Histological Findings and Amylase Contents of the Pancreas and Parotid Gland of Rats with Experimental Obstruction of the Common Bile Duct

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In 10 control rats and 9 rats with common bile duct obstruction, histological studies were conducted on the pancreas and the parotid. Amylase activity was measured in these tissues and serum. The following results were obtained

1. After common bile duct obstruction, the parotid gland showed almost normal histological findings.

2. Amylase content of the parotid gland was normal after the common bile duct obstruction.

3. Serum amylase was decreased in the activity after the common bile duct obstruction.

4. In mild change in histological findings of the pancreas after bile duct ligation, the parotid gland showed no histological or functional changes. These results would rule out an influence, if any, of common bile duct obstruction on histology and function of the parotid gland.

As a part of the experimental studies on the correlation between the pancreas and the parotid gland, the authors (Kakizaki et al. 1971, 1972a, b, c) have already shown the atrophic and degenerative changes of the parotid glands histologically and decrease of amylase content of the parotid glands and fall of bicarbonate output in the parotid saliva functionally when pancreatitis was produced experimentally in rats and dogs. In a few clinical cases in man, moreover, a tendency towards decrease of the amount of secretion of parotid saliva and amylase and bicarbonate concentrations in it was found in the presence of a pancreatic lesion. Usefulness of examination of parotid saliva for the diagnosis of pancreatic disease was suggested (Kakizaki et al. 1973).

In a previous experiment of ours (Kakizaki et al. 1971), however, we ligated the common bile duct at the point of inflow into the duodenum in order to produce pancreatitis experimentally and the changes of the parotid glands were studied.

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In this method, however, an effect, if any, of the ligation of common bile duct itself on the parotid gland cannot be neglected, in addition to the effect of pancreatitis. In order to study the effect of obstruction of common bile duct itself on the parotid gland, the present experiments were undertaken.

**Materials and Methods**

Nineteen male Wistar rats weighing 260-375 g were used.

*Preparation of the control group:* Ten control rats were laparotomized under ether anesthesia and blood samples were obtained from the abdominal vena cava inferior, for amylase measurement. The liver, pancreas and parotid glands were then removed and fat and blood vessels were removed as completely as possible. After washing with chilled physiological saline to remove adhering blood, they were again washed with chilled distilled water. Part of this was studied histologically and the rest of the tissue was used for amylase measurement.

*Histological examinations of the liver, pancreas and the parotid glands:* The liver, pancreas and parotid glands removed by the method described above were immediately fixed in 10% formalin and used for histological examination by hematoxylin-eosin, elastica-Masson and Sudan III method.

*Measurement of amylase in the pancreas and parotid glands:* The pancreas and parotid glands obtained were immediately homogenized in the Potters homogenizer with 10 ml chilled distilled water, under the ice-cold condition. The specimen was sufficiently lyophilized in a lyophilizer to obtain pancreatic and parotid powder. Ten mg of the powder were dissolved in 5 ml Tris-HCl buffer (pH 8.0) and the amylase activity of this solution was measured by the method of Caraway (1959). The amylase activity was expressed as the unit per mg of the lyophilized powder (u/mg).

*Serum amylase measurement:* Serum amylase activity was measured by the Caraway method (Caraway 1959).

*Experimental preparation of common bile duct obstruction:* In order to prepare obstruction of the common bile duct, 9 rats were laparotomized under ether anesthesia and the common bile ducts were ligated at the hepatic hilus (common bile duct at a portion superior to the inflow of pancreatic duct) with a fine silk suture, and the wound was closed. They were laparotomized under ether anesthesia 96 hours postoperatively. Blood amylase activity was determined by the same method as in the control group, and the liver, pancreas and parotid glands were used for histological examinations, while the pancreas and parotid glands were used for amylase measurement.

**Results**

*Histological findings*

*Liver:* In the obstruction group, a dilatation of the common bile duct above the site of ligation was apparently observed. Histologically, a dilatation of bile ducts within the Glisson's sheath was noted. However, no dilatation of bile duct was noted in the acinus, and a formation of bile thrombus was absent. Besides, some irregularity of the hepatic cell column was noted, with sporadic appearance of fat droplets within the hepatic cells. Infiltration by inflammatory cells was extremely rare.
Pancreas and Parotid of Rats with Bile Duct Obstruction

Pancreas: While no findings seen upon ligation of the common bile duct at the inflow to the duodenum such as dilatation of the duct system and acinar atrophy (Fig. 1) (Kakizaki et al. 1971) were noted, pathological findings in the obstruction group consisted of a mild decrease of zymogen granules in number in the glandular acinus and an appearance of fine granular fat droplets within the acinar cells. In the coarse connective tissue outside of the pancreatic parenchyma, round cell infiltration was occasionally noted but it is not so extensive (Figs. 2 and 3).

Fig. 1. Histological picture of the pancreas after ligation of the common bile duct at the inflow into the duodenum. Dilatation of the ductal system, atrophy of the acinus, disappearance of zymogen granules, and vacuolation of glandular cells. (HE stain)

Parotid gland: As was already reported (Kakizaki et al. 1971), atypical arrangement of glandular cells, vacuolation of glandular cells, disappearance of zymogen granules, narrowing of the glandular acinus and appearance of fat droplets within the glandular acinus cells were noted in the parotid of rats with experimentally produced pancreatitis as shown in Fig. 4. In the ordinary specimens in the obstruction group, on the contrary, an appearance of the parotid was almost the same as that of the normal parotid. Detailed examination, however, revealed the presence of fat droplets in some portion in the glandular acinar cells of the parotid (Fig. 5).

Amylase values in organs

Control group: As shown in Table 1, amylase value in the pancreas ranged from 130.7–179.1 u/mg with the average of 152.9 u/mg. Amylase value in the parotid gland, on the other hand, was 208.4–269.1 u/mg with the average of
Fig. 2. Histological picture of the pancreas after ligation of the common bile duct at the hepatic hilus. Mild decrease of zymogen granules and fine granular fat droplets in the glandular acinar cells. (HE stain)

Fig. 3. Histological picture of the pancreas after ligation of the common bile duct at the hepatic hilus. Round cell infiltration in the coarse connective tissue outside of the pancreatic parenchyma. (HE stain)
Fig. 4. Histological picture of the parotid gland in acute pancreatitis. Atrophy and irregular arrangement cells, vacuolation of glandular cells, disappearance of zymogen granules, narrowing of glandular acinus, and appearance of fat droplets in the glandular cells. (HE stain)

Fig. 5. Histological picture of the parotid in the obstruction group. Histological findings are almost similar to the normal parotid gland. As the only abnormal finding, fat droplets are noted in the glandular acinus. (HE stain)
236.1 u/mg. Amylase value in the parotid gland was definitely higher than that in the pancreas.

**Obstruction group:** As shown in Table 2, the amylase value in the pancreas was 32.8–81.9 u/mg with the average of 57.3 u/mg while amylase value in the parotid gland was 170.8–253.1 u/mg with the average of 215.2 u/mg. The amylase value in the parotid was definitely higher than that in the pancreas.

### Table 2. Organ and serum amylase content (Obstruction group)

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Body weight (g)</th>
<th>Organ amylase content (u/mg)</th>
<th>Serum amylase value (u)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before surgery</td>
<td>After surgery</td>
<td>Pancreas</td>
</tr>
<tr>
<td>11</td>
<td>350</td>
<td>340</td>
<td>52.0</td>
</tr>
<tr>
<td>12</td>
<td>295</td>
<td>290</td>
<td>38.4</td>
</tr>
<tr>
<td>13</td>
<td>320</td>
<td>315</td>
<td>78.0</td>
</tr>
<tr>
<td>14</td>
<td>305</td>
<td>290</td>
<td>49.7</td>
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<tr>
<td>15</td>
<td>330</td>
<td>310</td>
<td>56.1</td>
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<tr>
<td>16</td>
<td>320</td>
<td>300</td>
<td>81.9</td>
</tr>
<tr>
<td>17</td>
<td>310</td>
<td>300</td>
<td>53.0</td>
</tr>
<tr>
<td>18</td>
<td>280</td>
<td>245</td>
<td>33.8</td>
</tr>
<tr>
<td>19</td>
<td>355</td>
<td>325</td>
<td>73.8</td>
</tr>
<tr>
<td>Average</td>
<td>316</td>
<td>302</td>
<td>57.3</td>
</tr>
</tbody>
</table>

Comparison between control and obstruction group (Table 3): In the pancreas of the control group, the value was 152.9±15.9 u/mg, and the corresponding value in the obstruction group was 57.3±16.2 u/mg, indicating a marked decrease in the latter, with a significant difference at P<0.001. In the parotid gland, on the other hand, the value in the control group was 236.1±21.5 u/mg, while that in the obstruction group, 215.2±26.1 u/mg, without a significant difference. Fig. 6 shows such relationship distinctly. In the obstruction group, amylase value in the
TABLE 3. Amylase content of the pancreas and parotid (Comparison between control and obstruction group)

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Obstruction group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of rat</td>
<td>Amylase value (u/mg)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>10</td>
<td>152.9±15.9</td>
</tr>
<tr>
<td>Parotid</td>
<td>10</td>
<td>236.1±21.5</td>
</tr>
</tbody>
</table>

Fig. 6. Amylase content in the pancreas and parotid. (Comparison between control and obstruction group)

pancreas was lower than in the control group but the amylase in the parotid was similar between these two groups.

Serum amylase value

**Control group:** As shown in Table 1, serum amylase was 1,218–1,500 u with the average of 1,372 u.

**Obstruction group:** As shown in Table 2, serum amylase was 834–1,440 u with the average of 1,030 u.

**Comparison between control and obstruction group (Table 4):** The value in the control group was 1,371.7±86.1 u, and that in the obstruction group, 1,029.8±179.5 u with significant difference at P<0.001. As is evident in Fig. 7, serum amylase was found to be decreased in the obstruction group.
Histologically, mild pathological changes are noted in the pancreas in the obstruction group. In the parotid gland, however, histological findings were almost similar to those in the normal glands. In the glandular acinar cells of both organs, however, fat droplets were seen in some areas. Amylase content in the pancreas was decreased in the obstruction group, but the amylase in the parotid gland was similar to that in the controls. Serum amylase value was found to be decreased in the obstruction group.

**DISCUSSION**

Using rats the authors studied the influence of acute pancreatitis on the parotid gland, demonstrating atrophic and degenerative changes of the parotid gland along with the decrease of amylase value in the parotid gland in rats with experimentally produced pancreatitis (Kakizaki et al. 1971, 1972b). In these experiments, the common bile duct was ligated at the site of inflow into the duodenum for the preparation of acute pancreatitis. With the use of this method, however, advanced acute pancreatitis develops, along with obstruction of the common bile duct. Consequently, it becomes necessary to study the influence of
the common bile duct obstruction itself on the parotid gland. The common bile duct was ligated at the hepatic hilus (superior to the site of inflow of pancreatic duct) with a fine silk suture. After 96 hours histological changes of the parotid gland, and changes of amylase content of the parotid gland and in serum were studied. The mild decrease of zymogen granules in the glandular acini and round cell infiltration in the coarse connective tissue outside of the pancreatic parenchyma were found histologically. The parotid gland, however, gave no histological changes and showed almost similar findings to those in the normal gland. Such mild changes in the pancreas were probably produced by some influence of the operative procedure along with some infection. Fine granular fat droplets also appeared in the liver, pancreas and parotid. As the cause of these changes, metabolic disturbance in the liver and digestive disturbance due to the common bile duct obstruction are thought to be most likely. As to amylase content of organs, pancreatic amylase content was decreased in the group of the common bile duct obstruction, but amylase content of the parotid was similar to that in the controls. In the present experiment, mild histological changes were seen in the pancreas and pancreatic amylase content was decreased. In the pancreatic change of this extent, parotid gland was free of changes histologically and functionally. The common bile duct obstruction itself was thus shown to influence the parotid gland scarcely.

In the past, a decrease of serum amylase level was reported to occur in liver diseases (Cummins and Bockus 1951), and a rise of serum amylase in diseases of the bile ducts (Lewison 1941; Smith et al. 1952; Pfeffer et al. 1953). In the present study, serum amylase level was definitely decreased in the common bile duct obstruction. Considering these together with the former experimental results (Kakizaki et al. 1972b, c), the changes of serum amylase level appear to be not so valuable in the diagnosis of a pancreatitis.

References


