HISTOCHEMICAL STUDIES ON STEROID DIABETES OF GUINEA PIGS WITH SPECIAL REFERENCE TO LANGERHANS ISLETS OF PANCREAS

ATSUSHI AOKI

Central Laboratory, Gifu University Hospital and Department of Pathology, Gifu University School of Medicine, Gifu

SYNOPSIS

The islets of Langerhans in guinea pigs with steroid-induced diabetes (by injecting 5 mg of cortisol twice daily) were studied histologically and histochemically at various time intervals. The islets in those animals marked hypertrophy with hyperplasia. Hydropic degeneration and glycogen infiltration were also observed in the beta cells in various degrees. The alpha cells of the animals with prolonged severe diabetes also showed degranulation and glycogen infiltration, which had not yet been reported and represented unique findings in steroid-induced diabetes. Histochemical studies showed a definite increase of glucose-6-phosphatase activity after 3 days of cortisol treatment. The activities of glucose-6-phosphate dehydrogenase and lactic dehydrogenase increased in both alpha and beta cells. In the early stage of diabetes acid phosphatase activity was either normal or slightly increased, but later decreased in both alpha and beta cells. These altered enzymatic activities returned to normal one week after cessation of steroid treatment. Several other enzymes examined histochemically showed no significant changes. The histochemical findings in the alpha and beta cells in this experiment suggest a new interpretation of the functional correlation between the two types of cells. The significance of these enzymes and their possible role in the functional activity of the islet cells are discussed.

It is well known that adrenal steroids increase gluconeogenesis and glycogen synthesis in the liver and induce hyperglycemia. Recently, the clinical use of the adrenal steroids in large doses in patients with various kinds of diseases has posed new problems, because it has not infrequently induced steroid diabetes as an iatrogenic disorder.

Moreover, there have been many attempts to induce steroid diabetes experimentally since Ingle (1941) induced diabetes in force-fed rats by treatment with cortisone.

Histological changes found in the pancreas of the animals with experimental steroid diabetes have been reported to be hypertrophy and hyperplasia of the islets of Langerhans, and degranulation and hydropic degeneration of the beta cells (Hausberger and Ramsay, 1953; Hausberger and Ramsay, 1955; Lazarus and Bencosme, 1955; Bencosme and Lazarus, 1956; Lazarus and Bencosme, 1956; Volk and Lazarus, 1958; Lazarus and Volk, 1959). However, the histological findings in the islet cells described in the above papers were of those in the beta cells, and no changes in the alpha cells have been reported.

With the progress of enzyme histochemistry, many new enzymes have been found in the
islet cells of various species (Gössner, 1959; Lazarus, 1959 a; Lazarus and Bradshaw, 1959; Lazarus, 1959 b; Gepts and Toussaint, 1963). The effect of the adrenal steroids on these enzymes has been reported by Lazarus (1959 b), Lazarus and Barden (1961), Gepts and Toussaint (1963) and Hellerström et al., (1965). However, the results of their investigations were not always consistent, because of the difference of species used in their experiments.

Some investigators tried to observe the effect of stimulation of beta-cells by glucose loading (Smith and Lacy, 1962) or by the administration of sulfonylurea (Lazarus 1959 b) on the activities of enzymes in the islet cells on the theory that the enzymes may be responsible for the synthesis and/or release of insulin. Lazarus (1959 a, 1959 b) and Lazarus and Barden (1961) postulated that glucose-6-phosphatase in the beta cells might be responsible for insulinogenesis and that adenosine-triphosphatase might be related to release of insulin in the beta cells. The animals used in these investigations were mostly rabbits, rats or mice. No histochemical observation on enzymes in the islets of guinea pigs has yet been reported. The present investigation is designed to demonstrate several enzymes histochemically in the islet cells of untreated and steroid-treated guinea pigs in order to analyze a possible relationship between enzyme activities and the function of the islet cells. In addition, both alpha and beta cells in the pancreatic islets of guinea pigs with steroid diabetes have been studied histologically.

MATERIALS AND METHODS

Male and female guinea pigs weighing 500–700 gm were used in the experiment. Most of them were obtained from commercial farms and reared in the laboratory for about two weeks prior to the experiment. Some of them were born and grown in my laboratory, although their parents had been obtained commercially. They were freely fed CR-1 diet (Nihon Haigoshiryo Co.) and given additional vegetables, and water ad libitum.

They were kept in individual cages at least one week prior to the beginning of the experiment.

Diabetes was induced by the method of Hausberger and Ramsay (1955); i.e. 5 mg of 17-hydroxycorticosterone acetate (Cortisol, Scheroton F, Schering Co.) was injected subcutaneously twice daily for various periods.

Urinary glucose was examined with Testape (Lilly Co.) twice a day just prior to steroid injections. The severity of glycosuria (−,+,++,++++) was determined by comparing the color on the tape with the attached color table.

Body weight was checked early every other morning. Blood glucose was determined by the method of Nelson-Somogyi (1952) on blood drawn from the femoral veins at the time of sacrifice.

Two animals were sacrificed on the 3rd day, 3 to 4 hrs. after the last injection. Four, 3 and 4 of these guinea pigs were killed on the 15th, 21st and 30th day, respectively, and 3 after treatment for more than 30 days. Two animals were sacrificed one week after cessation of treatment which had lasted for 30 days.

Immediately after death, the pancreas and liver were removed, frozen by liquified carbon dioxide, and cut into thick slices of 10μ with a cryostat for enzyme histochemistry. The pancreas was fixed in Bouin’s solution, dehydrated, embedded in paraffin and then cut into sections of 4μ thick. The sections were stained with Gomori’s chromehematoxylin phloxin stain (1941), aldehyde-fuchsin trichrome stain (Gomori, 1950 a) and periodic acid Schiff (McManus, 1948). The Bouin-fixed pancreatic tissues of the animals treated with steroid for a long time were cut serially and stained by Gomori’s method or PAS stain alternately to detect glycogen infiltration in the islet cells. The PAS-positive granules in the pancreas were also examined by diastase-digestion to determine whether or not they were glycogen. A particle of the liver was also fixed in Carnoy’s solution and stained with PAS for glycogen. Some of the fresh frozen sections of the pancreas and liver were fixed
Table 1. Methods utilized in histochemical observation of various enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Method</th>
<th>Fixation</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fixatives</td>
<td>Time (min.)</td>
<td>Temp. (°C)</td>
</tr>
<tr>
<td>Glucose-6-phosphatase</td>
<td>Wachstein &amp; Meisel (1956)</td>
<td>Post fixed 6% neutral formalin</td>
<td>30</td>
</tr>
<tr>
<td>Phosphorylase</td>
<td>Takeuchi &amp; Kuriaki (1955)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acid Phosphatase</td>
<td>Gomori (1950 b)</td>
<td>Cold acetone</td>
<td>30</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>Gomori (1952)</td>
<td>Cold acetone</td>
<td>30</td>
</tr>
<tr>
<td>Adenosine triphosphatase</td>
<td>Padycula &amp; Herman (1955)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5-Nucleotidase</td>
<td>Wachstein &amp; Meisel (1957)</td>
<td>Post fixed 6% neutral formalin</td>
<td>30</td>
</tr>
<tr>
<td>Succinic dehydrogenase</td>
<td>Nachlas et al. (1957)</td>
<td>Post fixed 6% neutral formalin</td>
<td>30</td>
</tr>
<tr>
<td>Lactic dehydrogenase</td>
<td>Hess et al. (1958)</td>
<td>Post fixed 6% neutral formalin</td>
<td>30</td>
</tr>
<tr>
<td>α-Glycerophosphate dehydrogenase</td>
<td>Hess et al. (1958)</td>
<td>Post fixed 6% neutral formalin</td>
<td>30</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>Hess et al. (1958)</td>
<td>Post fixed 6% neutral formalin</td>
<td>30</td>
</tr>
<tr>
<td>Isocitric dehydrogenase</td>
<td>Hess et al. (1958)</td>
<td>Post fixed 6% neutral formalin</td>
<td>30</td>
</tr>
</tbody>
</table>

in formol and stained with Sudan III for lipids.

Fresh frozen sections of 10µ thick were used for histochemical demonstration of various enzymes by the methods listed in Table 1. Sections from normal guinea pigs of the same sex were used as controls for enzyme histochemistry.

**EXPERIMENTAL RESULTS**

Glycosuria first appeared on the 3rd to 5th day of treatment. In general, severe diabetes occurred more in males than in females, and the severity of diabetes which developed in female animals was variable. Glycosuria disappeared within 2 to 3 days after the treatment was discontinued. So-called “meta-diabetes” was not observed. The severity of glycosuria during the treatment, body weight at the beginning and end of the experiment, and blood sugar levels at the time of sacrifice are shown in Table 2. A typical response of body weight and urinary glucose of an animal manifesting severe steroid diabetes is shown in Figure 1.

**Histological findings in the pancreas**

Slight degranulation and hypertrophy of the beta cells appeared as early as 3 days after treatment was started. In animals killed after 7 days of treatment, degranulation of the beta cells was witnessed, and the islets became larger than normal. Figure 2 shows a normal guinea pig islet, stained with Gomori’s chrome-hematoxylin phloxine method. The changes may be due to the proliferation of beta cells. Some mitotic figures are seen in the beta cells (Fig. 3). Small islets are intermingled with hypertrophied islets. Hypertrophy of the islets and degranulation of the beta cells increased after 15 days of treatment, and the cytoplasm of the beta cells became pale and sparsely vacuolated. At this stage the ratio between alpha and beta cells was decreased due to hyperplasia of the latter (Fig. 4). After 3 weeks of treatment, hyperplasia of the islets became predominant. Beta granules were markedly decreased, and vacuolated beta cells were sparse. Some of the alpha cells were slightly degranulated (Fig. 5). In the animals
treated for more than 30 days, vacuolation of the beta cells increased and only a few granulated beta cells were found in an islet. The nuclei of the vacuolated beta cells became rather small or pyknotic, or degenerated. The area of Langerhans islets occupying a definite microscopic field increased tremendously. Hypertrophy and hyperplasia of the islets were more exaggerated. The alpha cells were also degranulated to varying degrees. Some degranulated alpha cells were difficult to differentiate from degranulated beta cells (Fig. 6, 7).

In the animals killed one week after the discontinuation of treatment, granules of the beta cells were restored to an almost normal appearance, though vacuolated beta cells were still seen occasionally (Fig. 8).

Histochemical findings

(1) Glycogen

Pancreas: Glycogen was not demonstrated in the islet cells of untreated guinea pigs, but it was present in the beta cells of the cortisol-treated animals after 3 weeks of treatment. However, no glycogen was found in the epithelial cells of the ducts of the exocrine glands at that time. Much glycogen was deposited in the beta cells of animals with severe diabetes of more than one month's duration, and a small amount of glycogen was also observed in the epithelial cells of the ducts (Fig. 9). Careful examination of serial sections stained alternately with Gomori’s and PAS stain revealed a deposition of glycogen in the alpha cells, somewhat less than that in the beta cells (Fig.

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Table 2. Protocol of animal treated with cortisol

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Sex</th>
<th>Total doses of injected cortisol (mg)</th>
<th>Body weight (g)</th>
<th>Urinary glucose (Tes-tape)</th>
<th>Duration of diabetic state (days)</th>
<th>Blood sugar level at sacrificing (mg/dl)</th>
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<tr>
<td>1</td>
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<td>30</td>
<td>710</td>
<td>715</td>
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<tr>
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<td>F</td>
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<td>680</td>
<td>680</td>
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<td>840</td>
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<td>F</td>
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<td>780</td>
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<td>630</td>
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<td>710</td>
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<td>210</td>
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<td>310</td>
<td>610</td>
<td>730</td>
<td>★★ ~ —</td>
<td>28</td>
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Hydrocortisone 2 to 5mg daily

Fig. 1. Body weight urinary glucose in a guinea pig treated with hydrocortisone acetate (cortisol)

10, 11). Vacuolation of the beta cells did not always parallel the degree of glycogen infiltration.

Liver: A moderate amount of glycogen was stored in the liver cells in the centrolobular area in normal guinea pigs (Fig. 20). The liver cells were diffusely hypertrophied as early as three days following treatment with cortisol, and intracytoplasmic glycogen increased even in the cells in the peripheral zones of the lobule, so that increased staining occurred throughout the lobule (Fig. 21).

(2) Lipid

Pancreas: Lipid was not demonstrable in the islets of either the untreated or the cortisol-treated guinea pigs.

Liver: No lipid was demonstrated in the liver cells of either the cortisol-treated guinea pigs or the controls. However, a small amount of lipid was seen in the Kupffer's cells on the peripheral zone of the lobules of animals treated for more than one month.

(3) Enzymes
(a) Glucose-6-phosphatase (G-6-Pase)

Pancreas: Moderate activity of this enzyme was demonstrated in the beta cells of normal guinea pigs (Fig. 12). No positive reaction was obtained in the acinar cells or duct epithelium. The activity in the beta cells definitely increased after 3 days of cortisol treatment, but the increased activity had returned to normal one week after discontinuation of the treatment (Fig. 13).

Liver: G-6-Pase activity was found in considerable amounts throughout the lobule, though it appeared to be more concentrated around the central vein than in the periphery of the lobules in the control animals (Fig. 22):
Table 3. Histochemical reaction in pancreatic islet cells and liver cells of cortisol treated and control guinea pigs

<table>
<thead>
<tr>
<th>Kinds of histochemical reaction</th>
<th>Tissue</th>
<th>Pancreatic islets of Langerhans</th>
<th>Liver</th>
<th>Change after cortisol treatment</th>
<th>Grade of normal reactivity</th>
<th>Changes after cortisol treatment</th>
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<tr>
<td></td>
<td></td>
<td>Relative activity of A-and B-cells in the control</td>
<td>Grade of normal reactivity</td>
<td>Change after cortisol treatment</td>
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<td></td>
<td></td>
<td>O</td>
<td>O</td>
<td>O ~ / /</td>
<td>moderate</td>
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<td></td>
<td></td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O ~ / /</td>
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<tr>
<td></td>
<td></td>
<td>B-cells only</td>
<td>high</td>
<td>/ /</td>
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<td>O</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>B = A</td>
<td>high</td>
<td>~ \ \</td>
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<tr>
<td></td>
<td></td>
<td>O</td>
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<td>O</td>
<td>moderate</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>B = A</td>
<td>moderate</td>
<td>~</td>
<td>high</td>
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<td></td>
<td></td>
<td>B = A</td>
<td>low</td>
<td>~</td>
<td>moderate</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>B = A</td>
<td>low</td>
<td>~</td>
<td>high</td>
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<tr>
<td>α-GlycD</td>
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<td>B = A</td>
<td>low</td>
<td>~</td>
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<tr>
<td>G-6-PD</td>
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<td>~</td>
<td>moderate</td>
<td></td>
</tr>
<tr>
<td>I D</td>
<td></td>
<td>B = A</td>
<td>moderate</td>
<td>~</td>
<td>moderate</td>
<td></td>
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</tbody>
</table>

/ / or \ \: marked increase or decrease

~: no detectable change

A: alpha cells

The activity increased in the cortisol-treated animals (Fig. 23).

(b) Phosphorylase

Pancreas: No activity of this enzyme was demonstrated in the islets of untreated guinea pigs. Even the islets with heavy deposits of glycogen following a long-term administration of cortisol were free of this enzyme.

Liver: Slight activity of this enzyme was demonstrated in the intact liver cells of the centrolobular zone. The activity disappeared soon after the beginning of cortisol treatment.

(c) Acid phosphatase (AcPase)

Pancreas: AcPase activity was high in the islet cells of untreated animals, moderate in the duct epithelium, and low in the acinar cells, in which it was concentrated in the secretory poles (Fig. 16). The activity in the islet cells did not change during the early stage of cortisol treatment, but it decreased after 3 weeks of treatment, and the decrease in both alpha and beta cells was apparent in the animals treated for more than one month (Fig. 17).

Liver: High AcPase activity was found uniformly throughout the lobules of normal liver (Fig. 24), but it tended to decrease as early as one week after the beginning of cortisol treatment (Fig. 25).

(d) Alkaline phosphatase

No alkaline phosphatase activity was demonstrated in the pancreas of normal guinea pigs except in excretory ductules. A high concentration of this enzyme was found in the bile capillaries on the periphery of the hepatic lobules. However, no significant change in activity was produced by cortisol treatment.

(e) 5-Nucleotidase

Pancreas: Marked activity of this enzyme was demonstrated in the excretory ductules and vascular walls of the untreated animals. The activity in the acinar and islet cells was scanty and was not changed by treatment with cortisol.
Liver: The activity was intense in the vascular walls and moderate in normal liver cells throughout the lobule, and it was not altered by treatment with cortisol.

(f) Adenosine triphosphatase (ATPase)

Pancreas: In the untreated animals the activity of this enzyme was high in the excretory ductules and vascular walls and moderate in the islet and acinar cells. It was not changed by cortisol treatment.

Liver: The activity was intense in the vascular walls and moderate in normal liver cells throughout the lobule, and it was not altered by treatment with cortisol.

(h) Isocitric dehydrogenase (ID)

Pancreas: The activity of this enzyme was noted throughout the untreated pancreas. It was high in the ductules, moderate in the acinar cells, low in the islet cells, and was not changed by treatment with cortisol.

Liver: In the control animals moderately intensive ID activity was distributed throughout the hepatic lobule, and it was more intense in the cells on the periphery of the lobule. It increased evenly throughout the lobule as early as one week after the beginning of treatment.

(i) \(\alpha\)-Glycerophosphate dehydrogenase (\(\alpha\)-GlycD)

Pancreas: The activity of this enzyme was intense in the acinar cells and moderate in the islet cells in the control guinea pigs. Treatment with cortisol did not influence the activity in either cells.

Liver: Staining was diffuse in normal liver cells and a minimal increase was observed after treatment with cortisol.

(j) Lactic dehydrogenase (LD)

Pancreas: Moderately intense staining was seen in the acinar cells of the pancreas, while that in the islet cells was less intense in the untreated animals (Fig. 18). However, administration of cortisol to the islet cells increased the reactivity to the level seen in the acinar cells (Fig. 19).

Liver: The activity of this enzyme in normal liver cells was moderately intense. It was not altered or was slightly decreased by cortisol treatment.

(k) Succinic dehydrogenase (SD)

Pancreas: Activity of this enzyme was not noted throughout the untreated pancreas. It was high in the ductules, moderate in the acinar cells, low in the islet cells, and was not changed by treatment with cortisol.

Liver: The staining was distributed in the liver cells of the centrolobular zone in the controls. It increased following treatment to the extent that it appeared also in the cells in the periphery of the lobules.

DISCUSSION

Hausberger and Ramsay (1953, 1955) first induced steroid diabetes in guinea pigs. They found hypertrophy of the pancreatic islets due to hyperplasia of beta cells. They also reported degranulation of the beta cells frequently associated with hydropic degeneration.

The lesions of the beta cells in the present investigation correspond roughly to those described by Hausberger and Ramsay. How-
ever, degranulation and glycogen infiltration in the alpha cells were also clearly demonstrated in this experiment. No descriptions have been published of the lesions in the alpha cells in rats, rabbits or dogs due to steroid-induced diabetes. Only one report is available in which glycogen infiltration in the alpha cells was noted in experimental diabetes (Volk and Lazarus, 1963). However, in this case diabetes was not induced by steroid but by growth hormone injected into partially pancreatectomized dogs. Accordingly, the lesions in the alpha cells of animals with steroid diabetes described in this paper seem to represent unique findings.

Vacuolation of the beta cells in the islets of Langerhans in human subjects or animals with experimental diabetes has been considered to represent a degenerative process due to exhaustion of the cells and has been designated as vacuolar or hydropic degeneration (Weichselbaum and Stangl, 1901; Allen, 1922; Ham and Haist, 1941; Luckens and Dohan, 1942). In subsequent years, Toreson (1951), Lazarus and Volk (1957, 1958 a, b) have ascribed the lesion to a mere glycogen infiltration which was found in the renal tubules, myocardium or other tissues of diabetic animals as a manifestation of hyperglycemia, since glycogen was demonstrable in the vacuolated cytoplasm of beta cells with no regressive changes in their nuclei and since a similar infiltration of glycogen was also observed in the duct epithelium. In the present study the nuclei of beta cells with extensive vacuolation did show regressive changes and not infrequently contained less glycogen, while a large amount of glycogen was found in the beta cells with minimal cytoplasmic vacuolation and without nuclear degeneration. It is known that tissue glycogen is lost to some extent during the fixation and preparation of microscopic slides. The loss seems to be caused by the kind of fixative, degree of polymerization of glycogen, and perhaps other unknown factors. Therefore, vacuolation of the beta cells with less glycogen infiltration may be due to the artificial loss of glycogen. However, it is more likely, on the basis of the findings in the present study, that glycogen infiltration and hydropic degeneration in the beta cells are not equivalent and that the latter represents a degenerative change.

Intracytoplasmic glycogen in alpha cells was less densely packed in alpha cells than in beta cells and was observed in this study only in animals with prolonged severe diabetes. These findings are in accord with the observations in growth hormone-induced diabetes in dogs reported by Volk and Lazarus (1963). They suggested that the difference in severity of glycogen infiltration between the beta and alpha cells depended on quantitative rather than qualitative difference of cellular metabolic systems for the disposal of available glucose. However, in this study both qualitative and quantitative differences in the activities of enzymes which participated in carbohydrate metabolism were found existing between the alpha and beta cells. G-6-Pase was not present in the alpha cells and there was less G-6-PD activity in them than in beta cells of both intact and diabetic animals.

It is a well established fact that the beta cells produce insulin as their hormone and that insulin or a precursor is usually stored within beta granules. Loss of granules from beta cells means diminished insulin storage and may result either from decreased insulin production or from increased insulin liberation.

As mentioned above, in the hyperglycemic state experimentally induced by adrenocortical hormone, there is a decrease or a loss of beta granules. This finding indicates that cortisol administration may cause an increase of production and secretion of insulin by the beta cells.

The author supposes that excessive insu-
linogenesis of beta cells caused by steroid treatment may lead to their exhaustion and thus derange the cellular metabolism of glucose which is probably in turn responsible for the earlier and more severe glycogen infiltration in beta than in alpha cells.

This later and slighter glycogen infiltration in alpha cells may also depend on the difference in the enzymatic system and glucose metabolism between alpha and beta cells. The loss of granules from alpha cells can also be used as an indicator of diminished glucagon storage. This may result either from diminished glucagon synthesis or from increased glucagon secretion.

The results of the present experiment thus show that adrenocortical hormone injected into guinea pigs tends to increase not only the production and liberation of insulin by beta cells, but also output of glucagon by the alpha cells.

It is difficult to evaluate the biological significance of the enzymes that are histochemically demonstrable in the islets, since the number of demonstrable enzymes is limited and their activities vary with the animal species. However, the kinds of enzymes and their activities in the islets differ from those in the acinar cells. These differences seem to be related to different functions between the islets and exocrine tissues. Therefore many investigators have tried to demonstrate enzymes localized in Langerhans islets (Lazarus, 1959 a; Lazarus and Bradshaw, 1959; Gepts and Toussaint, 1963; Hellman and Hellerstrom, 1962; Goessner, 1959). The activities of some enzymes in beta cells in animals given cortisol, sulfonylurea and glucose have also been investigated (Lazarus, 1959 b; Gepts and Toussaint, 1963; Lazarus and Barden, 1961; Hellstrom et al., 1965).

Lazarus has presumed that a decrease in G-6-Pase activity led to an increase of the concentration of glucose-6-phosphate in the beta cells and stimulates insulin output, and he suggested a possible relationship between AcPase in the islets and insulin synthesis (1959 b). Lazarus and Barden also speculated on the possible relationship between extra-mitochondrial ATPase and mechanism of insulin release (1961), since extramitochondrial ATPase in beta cells disappears with cortisol treatment along with a decrease of beta granules. However, they could not demonstrate any changes in G-6-Pase and of AcPase activities following cortisol treatment (1959 b). Lazarus and Barden (1965) postulated a relationship between G-6-Pase and insulin synthesis rather than insulin output on the basis of their electron microscopic study of histochemistry, which revealed that G-6-Pase could not be demonstrated in the beta granules but was present in the nuclei and the inner surface of the endoplasmic reticulum. The present study revealed an increase in G-6-Pase in the beta cells prior to the appearance of glycosuria and its persistence after degranulation of the beta cells in guinea pigs treated with cortisol. This result is inconsistent with the findings of Lazarus in rabbits. However, since G-6-Pase activity was elevated also in the islets of hyperglycemic obese mice with a high serum level of insulin-like activity (Hellman and Hellerstrom, 1962), the increase in activity of this enzyme during cortisol treatment in guinea pigs may well have been related to hyperactivity of the beta cells.

The function of AcPase in pancreatic islets has not yet been fully clarified. Lazarus (1959 b) reported no change in the activity of this enzyme in the islet cells of rabbits after treatment with sulfonylurea, while a slight decrease in activity was reported following treatment with cortisol in rats by Gepts and Toussaint (1963). Recently Lazarus et al., (1966) revealed electron microscopically that AcPase activity was localized in the Golgi complex, and not to beta granules. From this result and from the
findings that beta granules are formed in endoplasmic reticulum and that no alteration of this enzyme is observed after stimulation of beta cells, they concluded that there was no direct relationship between AcPase and the formation or release of granules in the beta cells. However, if Vorbrodt's hypothesis (1958) that AcPase is related to the process of protein synthesis is universally assumed to be valid, then this enzyme in the islet may play some role in the synthesis of insulin.

Gössner (1959) reported that AcPase was absent in the islets of a patient with severe juvenile diabetes, while in senile diabetes less AcPase was demonstrated than in controls. This finding is very interesting in connection with the above hypothesis of Vorbrodt. In the present study, the activity of AcPase in the islets of cortisol-treated guinea pigs appeared to be normal or slightly increased in the early phase, and was decreased by prolonged treatment. These findings also support the hypothesis that AcPase may play a role in the synthesis of insulin.

The biological significance of ATPase in the islets is still obscure. Lazarus and Barden (1961) demonstrated that the activity of extramitochondrial ATPase was confined to beta granules and that this activity disappeared along with degranulation of the beta cells upon adrenal steroid administration. They speculated on the possible relationship between this enzyme and the mechanism of insulin release. But this enzyme was not found in the islets of hyperglycemic obese mice. Hellerström et al. (1965) concluded that the decrease in ATPase activity in their cortisol-treated rats was not specific for the islet cells since a similar decrease in the exocrine tissue of the pancreas was also demonstrated quantitatively. In the present study, ATPase activity in the islet cells of guinea pigs was weak and no alteration of the activity was induced by cortisol.

Phosphorylase was not demonstrated in the islets of hyperglycemic obese mice (Hellman and Hellerström, 1962), neither was it detected in the islets of normal guinea pigs nor in those with glycogen infiltration in the cortisol-treated animals. It is well-known that there are two pathways through which glycogen is synthesized in tissue: one is by amylophosphorylase and the other is via UDPG (uridine-5-diphosphate glucose) from glucose-1-phosphate. The latter may be responsible for glycogen infiltration in the islet cells in steroid-induced diabetes.

Gepts and Toussaint (1963) and Hellerström et al. (1965) reported that the histochemical reaction of 5-nucleotidase in rat islets with no G-6-Pase activity was intensified by cortisone treatment and thereby suggested a close relationship between 5-nucleotidase and the specific function of the islet beta cells. However, the present study made using guinea pigs did not demonstrate any increase in activity of this enzyme, which was low in the islets of untreated animals. It is impossible therefore, to attach any important significance of this enzyme in the function of the islet cells of rats.

Oxidation of glucose in beta cells is mostly done by the hexose monophosphate shunt rather than the Embden-Meyerhof pathway. Intense activities of some dehydrogenases related to the former pathway were demonstrated in the islet cells (Lazarus and Bradshaw, 1959). Wilson and Siperstein have suggested (1959) that this pathway may not only supply energy for metabolic processes but may possibly be a source of TPNH, which is supposed to be related to the protein synthesis of cells. Therefore, Lazarus and Bradshaw (1959) postulated the possible role of this metabolic system in controlling beta cell insulin synthesis. Smith and Lacy (1962) demonstrated an increase in the activities of these dehydrogenases in rabbits receiving repeated injections of glucose. They also found a similar increase in the
exocrine tissue of the pancreas and other organs and suggested that alteration in these enzyme activities was not specific for islet cells. Gepts and Toussaint (1963) have criticized Lazarus' hypothesis because they observed a decrease in the activities of these dehydrogenases in cortisone-treated rats, but their animals did not develop diabetes. In the present experiment ID, α-GlycD and SD activities were not changed by cortisol treatment; however, G-6-PD and LD activities were definitely increased. An increase in G-6-PD activity which takes place as the first step of the hexose monophosphate shunt may accelerate glucose utilization in pancreatic beta cells via this metabolic pathway and may be responsible for increasing insulin synthesis as Lazarus and Bradshaw (1959) suggested.

In guinea pigs with steroid-induced diabetes, G-6-PD and LD activities increased in both alpha and beta cells. ACPase was normal or slightly increased in alpha and beta cells during the initial stage of steroid-induced diabetes in guinea pigs, and later decreased markedly. These histochemical findings are very important to the functional relationships between alpha and beta cells.

The problems of glucagon and insulin still remain synthetically linked. Much of the evidence in favour of the hormonal nature of glucagon has been obtained from experiments indicating that the alpha cells secrete a hormone which antagonizes hypoglycemic action of insulin. On the other hand, it is said that glucagon is produced during hyperglycemia rather than during hypoglycemia. If this is true, glucagon could possibly be physiologically synergistic with insulin. The histochemical results of the present study support the viewpoint that the function of alpha cells is completely synergistic with that of beta cells.

Finally the activities of various enzymes seem to be less accurately estimated by histochemical staining reactions than by biochemical techniques. Moreover, the functions of the enzymes demonstrated in the islet cells have been speculated on only by comparing histochemical and biochemical findings. However, the alterations in the activities of some enzymes shown in the present investigation indicate a close relationship between them and the increased function of the islet cells, both alpha and beta cells, in the progress of steroid-induced diabetes in guinea pigs.

**SUMMARY**

Diabetes was induced in guinea pigs by the daily subcutaneous injection of 10 mg of cortisol. Histological and histochemical examinations revealed the following results.

1) The islets of Langerhans showed marked hyperplasia in steroid-induced diabetes.

2) Degranulation and glycogen infiltration of the beta cells appeared in the initial stage of the diabetes, and similar but less definite findings were later noted in the alpha cells in animals with long-standing diabetes.

3) Intracytoplasmic vacuolation of the beta cells should be distinguished from glycogen infiltration.

4) Phosphorylase activity was not demonstrated histochemically in the islets of Langerhans in either normal or diabetic animals.

5) G-6-Pase activity in the beta cells was increased by steroid treatment.

6) G-6-PD and LD activities were increased in both alpha and beta cells.

7) AcPase in alpha and beta cells was normal or slightly increased in the early stage of steroid-induced diabetes, but later decreased.

8) These enzymatic activities returned to normal one week after the cessation of cortisol treatment.

9) Other enzymes; Histochemical studies showed no significant changes of ATPase, 5-nucleotidase, SD, α-Glyc D or ID activity in the islets.
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Present address: *Department of Pathology, Kyoto University School of Medicine
**Chief of Clinical Pathology, Kansaidenryoku Hospital, Osaka.

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Fig. 2. Pancreas of a normal guinea pig. The size of the islet and the density of granules of the cells in this picture indicate a normal level. Granules of beta cells look dense and those of alpha cells bright. Gomori's chrome-hematoxylin and phloxine (CHP) stain. ×200.

Fig. 3. Pancreatic islet of a diabetic guinea pig treated with cortisol for 7 days. The beta cells are hyperplastic and degranulated, and mitotic figures (arrows) are seen. CHP stain. ×200.

Fig. 4. Pancreatic islet of a diabetic guinea pig treated with cortisol for 14 days. Marked degranulation, hypertrophy and few vacuolated cells are seen in the beta cells. This islet is composed almost of beta cells. CHP stain. ×200.

Fig. 5. Pancreatic islet of a diabetic guinea pig treated with cortisol for 21 days. The beta cells are almost completely degranulated and slightly vacuolated. The alpha cells are slightly degranulated. Arrows indicate degranulated alpha cells. CHP stain. ×200.

Fig. 6. Pancreas of a diabetic guinea pig treated with cortisol for 40 days showing a marked increase in size and number of islet. CHP stain. ×40.

Fig. 7. Higher magnification of the pancreas shown in Figure 6. Most of beta cells are almost completely vacuolated and their nuclei are small or pyknotic. A small number of atrophic alpha cells are seen. CHP stain. ×200.
Fig. 8. Pancreatic islet of a guinea pig 7 days after cessation of 30-day cortisol treatment. The alpha and beta cell granules are restored to almost normal appearance, though a few vacuolated beta cells yet remain. CHP stain. ×200.

Fig. 9. Pancreatic islet of a guinea pig with cortisol treatment for 40 days, showing a large amount of glycogen infiltration. PAS stain. ×200

Fig. 10, 11. Serial sections of a pancreatic islet with cortisol treatment for 40 days. Glycogen clearly deposits in the alpha cells. Arrows indicate alpha cells with glycogen granules.

Fig. 10: CHP stain. Arrows show alpha cells, ×400. Fig. 11: PAS stain. ×400.

Fig. 12. G-6-Pase activity distributed in the pancreas of a control guinea pig. The activity almost entirely confined to the beta cells. The alpha cells and acinar tissues remain unstained. ×200.

Fig. 13. G-6-Pase activity increase in the beta cells of a guinea pig treated with cortisol for 7 days. ×200.
Fig. 14. G-6-PD activity distributing in the pancreas of a control guinea pig. Marked activity is shown in the beta cells as well as the duct epithelium and less activity in the alpha cells. ×200.

Fig. 15. G-6-PD activity increased in the beta and alpha cells of a guinea pig treated with cortisol for 15 days. ×200.

Fig. 16. AcPase activity distributing in the pancreas of a control guinea pig. The activity is intense in the islet cells, ductules, and secretory pole of acinar cells. ×200.

Fig. 17. Pancreas of a guinea pig treated with cortisol for 30 days showing decreased activity of AcPase in the islet cells. ×200.

Fig. 18. Activity of LD distributing in the pancreas of a control guinea pig. Its intense activity is seen in the acinar tissue and duct epithelium, while it is less active in the islet cells. ×200.

Fig. 19. Pancreas of a guinea pig treated with cortisol for 15 days. Activity of LD in the islet cells increased up to almost the same level as seen in the acinar cells. ×200.
Fig. 20. Glycogen is moderately demonstrated in the liver cells on the centrolobular portion of a normal guinea pig. PAS stain. ×200.

Fig. 21. Liver glycogen increased throughout the lobule and the liver cells diffusely hypertrophied after cortisol treatment for 7 days. PAS stain. ×200.

Fig. 22. G-6-Pase activity distributing in the liver of a control guinea pig. Marked activity is confined to the cytoplasm of the hepatic cells. ×200.

Fig. 23. The liver of a guinea pig treated with cortisol for 15 days showing marked increased activity of G-6-Pase. ×200.

Fig. 24. AcPase activity distributing in the liver of a control guinea pig. Moderate activity is found in the liver cells throughout the lobule. ×200.

Fig. 25. The liver of a guinea pig treated with cortisol for 30 days showing marked decreased activity of AcPase. ×200.