Experimental Studies on the Pathophysiological Changes in the Pancreas of Rat following Bilateral Ligation of the Parotid Gland Duct

Goro Kakizaki, Masami Sasahara, Takayuki Saito, Takehiro Soeno, Yoshiyuki Fujiwara, Takamasa Nihei, Takuzo Ishidate* and Akira Senoo*

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Kakizaki, G., Sasahara, M., Saito, T., Soeno, T., Fujiwara, Y., Nihei, T., Ishidate, T. and Senoo, A. Experimental Studies on the Pathophysiological Changes in the Pancreas of Rat following Bilateral Ligation of the Parotid Gland Duct. Tohoku J. exp. Med., 1976, 118 (4), 331-348 — The blood sugar level, blood insulin level, serum amylase level and organ amylase level in the pancreas were measured in a total of 110 rats examined at 12, 24, 48, 72 and 96 hr, and 1, 2, 3, 4, and 5 weeks after bilateral ligation of the parotid gland ducts. In parallel with this functional study, light microscopic study of the pancreas and the parotid gland and electron microscopic observations of the pancreas obtained from the rats were performed at the respective periods. The following results were obtained: 1) The serum amylase level was most elevated in the group examined at 12 hr after ligation and it decreased gradually and returned to the normal level at 96 hr after ligation and thereafter. 2) As for the pancreatic exocrine function, the organ amylase level of the pancreas was significantly elevated in the groups examined at 24 hr to 3 weeks after ligation. Electron-microscopically, hyperfunctional state in the pancreatic acinar cells was recognized as evidenced by the dilatation of the granular endoplasmic reticulum. 3) With regard to the endocrine function of the pancreas, the blood sugar level and blood insulin level did not show any significant changes after ligation as compared with the control group. Morphologically, electron microscopy showed a decrease in electron density and swelling of the cored granules, disintegration and fusion of their limiting membranes in the beta cells. These changes are interpreted as indicating the discharge of secretory granules in the beta cells. It is concluded that an impairment of the parotid glands influences the function of the pancreas to a certain degree.

 ultrastructure of pancreas; amylase; insulin; blood sugar; parotid gland

In a series of studies on the correlation between pancreas and parotid gland, the present authors have experimentally evidenced that in the presence of pancreatitis, the parotid gland showed atrophy in histology as well as decreased parotid salivary output, declined maximal bicarbonate concentration and diminished amylase content in the saliva secreted by the parotid gland (Kakizaki et al. 1971, 1972a, b, c, 1973b; Ishidate et al. 1974). This observation has been applied in clinical cases, and a new diagnostic test of pancreatic lesion by the
examination of the saliva secreted by the parotid glands had proved valuable in
clinical cases (Kakizaki et al. 1973a, 1974a, b, 1975). In this paper, one of the
consecutive themes, the pathophysiological behavior of the pancreas associated
with an impairment of the parotid glands has been evaluated.

**MATERIALS AND METHODS**

A total of 110 male Wistar rats weighing 340 to 375 g were used.

*Experimental methods*

*Control group.* 10 rats fasted for 12 hr were used. Under deep ether anesthesia,
a laparotomy was made to expose the inferior vena cava, from which blood was with-
drawn for determination of the blood sugar, serum amylase and blood IRI (immunoreactive
insulin) levels. Then the pancreas and the parotid glands were extirpated and freed from
the neighboring fatty tissue and blood vessels. They were washed in chilled physiological
saline solution to remove adhering blood, then washed with chilled distilled water. Part
of this was used for determination of the organ amylase level in the pancreas and the
rest of the tissue was prepared for light and electron microscopic examinations.

*Bilateral ligation of the parotid gland duct.* A total of 100 rats were used. Under
ether anesthesia the ducts of the parotid glands on both sides were ligated with fine silk.
They were divided into 10 groups, each consisting of 10 rats. Each group was laparo-
tomized under deep ether anesthesia at 12 hr, 24 hr, 48 hr, 72 hr, 96 hr, 1 week, 2 weeks, 3
weeks, 4 weeks, and 5 weeks after ligation. As in the control group, blood was with-
drawn from the inferior vena cava for determination of the blood sugar, blood IRI and
serum amylase levels. Some portions of the extirpated pancreas and parotid gland were
prepared for light and electron microscopic examinations and the remaining portions for
determination of the organ amylase level of the pancreas.

*Methods of examination*

*Blood sugar.* The blood sugar level was estimated by the Optoloidine boric acid
(OTB) method (Genba et al. 1963).

*Blood IRI.* Blood IRI was determined by Insulin-kit (Wide and Porath 1966). As
gamma-counter, Aloka JDC0751, Autowell Gamma System was employed.

*Serum amylase.* This was determined by the Caraway method (1959).

*Pancreatic organ amylase level.* A portion of the pancreas obtained according to
the method described above was homogenized in 10 ml of chilled distilled water utilizing a
Potter homogenizer. Then the homogenate was freeze-dried to obtain a powdered
pancreas. 10 mg of the powdered pancreas was dissolved in 5 ml of Tris-HCl buffer (pH
8.0) and the amylase level of this solution was estimated by the Caraway method (1959).
The amylase activity was expressed as units per mg of the powdered pancreas (U/mg).

*Light- and electron-microscopic examination of the pancreas and the parotid glands.*
Pieces of the pancreas and parotid glands extirpated were treated with 3% glutaraldehyde
solution (pH 7.3) after the method of Sabatini et al. (1963), at 4°C for 3 hr. After
 prefixed, the tissue was washed in several changes of isotonic phosphate buffer solution,
and was postfixed for 2 hr in an ice-cold (4°C) solution of 1% OsO₄ buffered at pH 7.3
according to the method of Millonig (1961). The fixed material was dehydrated rapidly in
an ethanol series, and embedded in Epon 812 (Luft 1961). Before preparing ultrathin sections,
1 μm-thick sections were cut with glass knives and stained with toluidine blue for light
microscopy. The double staining method with uranyl acetate and lead citrate was
applied to the ultrathin sections on the grids. Sections were examined and photographed
with an HU-12 or a JEM 100B electron microscope at an accelerating voltage of 75 or
80 kV. In addition, other sections stained with hematoxylin-eosin were made for light microscopic examination.

**RESULTS**

*The changes in the blood sugar level*

Table 1 shows the fasting blood sugar levels (lowest, highest and mean values) estimated at the respective intervals in the control and ligated groups. Statistically there is no significant difference between control and ligated groups (Table 1). It was found that the ligation of the parotid gland ducts does not influence the blood sugar level.

**TABLE 1. Changes of the blood sugar values (mg/100 ml)**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Experimental (time after ligation)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Lowest value</td>
<td>112</td>
<td>133</td>
</tr>
<tr>
<td>Highest value</td>
<td>204</td>
<td>246</td>
</tr>
<tr>
<td>Mean±S.D.</td>
<td>149</td>
<td>173</td>
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<tr>
<td></td>
<td>±26</td>
<td>±35</td>
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</table>

*The changes in the blood IRI level*

The blood IRI levels (lowest, highest and mean values) in the control and ligated groups estimated at the respective intervals are shown in Table 2. Statistical analysis did not reveal a significant difference between these two groups (Table 2). Thus the ligation of the parotid gland duct was found to have almost no influence on the blood IRI level.

**TABLE 2. Changes of the serum IRI values (U/ml)**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Experimental (time after ligation)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Lowest value</td>
<td>20.5</td>
<td>21.3</td>
</tr>
<tr>
<td>Highest value</td>
<td>43.8</td>
<td>34.5</td>
</tr>
<tr>
<td>Mean±S.D.</td>
<td>30.5</td>
<td>25.4</td>
</tr>
<tr>
<td></td>
<td>±6.6</td>
<td>±4.7</td>
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</table>

*The changes in the serum amylase level*

The lowest, highest and mean values for serum amylase estimated at the respective intervals in the control and ligated groups are shown in Table 3. Statistical analysis revealed that the serum amylase levels in these groups examined at 12, 24, 48, and 72 hr after ligation were markedly elevated as compared with those in the control group (Table 3). The difference between these
The changes in the organ amylase level in the pancreas

The amylase level in the pancreas (lowest, highest and mean values) estimated at the respective intervals after ligation in the control and ligation groups are shown in Table 4. In comparison with the control group, the organ amylase level was significantly elevated in the ligated groups sacrificed at 24, 48, 72, and 96 hr, 1 week, and 3 weeks after ligation (p<0.001). Thus the organ amylase level in the pancreas was found to be significantly elevated from 24 hr to 3 weeks after ligation of the parotid gland duct and to return to normal level thereafter.

The light microscopic findings

The parotid gland. Fig. 1 shows the histological picture of the parotid gland in a control rat. In contrast, the histological patterns in the ligated groups are shown in Table 5. From the early stage, atypical arrangement of the glandular cells, narrowing of the acini, dilatation of secretory ducts, and proliferation occurred markedly. Desquamation of the glandular cells, vacuolization and disappearance of zymogen granules in the glandular cells, and exudation in the acini and neighboring fatty tissue occurred moderate in degree. Figs. 2 and 3 show histological pictures of the parotid gland 96 hr and 2 weeks after ligation respectively. Thus, the parotid gland was markedly atrophied following ligation of the ducts.

The pancreas. The histological picture of the pancreas in a control rat is shown in Fig. 4. In the ligated group, the pancreas shows in histology a moderate decrease in amylase level immediately after ligation of the parotid gland duct, then decreases gradually to return to approximately the normal level within 96 hr.
Fig. 1. Photomicrograph of the parotid gland of the control group (H.E. stain).

**Table 5. Histological findings of the parotid gland**

<table>
<thead>
<tr>
<th>Histological findings</th>
<th>Time after ligation</th>
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<tbody>
<tr>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Atypical arrangement of glandular cells</td>
<td>±</td>
</tr>
<tr>
<td>Dissociation of the glandular cells</td>
<td>–</td>
</tr>
<tr>
<td>Vacuolation of glandular cells</td>
<td>–</td>
</tr>
<tr>
<td>Disappearance of zymogen granules</td>
<td>–</td>
</tr>
<tr>
<td>Exudation into the acinus</td>
<td>–</td>
</tr>
<tr>
<td>Narrowing of the acinus</td>
<td>–</td>
</tr>
<tr>
<td>Ductal dilatation</td>
<td>–</td>
</tr>
<tr>
<td>Exudation and cell infiltration in interstitial tissue</td>
<td>–</td>
</tr>
<tr>
<td>Exudation in periglandular fatty tissue</td>
<td>–</td>
</tr>
</tbody>
</table>

Atypical arrangement of acinar cells and a slight hypertrophy of the islets of Langerhans in the groups examined at 96 hr, 1 week and 2 weeks after ligation. In the remaining ligated groups, there was no marked change in histology (Table 6). Fig. 5 shows the histological findings of the pancreas 2 weeks after ligation. It is thus evidenced that the pancreas showed slight histological changes from 96 hr to 2 weeks after ligation of the parotid gland duct.

**Electron microscopic findings of the pancreas**

**Pancreatic acinar cells.** Fig. 6 shows an electron micrograph of acinar cells in normal pancreas. In the ligated groups, on the other hand, dilatation of granular endoplasmic reticulum began to occur slightly 48 hr after ligation. In
Fig. 2. Photomicrograph of the parotid gland 96 hr after ligation. Atypical arrangement of acinar cells, narrowing of the acinus, ductal dilatation, exudation and cell infiltration in interstitial tissue are noticed (H.E. stain).

Fig. 3. Photomicrograph of the parotid gland 2 weeks after ligation. Ductal dilatation, narrowing of the acinus, disappearance of zymogen granules, exudation and cell infiltration in interstitial tissue are markedly noticed with dissociation of the glandular cells (H.E. stain).

the groups examined at 1 week and 2 weeks after ligation the dilatation of the granular endoplasmic reticulum was pronounced and in addition, increase of heterochromatin in the nuclei and irregularities of the nuclear border were also evidenced. Among ligated groups, the changes in the group examined at 2 weeks were most marked. In the group sacrificed at 5 weeks such changes could be evidenced in several places but in general this group showed approximately the same findings
Pancreas of Rat with Bilateral Ligation of Partoid Gland Duct

Fig. 4. Photomicrograph of the pancreas of the control group (H.E. stain).
Fig. 5. Photomicrograph of the pancreas 2 weeks after ligation. A slight hypertrophy of the islets of Langerhans and a moderate disarrangement of acinar cells are noticed (H.E. stain).

as in the control group. In all ligated groups, the decrease of zymogen granules was not evidenced (Table 7).

Figs. 7, 8 and 9 show the electron micrographs of the pancreatic acinar cells in the ligated groups examined at 1 week, 2 weeks and 5 weeks after ligation, respectively.

The endocrine cells of the pancreas. Fig. 10 shows an electron micrograph of the beta cells of the pancreatic islet in a control rat. In the islets of Langerhans in the ligated group, alpha cells did not show any marked changes. In the beta
cells, however, a decrease in electron density of the granules began to occur and they were swollen frequently at 48 hr after ligation. The limiting membranes of the granules were frequently disintegrated and fused. In several places, the cell showed relatively well developed Golgi apparatus. These changes were most

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<tr>
<th>Histological findings</th>
<th>Time after ligation</th>
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<tr>
<td></td>
<td>12  24  48  72  96 hr  1  2  3  4  5 weeks</td>
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<tr>
<td>Enlargement of the Langereis' islet</td>
<td>– – – + + + ± – –</td>
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<tr>
<td>Hydropic changes of islet cells</td>
<td>– – – – – – – – – –</td>
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<tr>
<td>Atypical arrangement of the glandular cells</td>
<td>– – – + + + ± – –</td>
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<tr>
<td>Vacuolation of glandular cells</td>
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<tr>
<td>Disappearance of zymogen granules</td>
<td>– – – – – – – – – –</td>
</tr>
<tr>
<td>Exudation into the acinus</td>
<td>– – – – – – – – – –</td>
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<tr>
<td>Narrowing of acinus and ductal dilatation</td>
<td>– – – – – – – – – –</td>
</tr>
<tr>
<td>Exudation and cell infiltration of interstitial tissue</td>
<td>– – – – – – – – – –</td>
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<tr>
<td>Exudation in peripancreatic fatty tissue</td>
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<th>Electron microscopic findings</th>
<th>Time after ligation</th>
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<tbody>
<tr>
<td></td>
<td>12  24  48  72  96 hr  1  2  3  4  5 weeks</td>
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<tr>
<td>Dilatation of granular endoplasmic reticulum</td>
<td>– ± + + + # # + ± ±</td>
</tr>
<tr>
<td>Decrease of the zymogen granules</td>
<td>– – – – – – – – – –</td>
</tr>
<tr>
<td>Increase in Golgi apparatus</td>
<td>– – – + + # # + ± ±</td>
</tr>
<tr>
<td>Irregularity of nuclear wall</td>
<td>– – – – + # # + ± ±</td>
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<tr>
<td>Increase in heterochromatin of nuclei</td>
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<tr>
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<td>12  24  48  72  96 hr  1  2  3  4  5 weeks</td>
</tr>
<tr>
<td>Dilatation of granular endoplasmic reticulum</td>
<td>– – – – – – – – – –</td>
</tr>
<tr>
<td>Decrease in electron density and swelling of cored granule</td>
<td>± + # # ± ±</td>
</tr>
<tr>
<td>Disintegration and fusion of limiting membrane of cored granule</td>
<td>± + # # ± ±</td>
</tr>
<tr>
<td>Increase in Golgi apparatus</td>
<td>– – – – – ± # # + ±</td>
</tr>
<tr>
<td>Irregularity of nuclear wall</td>
<td>– – – – – – – – – –</td>
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marked in the groups examined 2 weeks and 3 weeks after ligation, but they almost returned to the normal pattern in the group examined at 5 weeks (Table 8). Figs. 11 and 12 show the electron micrographs of the beta cells in the groups examined at 2 weeks and 3 weeks after ligation, respectively.

**DISCUSSION**

The present authors have been interested in the functional correlation between pancreas and parotid gland and have performed a series of studies on this problem. It has been experimentally demonstrated that histologically, a pancreatic lesion is associated with the atrophy of the parotid gland and that functionally, it is associated with decreases in amylase level and bicarbonate concentration in the saliva secreted by the parotid gland as well as with reduced salivary output (Kakizaki et al. 1971, 1972, b, c, 1973b; Ishidate et al. 1974). This has been evidenced also in clinical cases (Kakizaki et al. 1973a, 1974a, b, 1975). Among problems awaiting further investigation, the most important problem is the analysis of the mechanism of intercommunication between pancreas and parotid gland. Furthermore, studies on the pathophysiological changes of the pancreas associated with the primary lesion of the parotid gland appear to be of essential importance. Therefore, the present authors have performed experimental studies in rats to evaluate the latter problem.

Consequently, bilateral ligation of the parotid gland duct was done in rats and the blood sugar, blood IRI, serum amylase and organ amylase levels were estimated at varying intervals after ligation. Furthermore, light and electron microscopic studies of the pancreas and parotid gland extirpated at various intervals after ligation were performed in order to clarify the functional and morphological alterations of the pancreatic secretion following the parotid gland duct ligation.

With regard to the relationship between endocrine secretion of the parotid gland and sugar metabolism, numerous excellent studies have long since been published but their results have not been unanimous. Some reported that the blood sugar level decreased following extirpation or duct ligation of the parotid glands and conversely some others claimed that the blood sugar level was elevated. Thus there have been contradictory observations on the endocrine function of the parotid glands, and, still more, there are investigators who deny the endocrine function of this gland (Rauch 1959). According to the present study, we found that there were considerable variations of blood sugar level even among control rats, although we attempted to provide identical experimental conditions. Furthermore, it was found that following ligation of the parotid gland ducts, the level of blood sugar was almost identical with or somewhat higher than that in the control group and there was no significant difference between the two groups.

With regard to the insulin level in blood, it is generally known that the blood sugar level and insulin level change in parallel except under certain pathological conditions. On the other hand, we could not find the literature dealing with the changes in blood IRI following such an impairment of the parotid gland as
we have experimentally induced. In our results, the blood insulin level was slightly elevated in the groups examined at 3 weeks and 4 weeks after ligation of the parotid gland ducts as compared with the control group, whereas in the other ligated groups the blood insulin level was rather decreased from the control level. Statistical analysis did not find any significant difference between the control and ligated groups, indicating that the ligation of the parotid gland ducts did not affect the insulin secretion significantly.

Regarding the relationship between parotid gland and serum amylase level, it has been reported that the serum amylase level is elevated in patients with parotitis (Zelman 1944). Experimental extirpation of the parotid glands is also reported to be associated with elevation of the serum amylase level. In our experiments, the ligation of the parotid gland ducts induced a markedly elevated serum amylase level in the group examined at 12 hr after ligation, then the level decreased gradually returning to almost normal level in the group sacrificed at 96 hr after ligation. Beyond this period, the serum amylase level remained almost constant. When taking in account the changes of the organ amylase level in the pancreas as described below, this finding may have resulted from the temporary release of the amylase of the parotid gland into the blood following ligation, although the confirmation awaits the isolation of the amylase isozyme. Functional changes of the exocrine cells of the pancreas following impairment of the parotid glands have not been described in the literature. The present study evidenced that the organ amylase level in the pancreas was significantly elevated in the groups examined at 24 hr, 48 hr, 72 hr, 96 hr, 1 week and 3 weeks after ligation of the parotid gland ducts.

Histological changes in the pancreas and parotid glands following ligation of the parotid gland ducts should be then discussed. With the light microscopy, the parotid glands became markedly atrophic immediately after ligation. In the pancreas, an atypical arrangement of the acinar cells as well as a slight hypertrophy of the islets of Langerhans was observed at 96 hr to 2 weeks following ligation. It is known that the size of the islets of Langerhans varies in the same pancreas. There are reports dealing with the correlation between the size and number of the islets of Langerhans but the results are not consistent. Also it is known that there are considerable individual variations in the size and number of the islets of Langerhans. Therefore we should be cautious in the interpretation of the findings obtained. Electron microscopic studies of the changes in the pancreas in association with impairment of the parotid glands have not been reported so far as the present authors were able to survey. With regard to the secretory cycle in the pancreatic acinar cells, it is generally accepted that this cycle proceeds within a considerable short period, whereby the acinar cells take up amino acids, the raw materials of the enzyme proteins which they secrete, from the blood vessels, and synthetize protein in the granular endoplasmic reticulum, which is then transferred to the Golgi apparatus to be condensed to zymogen granules which are then secreted in the acinar lumen.
In this study, electron microscopic changes such as the dilatation of granular endoplasmic reticulum, increase in heterochromatin in the nucleus and irregularities of the nuclear border began to appear in the group examined at 48 hr after ligation, and became most pronounced at 2 weeks, and tended then to return gradually to normal structures. The authors are of the opinion that the changes occurring in the nuclei have resulted from the disadvantageous conditions acting upon the pancreas, and the cells are in the functional phase, that is, in the hyperfunctional state as presumed from the behavior of the granular endoplasmic reticulum. These morphological changes occur almost synchronously with the elevation of the organ amylase level in the pancreas as evidenced in the functional study.

In the islets of Langerhans, changes in the beta cells were pronounced after ligation. The cored granules became less electron dense and swollen; their limiting membranes were frequently disintegrated and fused together. The Golgi apparatus developed relatively well. These changes were particularly pronounced 2 to 3 weeks after ligation and tended to return gradually to the normal structure. It is accepted that the granules in the beta cells are insulin-containing secretory granules. The exact mode of granule formation in the beta cells is still unknown. Furthermore, the relationship of the granular endoplasmic reticulum and the Golgi apparatus in the beta cells has not been fully understood and it is still unclarified whether the secretory granules are formed in the granular endoplasmic reticulum or in the Golgi apparatus system as this is the case with the exocrine cells of the pancreas. The authors are inclined to interpret these electron microscopic changes of the beta cells as they are discharging secretory granules, that is, insulin is released into blood. As already described, the insulin level in blood following ligation of the parotid gland duct did not show a statistically significant changes, so that it cannot be correlated with the electron microscopic changes in the beta cells. In summary, it has been found that when the parotid glands are impaired, histological and functional changes of the exocrine and endocrine cells of the pancreas, though slight in degree, are induced.

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Histologic findings of the parotid gland and bicarbonate content in parotid saliva of 

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Fig. 6. Pancreatic acinar cell in control group. Cap, blood capillary; Er, granular 
endoplasmic reticulum; M, mitochondria; N, nucleus; Sg, secretory granules. 
Original magnification, ×3,000.

Fig. 7. Pancreatic acinar cell a week after ligation. The dilatation of the granular endo-
plasmic reticulum (Er) was pronounced and in addition, increase of heterochromatin 
in the nuclei (N) and irregularities of the nuclear border were also evidenced. Original 
magnification, ×5,000.
Fig. 8. Pancreatic acinar cell 2 weeks after ligation. The dilatation of the granular endoplasmic reticulum, irregularities of the nuclear border and increase of heterochromatin are markedly noticed. Original magnification, ×4,000.

Fig. 9. Pancreatic acinar cells 5 weeks after ligation. The photograph shows the dilatation of the granular endoplasmic reticulum in the left half and approximately normal granular endoplasmic reticulum in the right half. Original magnification, ×15,000.
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Fig. 10 Beta cells of the pancreatic islet in a control rat. Er, granular endoplasmic reticulum; G, Golgi apparatus; M, mitochondria; Sg, secretory granules. Original magnification, ×3,000.

Fig. 11. Beta cells of the pancreatic islet 2 weeks after ligation. A decrease in electron density and swelling of the granules are noticed. The limiting membranes of the granules are disintegrated and fused. Original magnification, ×4,000.
Fig. 12. Beta cells of the pancreatic islet 3 weeks after ligation. The photograph shows relatively well developed Golgi apparatus. Original magnification, $\times 5,000$. 