Histologic Findings of the Parotid Gland and Bicarbonate Content in Parotid Saliva of Dogs with Experimentally Produced Pancreatitis*

GORO KAKIZAKI,† NOBORU NOTO,† YOSHIYUKI FUJIWARA,†
EIICHI KATO,† TAKUZO ISHIDATE‡ and TAKAYUKI SAITO§
Department of Surgery,† and Department of Pathology,‡
Akita University School of Medicine, Akita, and Department
of Surgery, Tohoku University School of Medicine, Sendai§

KAKIZAKI, G., NOTO, N., FUJIWARA, Y., KATO, E., ISHIDATE, T. and SAITO, T. *Histologic Findings of the Parotid Gland and Bicarbonate Content in Parotid Saliva of Dogs with Experimentally Produced Pancreatitis.* Tohoku J. exp. Med., 1972, 108 (2), 165-177 — Degenerative changes of the parotid gland and a marked decrease of the maximum bicarbonate concentration in parotid saliva were found in dogs with acute experimental pancreatitis. ——— bicarbonate; experimental pancreatitis; parotid gland

In a series of experimental study on the correlation between the pancreas and parotid gland, the authors demonstrated in experiments of rats (Kakizaki *et al.* 1971, 1972 a, b) that acute pancreatitis caused atrophic changes and low amylase content of the parotid gland, and that histologic findings and amylase content of the parotid gland changed parallel to the severity of pancreatic lesions in the repairing period of acute pancreatitis and furthermore that perforative peritonitis and ileus themselves could not exert a direct effect upon histologic changes of the parotid gland.

In the present study of ours, experimental production of acute pancreatitis was made in dogs and histologic examination of the pancreas and parotid gland and biochemical examination of parotid saliva were done.

MATERIALS AND METHODS

Four mongrel dogs (Nos. 1,2,3 and 4) were used.

No. 2, a male, 11.6 kg of body weight, was examined for biochemical changes in saliva and blood before, 19 hours and 42 hours after the operation for production of acute pancreatitis, and for histologic changes of the pancreas and parotid glands 42 hours after the operation.

No. 3, a female, 11 kg of body weight, was used for biochemical study of saliva and blood before and 73 hours after the operation and for the histologic examination of the pancreas and parotid glands 73 hours after the operation.

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No. 4, a male, 10 kg of body weight, was examined for the same items as in No. 3, before and 28 hours after the operation.

No. 1, a male, 8 kg of body weight, was used for the histologic examination of the pancreas and parotid gland as a control (Figs. 1 and 2).

Methods for biochemical examination of saliva and blood, and for histologic examination

Collected saliva was subjected to the measurement of volume, pH, bicarbonate, sodium, chloride and potassium.

Blood drawn from the femoral vein was used for measurement of serum amylase, pH, hematocrit, electrolytes (sodium, chloride and potassium), total protein and plasma protein fractions.

Fig. 1. Photomicrograph of the pancreas from Dog No. 1. H.E. stain.

Fig. 2. Photomicrograph of the parotid gland from Dog No. 1. H.E. stain.

Bicarbonate was determined by titration method of Van Slyke *et al.* (1919), sodium and potassium by the method of flame photometer (Hald 1947), chloride by Schales and Schales' method (1941) and serum amylase values according to Somogyi's method (1938). pH was determined by the method of glass electrode (Andersen *et al.* 1960), hematocrit by the method of McGovern *et al.* (1955), total protein by light scattering method (Tietze and Neurath 1952) and plasma protein fractions according to cellulose acetate electrophoretic techniques (Kohn 1962).

The pancreas and parotid glands were fixed in 10% formalin and stained with hematoxylin-eosin for the histologic examination.

Method for collection of saliva

The animal was anesthetized with nembutal injected intravenously in a dose of 35 mg per kg of body weight, placed on its side and the other Stenson's duct was cannulated. The cannula was made by cutting off the tip of a hypodermic needle of suitable bore, and polishing the end. It was placed in the duct and fixed by silk and connected to the polyethylene tube. Then, pilocarpine was injected intramuscularly in a dose of 0.5 mg per kg of body weight. As soon as salivary secretion began, saliva was collected for forty to sixty minutes at every five minute intervals.

Method for production of acute pancreatitis

The abdomen was opened and the duodenum incised longitudinally about 5 cm in length. Paying attention not to injure the common bile duct, the ventral pancreatic duct was ligated at the site close to the duodenum by a silk thread. Then, polyethylene tube was inserted into the dorsal pancreatic duct from the dorsal duodenal papilla and a mixture consisting of 2 ml of bile aspirated from the gallbladder and 2 ml of Trypsin (10,000 unit), Mochida pharmaceutical company, were poured into the tube. As soon as the tube was drawn out, the dorsal pancreatic duct was ligated at the site close to the duodenum by a silk thread. After closure of the incised duodenum, the abdomen was closed.

RESULTS

Dog No. 2

Changes in chemical composition of blood and saliva: As shown in Table 1, serum amylase showed higher values 19 and 42 hours after the operation than before that. In plasma protein fractions, albumin and A/G were lower after the operation, but α_1 and α_2 globulins were higher after the operation. Serum pH, hematocrit, electrolytes and total protein showed no significant changes after the operation.

The salivary secretion after the injection of pilocarpine occurred from one, ten and four minutes before, and 19 and 42 hours after the operation, revealing that the beginning time of salivary secretion was delayed after the operation. As shown in Table 2, the total volume of saliva amounted to 22.0 ml for 40 minutes (0.5 ml for a minute), 9.8 ml for 35 minutes (0.28 ml for a minute) and 12.7 ml for 40 minutes (0.32 ml for a minute) before, 19 and 42 hours after the operation, indicating a marked reduction of salivary volume after the operation. The maximum bicarbonate concentration in saliva was 56 mEq/L, 52 mEq/L and 35 mEq/L before, 19 and 42 hours after the operation. Electrolytes and pH of saliva showed no changes after the operation.

Autopsy findings: A considerable amount of serous and bloody ascites was

TABLE 1. *Chemical composition*

Lapse of time after the operation for producing acute pancreatitis	Conditions for blood sampling	Serum amylase (mg/100 ml)	pH	Ht (%)
Before	Before injection of pilocarpine	1,609	7.6	36
	21 min. after injection of pilocarpine	2,048	7.6	44
19 hours	Before injection of pilocarpine	9,322	7.5	48
	28 min. after injection of pilocarpine	9,530	7.5	49
42 hours	Before injection of pilocarpine	11,698	7.6	39
	22 min. after injection of pilocarpine	11,842	7.6	42

TABLE 2. *Volume and chemical composition*

(A) (B)	Volume (ml)			pH			HCO ₃ (mEq/L)		
	Before	19 hours	42 hours	Before	19 hours	42 hours	Before	19 hours	42 hours
5	4.1	2.8	1.1	7.6	7.6	7.6	44	43.5	23
10	3.7	2.1	2.8	7.6	7.6	7.6	53	52	35
15	2.7	1.1	1.8	7.6	7.6	7.6	51	47	35
20	3.6	1.8	1.8	7.6	7.6	7.6	50	40	33
25	2.3		1.8	7.6		7.6	56		27
30	2.4	2.0	1.3	7.6	7.6	7.6	54	37	24
35	1.5		1.1	7.6		7.6	53		23
40	1.7		1.0	7.6		7.6	53		23

(A) Lapse of time after the operation for producing acute pancreatitis

(B) Time of

found in the abdominal cavity. Fatty necroses were present around the pancreas. The pancreas was edematously swollen and hemorrhagic. Both parotid glands were edematous and atrophic.

Histologic findings

Pancreas: In pancreatic head, there was bleeding around the pancreatic duct and in some of the acinus. The acinus was markedly edematous. In pancreatic body, necrobioses or necroses were obviously noticed and infiltration of granulocytes and round cells was moderately noticed in a part of peripancreatic fatty tissues and interstitial tissues. There were also fatty necroses in the peripancreatic tissues (Fig. 3). In pancreatic tail, infiltration of inflammatory cells and fatty necroses were limited in a part of the peripancreatic tissue.

Parotid glands: An obvious finding was an atypical arrangement of the

of blood of Dog No. 2

Electrolyte (mEq/L)			Total protein (g/100 ml)	A/G	Plasma protein fractions					
Na	Cl	K			albumin	α_1	α_2	β_1	β_2	γ
135	104	5.8	5.6	0.71	41.5	8.5	13.5	13.0	11.5	12.0
140	103	4.9	6.2	0.64	39.0	9.5	14.5	13.0	13.0	11.0
135	101	5.5	7.0	0.64	39.0	10.0	18.0	10.0	13.0	10.0
156	100	5.0	7.5	0.56	36.0	11.0	19.0	10.0	15.0	9.0
154	110	5.9	5.4	0.45	31.0	17.5	22.5	9.5	11.5	8.0
150	109	6.8	5.8	0.49	33.0	12.5	17.5	9.5	15.0	12.5

of saliva of Dog No. 2

Na (mEq/L)			Cl (mEq/L)			K (mEq/L)		
Before	19 hours	42 hours	Before	19 hours	42 hours	Before	19 hours	42 hours
108	113	120	77.5	67.6	77.5	8.4	8.4	8.0
106	90	124	63.5	43.0	83.5	5.6	5.6	4.8
113		150	67.5		65.5	5.6		5.0
122	92	140	67.5	46.5	60.0	5.6	5.0	7.0
123		133	62.5		63.5	5.6		6.4
124	67	140	64.0	44.5	47.5	5.8	6.0	3.6
125		133	67.6		37.5	5.4		3.6
125			64.0			4.8		

saliva sampling (min)

glandular cells in which vacuolation and disappearance of zymogen granules were found. The debris of epithelial cells was noticed markedly in peripheral parotid ducts (Fig. 4).

The results in Dog No. 2 were summarized as follows. In forty-two hours after experimental production of acute pancreatitis, histologic findings obviously showed necroses in the pancreatic body and degenerative changes in the parotid gland. The volume and maximum bicarbonate concentration of parotid saliva were markedly reduced. In nineteen hours after experimental production of acute pancreatitis, however, the maximum bicarbonate concentration of parotid saliva showed no significant difference in comparison with the control, though the flow of parotid saliva was reduced.

Fig. 3. Photomicrograph of the pancreatic body from Dog No. 2. The acini are broken down and necroses of glandular cells noticed extensively. The acini fell in necrobioses and relatively normal acini are irregularly remaining around necroses, and among them the infiltration of inflammatory cells accompanied by polynuclear leucocytes is noticed. (H.E. stain)

Fig. 4. Photomicrograph of the parotid gland from Dog No. 2. Obvious findings are the vacuolation of glandular cells and the disappearance of zymogen granules. (H.E. stain)

Dog No. 3

Changes in chemical composition of blood and saliva: Serum amylase was 1,958 mg/100 ml and 11,250 mg/100 ml before and 72 hours after the operation, respectively.

Fig. 5. Photomicrograph of the pancreatic tail from Dog No. 3. Necrosis is noticed similar to the histologic findings of Fig. 3. H.E. stain.

Fig. 6. Photomicrograph of the parotid gland from Dog No. 3. The vacuolation of glandular cells is scattered. H.E. stain.

The beginning of salivary secretion after injection of pilocarpine occurred from one and three minutes before and 73 hours after the operation. As shown in Table 3, the total flow was 36.0 ml for 50 minutes (0.72 ml for a minute) and 28.8 ml for 60 minutes (0.48 ml for a minute) before and 73 hours after the operation, respectively. The maximum bicarbonate concentration of saliva was 83 mEq/L and 41 mEq/L before and after the operation, respectively. The values for chloride showed a tendency to increase postoperatively and no significant changes were

TABLE 3. *Volume and chemical*

Time of saliva sampling (min)	Volume (ml)		HCO ₃ (mEq/L)	
	Before the operation	73 hours after the operation	Before the operation	73 hours after the operation
5	6.0	0.3	54	35
10	8.2	2.2	82	
15	4.7		83	
20	2.7	2.7	77	
25	2.4	3.9	66	41
30	1.9	3.3	61	39
35	2.2	2.7	60	38
40	2.5	3.1	52	36
45	2.8	3.0	82	34
50	2.6	3.0	82	34
55		2.3		34
60		2.3		33

TABLE 4. *Volume and chemical*

Time of saliva sampling (min)	Volume (ml)		HCO ₃ (mEq/L)	
	Before the operation	28 hours after the operation	Before the operation	28 hours after the operation
5	4.2	0.7	54	28
10	4.2	2.1	57	42
15	3.2	1.5	57	46
20	2.9	1.2	62	44
25	3.6	1.2	63	48
30	1.5	1.0	65	46
35	2.2	1.1	64	49
40	1.8	0.9	60	48
45	2.0	1.0	67	48
50	1.8	0.9	60	48
55	1.9	1.0	60	49
60		0.8		50

noticed in sodium and potassium before and after the operation.

Autopsy findings: There were a considerable amount of serous and bloody ascites and scattered areas of fatty necroses in the abdominal cavity. The consistency of the pancreas was hard, specially in the body and tail of the pancreas. An abscess, 5×5 cm in diameter, was found in the pancreatic tail. Both parotid glands were atrophic and edematous.

Histologic findings

Pancreas: There were marked edema, necroses, and abscess in a part of the pancreatic tail (Fig. 5). The body and head of the pancreas were edematous.

Parotid glands: The vacuolation of glandular cells was scattered, the grade of which was milder than that in Dog No. 2 (Fig. 6). There was an infiltration of polynuclear leucocytes into some of the periductal tissues.

The results in Dog No. 3 were summarized as follows. In seventy-three hours

composition of saliva of Dog No. 3

Na (mEq/L)		Cl (mEq/L)		K (mEq/L)	
Before the operation	73 hours after the operation	Before the operation	73 hours after the operation	Before the operation	73 hours after the operation
71	55	30.1	37.0	8.2	8.8
74		17.0		4.4	
78	82	14.6	37.6	5.4	6.0
62	76	12.5	37.6	5.8	6.2
49	86	10.3	37.6	7.4	6.4
35	65	7.6	29.2	7.5	4.6

composition of saliva of Dog No. 4

Na (mEq/L)		Cl (mEq/L)		K (mEq/L)	
Before the operation	28 hours after the operation	Before the operation	28 hours after the operation	Before the operation	28 hours after the operation
72	80	50	48	8.4	8.6
94		48		6.2	
	78	48	43	4.6	5.2
93	7	52	7	6.4	7
131					
137	73	52	40	6.8	5.6
128		43		6.8	

after experimental production of acute pancreatitis, obvious histologic findings were necroses in the pancreatic tail and degeneration in the parotid gland. Furthermore, the beginning of secretion of parotid saliva was retarded and both the volume flow and maximum bicarbonate concentration were markedly reduced.

Dog No. 4

Changes in chemical composition of blood and saliva: Serum amylase was 1,608 mg/100 ml and 4,026 mg/100 ml before and 28 hours after the operation, respectively.

The beginning of salivary secretion after injection of pilocarpine was from 3 minutes and 30 seconds preoperatively and 7 minutes postoperatively. As shown in Table 4, total volume of saliva was 29.3 ml for 55 minutes (0.53 ml for a minute) preoperatively and 13.4 ml for 60 minutes (0.26 ml for a minute) postoperatively. The maximum bicarbonate concentration was 67 mEq/L preopera-

tively and 50 mEq/L postoperatively. No significant changes were seen on electrolytes before and after the operation.

Autopsy findings: A small quantity of bloody ascites was found in the abdominal cavity. The head and body of the pancreas were edematous and firm, being accompanied by fatty necroses. There were no abnormal findings in the tail of the pancreas. Both parotid glands were somewhat atrophic.

Fig. 7. Photomicrograph of the pancreatic head from Dog No. 4. Necrosis accompanied by bleeding is present. H.E. stain.

Fig. 8. Photomicrograph of the parotid gland from Dog No. 4. There are vacuolation and atypical arrangement of glandular cells. H.E. stain.

Histologic findings

Pancreas: In the head of the pancreas, there were a marked edema and a few areas with necroses with infiltration of polynuclear cells (Fig. 7). In the body and tail of the pancreas, edema was also uniformly noticed.

Parotid glands: The vacuolation and atypical arrangement of glandular cells were noticed as seen in Dog No. 2 (Fig. 8).

The results in Dog No. 4 were summarized as follows. Histologically, necroses in the head of the pancreas and degenerative changes in the parotid gland were obviously noticed. The beginning of secretion of parotid saliva was retarded, volume flow was markedly reduced and the maximum bicarbonate concentration showed low values.

The results mentioned above lead to the following conclusions. When acute pancreatitis was produced in dogs, the parotid gland showed degenerative changes and the beginning of secretion of parotid saliva was retarded, and the volume and maximum bicarbonate concentration of the saliva were reduced.

DISCUSSION

The authors have demonstrated in rats that degenerative changes in histologic findings and low amylase content of the parotid gland were induced in acute experimental pancreatitis (Kakizaki *et al.* 1971, 1972 a, b). The present investigation was done to clarify the changes of bicarbonate content of parotid saliva of dogs with acute experimental pancreatitis.

For the measurement of the bicarbonate concentration, total carbon dioxide was determined by the method of Van Slyke *et al.* (1919). The total CO₂ measured was considered to be largely originated from bicarbonate (de Beer and Wilson 1932). It was found that the maximum bicarbonate concentration in parotid saliva was markedly reduced in dogs with acute pancreatitis.

Although there was a close relation between bicarbonate content and pH in saliva (Schneyer and Schneyer 1967), the regulation mechanism of the salivary concentration of bicarbonate itself has been difficult to identify. According to Schneyer and Schneyer (1967), bicarbonate of saliva can be derived from at least two sources: the cells of the salivary gland and the plasma. There are many evidences for a glandular source for salivary bicarbonate (Sand 1952, Coats and Wright 1957, Coats *et al.* 1958). Biochemical and histochemical studies (Van Goor 1948, Morris and Swayne 1964) have indicated significant carbonic anhydrase activity in many salivary glands. However, the results of studies on inhibitory effect of carbonic anhydrase on salivary bicarbonate and pH have been equivocal (Neidermeier *et al.* 1955, Chauncey and Weiss 1958, Yoshimura *et al.* 1959), so it may be assumed that a requirement for carbonic anhydrase in secretion of bicarbonate is not established at the present time (Schneyer and Schneyer 1967). On the other hand, there are also many experimental evidences indicating that salivary bicarbonate is derived from plasma bicarbonate (de Beer and Wilson 1932,

McClanahan and Amberson 1935, Sand 1952). However, it was reported that experimental metabolic acidosis gave inconsistent effects on the level of salivary bicarbonate (Sand 1952).

In the results of our present study it was worthy of note that bicarbonate concentration was decreased markedly in dogs with experimental acute pancreatitis despite of a constant value of pH in plasma and saliva. The decrease in salivary bicarbonate in these dogs may be caused by unknown mechanism, if any, which is related to degenerative changes of the parotid gland. And it was also noticed that the beginning of secretion of parotid saliva after pilocarpine injection was delayed and the volume flow of saliva was decreased in dogs with acute pancreatitis.

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