MN	RI	DR	
Periodontitis	1	0.41						0.43	0.50	0.51	0.65	0.80	1~4, 7
	2	0.41		0.62				0.42	0.47	0.48	0.63	0.78	extracted with pyridine
			0.41		0.63								→ treated with 70% ethanol
	3	0.40						0.44	0.49	0.48	0.66	0.78	→ dissolved in water
			0.42										
	4	0.40 (+GL)		0.61 (+RI)				0.42	0.48	0.49	0.64	0.76	
		0.41		0.61									
	5	0.42	0.43					0.43	0.47	0.50	0.63		5, 6
○	6	0.64						0.62			0.78	0.90	extracted with pyridine
			0.63					0.66					→ treated with ion-exchange
⊙	7	0.41						0.42	0.47	0.48	0.63	0.78	resins → dissolved in water
			0.41										
Alveolar abscess	8		0.45					0.45	0.49	0.51	0.65		8~10
								0.46					
	9	0.39 (+GL)				0.71		0.43	0.49		0.64		extracted with pyridine
			0.37	(+GL)			0.71						→ dissolved in water
	10	0.38						0.45	0.49		0.66		
			0.38										
	11	0.41						0.43	0.50	0.51	0.65	0.80	11~14
		0.42											
	12	0.40 (+GL)		0.67 (+RI)				0.44	0.49	0.48	0.66	0.76	extracted with pyridine
		0.41		0.66								0.78	→ treated with ion-exchange
	13	0.41						0.42	0.48	0.49	0.64	0.76	resins → extracted with 70%
	14	tailing		0.65				0.45	0.48	0.47	0.66		ethanol → dissolved in water
Gingival abscess	15	0.43				0.75		0.44	0.47	0.49	0.65	0.76	15, 16
			0.41				0.76					0.78	extracted with pyridine
	16		0.39					0.45	0.48	0.47	0.66		→ dissolved in water

* The majority of cases was indicated with aniline hydrogen phthalate

○ Solvent for chromatographic development: n-buthanol·pyridine·water (5 : 3 : 2)

⊙ Periodontitis associated with diabetes mellitus

A aniline hydrogen phthalate

B ammonia silver

from 0.37 to 0.45 and it was nearly corresponding to authentic glucose, it seemed to be glucose.

The other two sugar spots besides glucose also appeared in 3 cases of sample number 9, 12 and 14. In these spots, the one spot which was ranged from 0.65 to 0.67 was considered to be ribose and the other seemed to be rather desoxyribose than ribose, which showed Rf value of 0.71.

Quantitative analysis of carbohydrate in the samples:

The relation of the total weight of pus and exudate from the gingival pockets, and the relation of the amount of glucose in them (because the main sugar in gingival pocket fluid was detected to be glucose), to the severity of periodontitis (including diabetic complications), the pocket depth, sex and age were examined. Their results were detailed in Tables II and III.

1) The total weight of pus and exudate.

From Fig. 1 it was apparent that there was a tendency for the weight of pus to increase successively in proportion to the severity of periodontitis.

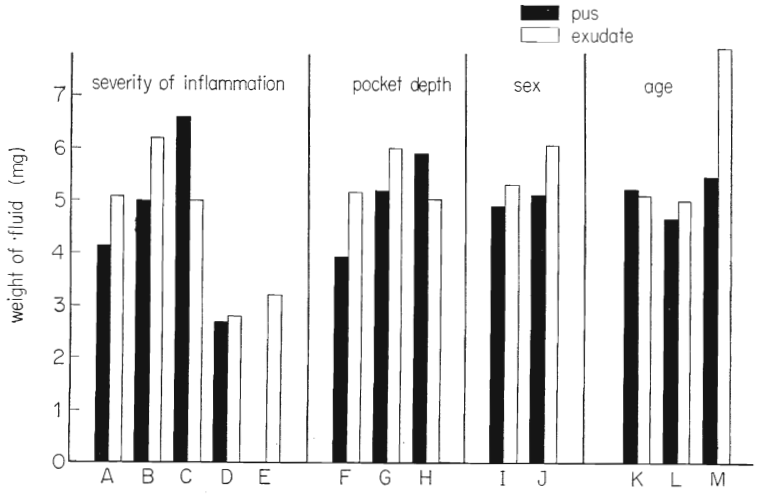

Fig. 1. Diagram showing the mean total weight of pus and exudate, according to severity of inflammation, pocket depth, sex, and age.

A: slight periodontitis
B: moderate periodontitis
C: marked periodontitis
D: periodontitis associated with diabetes mellitus
E: clinically healthy gingiva
F: 0～1 mm in pocket depth
G: 2～3 mm in pocket depth
H: 4～6 mm in pocket depth
I: male
J: female
K: 21～30 year
L: 31～40 year
M: 41～50 year

Table II. Individual values of total weight, glucose content in pus and exudate, according to severity of inflammation.

	sex	age	pocket depth (mm)	total weight (mg)		glucose content (γ)		glucose content (γ/mg)	
				A	B	A	B	A	B
slight periodontitis	♀	15	1	3.1	4.2	9.4	9.4	3.0	2.2
	♂	33	2	5.7	14.4	11.1	8.3	1.9	0.6
	♂	28	2	4.6	6.0	11.6	7.2	2.2	1.9
	♀	23	3	2.3	3.0	7.2	8.1	3.1	2.7
	♂	21	0	3.7	6.6	6.3	5.7	1.3	0.9
	♀	37	1	4.0	2.9	8.3	7.3	2.8	2.5
	♀	21	2	4.8	7.5	10.4	9.4	2.2	1.3
	♀	40	2	4.5	6.9	10.4	11.4	2.3	1.7
	♀	42	1	4.3	10.8	11.9	11.4	2.8	1.1
	♂	28	2	1.8	3.7	7.7	8.6	4.3	2.3
	♂	38	1	3.0	4.0	6.5	4.5	2.2	1.1
	♀	30	1	5.6	4.3	5.5	6.3	0.98	1.5
	♀	31	2	6.4	6.9	9.7	8.6	1.5	1.2
moderate periodontitis	♀	36	1	4.1		7.3		1.8	
	♂	39	2	5.8	2.6	9.7	9.7	1.7	3.7
	♂	45	1	3.1		9.4		3.0	
	♀	42	3	6.1	10.1	10.2	12.6	1.7	1.2
	♀	32	4	4.6	7.4	9.9	9.9	2.2	1.3
	♂	25	1	5.4	6.6	7.3	11.4	1.4	1.7
	♂	31	4	7.0		8.2		1.2	
	♀	34	2	3.9	4.6	9.1	7.4	2.3	1.6
	♀	48	2	7.8	6.1	12.2	10.2	1.6	1.7
marked periodontitis	♀	65	6	4.5	3.4	7.8	9.4	1.7	2.8
	♂	32	1	3.1	7.1	13.5	12.1	4.4	1.7
	♂	49	1	6.7	5.4	9.9	9.7	1.5	1.8
	♀	58	4	6.9	6.9	12.1	9.3	1.8	1.3
	♂	23	6	15.6	28.7	11.2	8.1	0.7	0.3
	♂	23	5	8.1	3.3	4.2	14.6	0.5	4.4
	♀	29	3	7.1	17.6	10.2	9.9	1.4	0.6
	♀	27	4	14.1	17.8	11.7	12.6	0.8	0.7
	♂	34	6	19.1		9.2		0.5	
	♀	37	3	8.3	10.0	10.6	10.2	1.3	1.0
	♂	25	4	4.5	3.9	10.6	9.1	2.4	2.3
periodontitis associated with diabetes mellitus	♀	60	3		0.7		3.5		5.0
	♀	49	2	2.4	4.5	8.1	5.5	3.4	1.2
	♂	40	1		1.2		2.5		2.1
	♀	60	3	3.1	3.9	7.0	11.0	2.3	2.8
	♂	59	2	8.9	15.8	6.4	9.5	0.7	0.6
	♀	46	1		3.3		9.5		2.9
	♂	30	1	1.6	3.1	6.0	9.5	3.8	3.1
	♀	24	1		1.8		6.4		3.6
	♂	48	1	3.7	6.7	11.3	10.8	3.1	1.6
clinically healthy gingiva	♂	21	1		3.1		2.4		0.8
	♂	22	1		2.7		4.7		1.7
	♂	19	0		4.2		1.6		0.4
	♂	19	1		5.5		2.4		0.4
	♂	21	0		5.1		0.4		0.1
	♀	22	1		2.8		4.9		1.8
	♀	20	1		4.1		4.2		1.0
	♀	24	0		2.5		1.3		0.5
	♀	20	0		3.2		4.2		1.3

A: pus B: exudate

Table III. Mean values with standard deviation of total weight, glucose content in pus and exudate, according to severity of inflammation, pocket depth, sex, and age.

		*	total weight (mg)		(γ) glucose content		(γ/mg) glucose content	
			M	S.D.	M	S.D.	M	S.D.
slight periodontitis	●	13	4.15	1.13	9.10	1.86	2.19	0.68
	○	13	5.09	1.71	8.84	1.93	1.62	0.66
moderate periodontitis	●	9	5.00	1.30	8.89	1.15	1.74	0.41
	○	6	6.18	1.18	10.76	1.29	1.50	0.74
marked periodontitis	●	11	6.59	1.54	10.37	1.31	1.13	0.62
	○	10	5.00	1.72	10.04	1.44	1.08	0.66
associated with diabetes	●	5	2.70	0.91	6.87	0.92	3.15	0.63
	○	9	2.81	1.41	9.15	2.09	2.51	0.81
clinically healthy gingiva	○	9	3.69	1.12	2.77	1.60	0.83	0.58
pocket depth 0 ~ 1 mm	●	11	3.94	1.03	7.77	1.56	2.08	0.74
	○	8	5.14	1.52	9.25	2.10	1.50	0.44
2 ~ 3 mm	●	13	5.18	1.69	9.95	0.91	1.93	0.51
	○	13	5.97	2.20	9.26	1.12	1.48	0.70
4 ~ 6 mm	●	9	5.93	1.59	10.10	1.59	1.30	0.72
	○	7	4.98	2.03	9.16	0.64	1.18	0.75
sex male	●	12	4.88	1.61	9.06	1.59	1.78	2.33
	○	10	5.27	1.84	9.18	1.74	1.93	0.57
female	●	21	5.10	1.85	9.63	1.63	1.84	2.18
	○	19	6.05	2.65	9.40	1.84	1.35	0.63
age 21~30 year	●	11	5.19	1.76	9.47	1.98	1.43	0.70
	○	11	5.08	1.78	9.87	1.64	1.46	0.69
31~40 year	●	13	4.63	1.41	8.99	1.44	1.69	0.64
	○	10	4.98	1.91	6.77	2.02	1.25	0.36
41~50 year	●	6	5.42	1.75	10.10	1.59	2.15	0.59
	○	5	7.86	2.44	9.16	0.64	1.50	0.32

●: pus ○: exudate *: subject's number

Statistically, a significant difference (P=0.05) was seen between slight and marked periodontitis. The weight also showed an increase in proportion to the increase of pocket depth.

As regards the amount of exudate from the gingival pocket, periodontitis represented a larger value than clinically healthy gingiva and statistically there was no significant difference in two groups (P=0.05). It was also observed that the weight tended to increase in slight periodontitis. The rela-

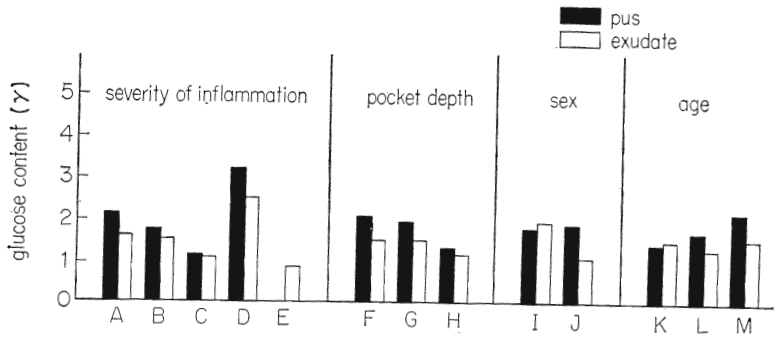

Fig. 2. Diagram showing the mean glucose content per 1 mg of pus and exudate, according to severity of inflammation, pocket depth, sex, and age.

A: slight periodontitis
B: moderate periodontitis
C: marked periodontitis
D: periodontitis associated with diabetes mellitus
E: clinically healthy gingiva
F: 0～1 mm in pocket depth
G: 2～3 mm in pocket depth
H: 4～6 mm in pocket depth
I: male
J: female
K: 21～30 year
L: 31～40 year
M: 41～50 year

tion between the pocket depth and the amount of exudate was similar to the finding obtained from the severity of periodontitis. As to sex and age differences, no difference in the amount of pus and exudate was noticed.

2) The amount of glucose in pus and exudate.

Fig. 2 summarized the difference of glucose content according to the severity of periodontitis, the pocket depth, sex and age. Namely, the glucose content per 1 mg of exudate was more increased in periodontitis than in clinically healthy gingiva, and statistically difference was significant ($P=0.05$). Next, the glucose content per 1 mg of pus and exudate respectively revealed a tendency to decrease proportionally to the severity of periodontitis. The mean glucose content in exudate, for example, was $1.62\pm0.66\,\gamma$ in slight periodontitis, $1.50\pm0.74\,\gamma$ in moderate periodontitis, and $1.08\pm0.66\,\gamma$ in severe periodontitis.

Meanwhile, periodontitis associated with diabetes showed more increase than periodontitis with regard to the glucose content in both pus and exudate, and it was observed that there was statistically a significant difference ($P=0.05$). Also there was a tendency for decrease of the glucose content in pus and exudate, as the gingival pocket depth increased. In regards to sex and age differences, a characteristic finding was not obtained.

3) The correlation between the glucose content per 1 mg of pus and exudate respectively in the same patients involving periodontitis.

As shown in Fig. 3, from all cases of periodontitis the relative coefficient

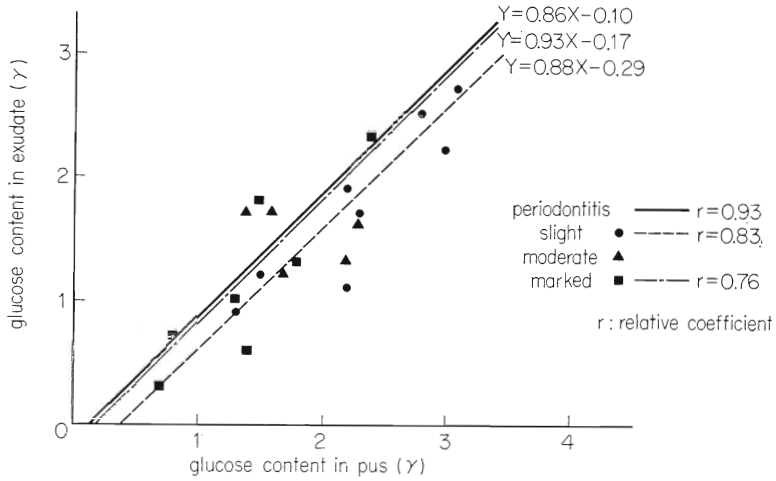

Fig. 3. Interrelation between individual values of glucose content per 1 mg of pus and that of exudate in same periodontitis.

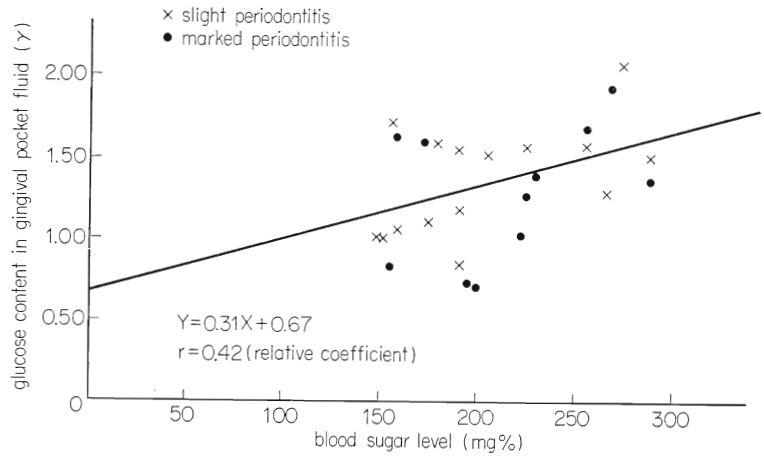

Fig. 4. Interrelation between individual values of glucose content per 1 mg of gingival pocket fluid and blood sugar level, in same periodontitis associated with diabetes mellitus.

r was 0.93. A statistical close correlation (P=0.05) was seen, between slight and marked periodontitis. The inclination (slope) of regression lines showed the value near approximately 1.

The relation between the blood sugar level and the glucose content in gingival pocket fluid:

1) Fig. 4 indicated the glucose content per 1 mg of exudate as the longi-

Table IV. Individual values of blood sugar level, glucose content, and glucose consumption, according to severity of periodontitis and diabetes

	slight periodontitis						marked periodontitis					
	sex	age	gingival pocket fluid's weight (mg)	blood sugar level (mg %)	glucose content in gingival pocket fluid (γ/mg)	glucose utilization in gingival tissue*	sex	age	gingival pocket fluid's weight (mg)	blood sugar level (mg %)	glucose content in gingival pocket fluid (γ/mg)	glucose utilization in gingival tissue*
blood sugar level 100 mg %	♂	54	3.2	158	1.71	8	♀	54	7.6	196	0.72	62
	♂	51	3.5	180	1.48	17	♂	38	16.2	156	0.82	41
	♂	54	3.7	175	1.11	36	♂	55	3.9	174	1.59	8
	♂	46	4.8	150	1.00	33	♂	58	3.9	159	1.61	1
	♂	38	6.2	192	0.85	55						
	♂	60	3.4	153	1.00	34						
	♂	33	6.9	192	1.17	39						
	♀	65	3.9	160	1.05	34						
	♂	62	4.0	192	1.55	19						
blood sugar level 200 mg %	♂	55	3.2	276	1.06	25	♀	38	19.0	226	1.26	44
	♂	48	4.0	288	1.50	47	♂	48	5.8	237	1.38	41
	♂	42	7.6	226	1.57	30	♂	59	5.8	258	1.67	35
	♂	60	5.7	207	1.52	26	♀	59	10.8	200	0.70	65
	♀	29	4.2	258	1.47	23	♀	57	7.4	224	1.01	54
	♂	50	3.9	267	1.28	52	♂	47	5.8	271	1.91	29
							♂	35	6.3	288	1.35	53

* $\frac{\text{blood sugar level minus glucose content}}{\text{blood sugar level}} \times 100$

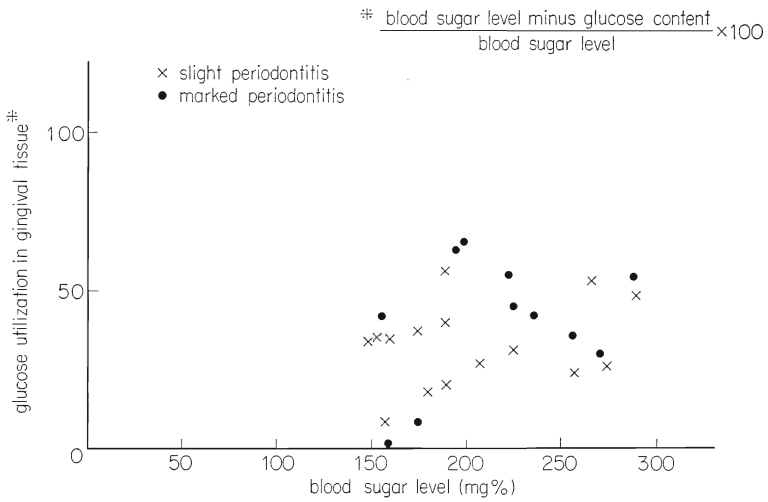

Fig. 5. Interrelation between individual values of glucose utilization in gingival tissue and blood sugar level, in same periodontitis associated with diabetes mellitus.

tudinal axis and the blood sugar level as the horizontal axis, plotting the individual value of examined subjects (Table IV). As a result, there was observed a tendency for increase of the glucose content in gingival pocket fluid in proportion to increase of the blood sugar. The relative coefficient r was 0.42, and the presence of a correlation was statistically demonstrated. The regression line became $Y=0.31X+0.67$. The inclination of the line, however, was gentle, and of all cases showing blood sugar levels higher than 250 mg%, only one case indicated over 2.00 γ/mg as the glucose content in exudate.

2) Table IV summarized the glucose content to be consumed in the gingival tissue. That is, the value of the blood sugar level minus the glucose content in exudate for anumerator and the blood sugar level as a denominator were given. By the ratio $\times 100$, the rate of glucose consumption in gingival tissue was calculated.

Fig. 5 represented the rate of the glucose utilization as the longitudinal axis and the blood sugar level as the horizontal axis plotting the individual value of subjects. As a result, the glucose consumption tended to increase proportionally to the increase of blood sugar level. This result became more increased in severe involved cases than in slight diabetes.

Discussion

I) Qualitative determination by paper chromatography.

(A) Preparation of sample.

When all samples in this report were extracted with pyridine, the most part of inorganic substances, amino acids, and proteins could be separated sufficiently from free sugars in the pus. Furthermore, for the complete removal of mucoid substances in alveolar abscess, 70% ethanol in accordance with Schultz-Haudts report[4] was utilized. As a result, both sugar spot's tailing and the sugar spots situated lower than the authentic sugar spots on the paper-chromatogram were not observed.

Next, the samples treated with ion-exchange resins as another fractionation showed satisfactory results such as fractionation mentioned above.

(B) Ribose and desoxyribose in the pus.

It is also suggested that a certain bacteroides in the gingival pocket, as reported by Macdonald et al.,[5] may decompose RNA or DNA, resulting in the appearance of ribose and desoxyribose or these sugars may be already contained in the exudate. In any cases, it is an interesting problem.

II) Total weight of pus and exudate.

The amount of pus increased proportionally to the severity of periodontitis and the increase of gingival pocket depth. This phenomenon may be reflected by an increased amount of pus accumulating in the pocket.

Brill[6] and Mann[7] reported that gingival inflammation brought about an increase in the amount of gingival pocket fluid. The result of this paper was also in agreement with these findings.

That is, the gingival pocket fluid was more increased in periodontitis than in clinically healthy gingiva.

Meanwhile, the finding that there was an increased amount in slight periodontitis and a slightly decreased amount in severe periodontitis, as shown in Fig. 1, was essentially similar to the result by Sueda[3].

III) Glucose concentration in the pus and the exudate.

Brill et al.[1] observed that intravenously injected fluorescent substance appeared in the gingival pockets of dogs, and Brill et al.[8] also found that the gingival pocket fluid included high molecular substances such as different proteins in blood serum.

From these reports, then, it can be suspected that tissue fluid originating from blood plasma may flow into the gingival pocket.

As illustrated in Fig. 2, the mean glucose content in clinically healthy gingival pockets was 0.83 γ/mg, and this value was almost equal to the blood sugar level or the glucose concentration in blood plasma of healthy persons (70–110 mg/dl). It was very impressive that the normal gingival pocket fluid contained the same concentration of glucose, as did plasma.

Tsunemitsu[9] and Fosdick et al.[10] noted that in periodontitis the fasting blood glucose level was approximately the same as that in healthy

persons and on the other hand, the mean 2 hour's blood glucose level after a meal was higher than the normal value in many cases.

Because the collection of samples in this report were performed at 2–3 hours after a meal, it can be pointed out that the increase of blood glucose level in periodontitis as compared with that of clinically healthy gingiva may be related to the increased glucose concentration in gingival pocket fluid.

Therefore, on diabetic patients with hyperglycemia, the effect of blood sugar level upon the glucose concentration in gingival pocket fluid was studied in this paper. The result which showed more increased glucose content in gingival pocket, proportionally to the increase of blood glucose concentration, may be influenced by the increased blood glucose level.

It was evident from Fig. 4 that in diabetic cases involving hyperglycemia greater than 250 mg%, it was only one case that glucose concentration in gingival pocket fluid was higher than 2.00 γ/mg.

Fig. 5 summarized that the rate of glucose consumption in the gingival tissue tended to rise in proportion to the increase of blood glucose level.

These findings lead us to assume the existence of a certain specific metabolic process in the gingival tissue.

It has been already reported[11,12] that oxygen consumption in the gingival tissue increased in slight periodontitis and decreased in severe periodontitis.

In contrast, however, it has been known in recent research[13] that glycolysis more increased in severe periodontitis in comparison with in slight periodontitis.

Besides, Glickman et al.[14] or Weinmann et al.[15] microscopically observed that the glycogen concentration in gingival epithelial tissue showed a tendency to increase due to the severity of periodontitis. Accordingly, such as the increased glycolysis and glycogen concentration can be regarded as etiological factors in the production of increased glucose uptake into the gingival tissue.

In conclusion, there was a close correlation between the glucose concentration of pus and that of exudate from the gingival pocket in the same patient, and the regression coefficient was nearly 1. From this result, it is postulated that the glucose concentration of pus may be equal to that of exudate since tissue fluid always exude into gingival pocket.

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