Sexual Disturbances in Diabetes*

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Histological, histochemical and biochemical investigations were performed on the adenohypophysis, thyroid, adrenal and gonad of diabetic patients and of experimental diabetic animals. The characteristic changes in the adenohypophysis of diabetics were remarkable increase of alpha cells and distinct decrease of delta cells. In most of the cases, the follicles of the thyroid were large and their epithelial cells were low and flat. The characteristic feature of the adrenal cortex was hyperplasia of the zona fasciculata. The testicular canaliculi were atrophied with thickened basement membranes. Spermatogenesis ceased almost completely in many tubules. The basement membrane of the diabetic testis was stained stronger than the normal with PAS. Spermatogenic cells were stained with Alcian blue; especially the cells near the basement membrane had strong affinity to the dye. A remarkable metachromasia with toluidine blue in the diabetic testicular tissue was recognized at pH 1.5 or 3.0. A significant increase in the values of QO2 and QCO2 of testicular tissues in diabetics was demonstrated. Almost all diabetics showed decreased urinary excretion of pituitary gonadotropin. The present investigation suggested that these changes were due to deficiency of TSH, ACTH and gonadotropin resulting from the degeneration of the adenohypophysis in diabetics. However, the sexual disturbances in diabetics might be due not only to deficient pituitary gonadotropin, but also to degenerative changes of the nerves innervating the gonads.

Sexual disturbances in diabetes are frequently observed. Impotency is the most frequent complaint of male patients suffering from diabetes. In spite of a considerable literature dealing with the sexual disturbances in diabetics (by Foglia et al., Schöffling et al., Willianueva, and others), the mechanism of impairment of sexual function in diabetics is still unknown. The present investigation is intended to clarify the mechanism of the disorders.

MATERIALS AND METHODS

Mature male dogs and rats of Wistar strain were used in the present experiments. The animals were treated with alloxan injections or submitted to...
subtotal pancreatectomy (95% pancreatectomy). Only those cases which had characteristic signs of diabetes and glucosuria were employed throughout the experiment. On the other hand, specimens of well preserved pituitaries, thyroids, adrenals and gonads obtained from routine autopsy cases with diabetes at the Department of Pathology, Tohoku University School of Medicine, were selected for study, and 11 specimens from non-diabetic cases were used as controls. The cases with history of endocrine diseases or hormonal therapy were excluded from the study. Determinations of urinary excretion of pituitary gonadotropin in 15 diabetics and urinary steroid excretion in 19 diabetics were also performed.

**Histological examinations**

The pituitaries were fixed in 10% neutral formol saline and paraffin sections were prepared at a thickness of 4 µ in horizontal planes. Successive sections were stained with hematoxylin-eosin, PAS-orange G-methylblue, aldehyde-fuchsin-Goldner, Masson-Goldner and iron-PAS staining techniques. Specimens of the thyroids, adrenals and ovaries were stained with hematoxylin-eosin, elastica-Masson and PAS. The testicular tissues were fixed in Bouin's fixative and the specimens were stained with hematoxylin-eosin, van Gieson's and Mallory's techniques.

**Histochemical examinations**

Periodic acid Schiff (PAS), Alcian blue and toluidine blue techniques were employed for histochemical demonstration of polysaccharides and mucosaccharides of testicular tissues of diabetics according to the method described by McManus, Mowry, and Curran.

**Biochemical examinations**

Q_{O_2} (O_2 consumption) and Q_{CO_2} (CO_2 production) of testicular tissues from diabetic dogs and rats were measured without substrate by Warburg's manometric techniques as described by Usami.

**Endocrinological examinations**

Determinations of urinary excretion of pituitary gonadotropin were performed on 15 diabetic patients. Urinary gonadotropin was absorbed in Kaolin and finally evaluated by the increase in weight of infantile mouse uterus according to Matsushima's method. Determinations of urinary 17-KS and 17-OHCS were also performed on 19 diabetics; urinary 17-KS was determined according to the method of Drekter et al. and 17-OHCS according to the method of Porter and Silber.
RESULTS

1. Histological observations

The adenohypophysis in human diabetics: The results obtained are summarized in Table 1. Cell counts were performed on the pituitaries of 11 patients with clinical diabetes mellitus. In comparison to the overall control group, the proportion of chromophobes remained unchanged, but there was a significant increase in the percentage of acidophil cells and a significant decrease in the relative number of basophil cells (Figs. 1 and 2). In most of the cases, alpha cells represented the predominant cell type and many of these cells were large with likewise large nuclei and contained heavy granules. On the other hand, vesicles and degranulation were often found in basophil cells, especially in delta cells in many cases. Beta cell was the predominant cell type of basophil cells, and in some cases no delta cell was seen in the glands. Partial fibrosis and hyperemia were also found in some cases.

The thyroid gland in human diabetics: In most of the patients, the follicles of the thyroid were large and their epithelial cells were low and flat. The colloid was abundant and thick (Fig. 3). In some cases, follicular colloid overflowed into the interstitial tissue. These appearances indicated a hypofunction of the thyroid gland. But in a few cases, the follicles were small and follicular epithelial cells were tall and columnar. The colloid was decreased in amount, thin, pale and vacuolated or scalloped around the edges (Fig. 4). These appearances were interpreted as an expression of hyperthyroidism.

The adrenal glands in human diabetics: Characteristic features of the adrenal cortex in diabetes were hyperplasia of the zona fasciculata. The layer was generally composed of compact cells and partially of clear cells. Small vacuoles in these cells and disturbed cellular arrangements were frequent observations of this layer (Fig. 5). Small nodular foci of hyperplasia, composed of compact cells, were found in some cases (Fig. 6). Sclerosis of capsular arteries of the adrenal cortex was also recognized. No remarkable change was found, except hyperemia, in the zona glomerulosa, zona reticularis and adrenal medulla.

The testes in human diabetics: As the human diabetic cases on which histological examinations of the testis were performed generally belonged to advanced ages, senile atrophy of the organ was frequently observed. But this change was severer than in the non-diabetic aged. In only one case, aged 29, the tubules were atrophied and the basement membrane of the canaliculi was thickened. Spermatogenesis ceased almost completely in the majority of the tubules. The quantity of germinal tissue was considerably decreased and the Sertoli cells were prominent (Fig. 7). Edema of the interstitial tissue and sclerosis of arteries were observed. In some cases, the interstitial connective tissue was remarkably increased. In all the cases, changes of the Leydig cells were not so conspicuous.
TABLE 1. Histological changes of

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>Age (years)</th>
<th>Complication</th>
<th>Duration of illness (yrs)</th>
<th>Blood sugar (mg/dl)</th>
<th>Acidophil cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38</td>
<td>Coma</td>
<td>2</td>
<td>215</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>Coma</td>
<td>4</td>
<td>388</td>
<td></td>
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<td>3</td>
<td>73</td>
<td>Wilson’s syndrome</td>
<td>4</td>
<td>309</td>
<td></td>
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<tr>
<td>4</td>
<td>68</td>
<td>Wilson’s syndrome</td>
<td>9</td>
<td>407</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>67</td>
<td>Wilson’s syndrome</td>
<td>6</td>
<td>225</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>63</td>
<td>Aplastic anemia</td>
<td>unknown</td>
<td>275</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>66</td>
<td>Pulmonary cancer</td>
<td>1.5</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>57</td>
<td>Sepsis</td>
<td>unknown</td>
<td>212</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>65</td>
<td>Sepsis</td>
<td>5</td>
<td>194</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>Pyelonephritis</td>
<td>4</td>
<td>360</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>52</td>
<td>Papillary necrosis</td>
<td>unknown</td>
<td>unknown</td>
<td></td>
</tr>
</tbody>
</table>

* Increase, ‡ No Change, ↓ Decrease, ++ Partial increase

The testes in diabetic animals: Histological examinations of the testes were performed on diabetic dogs and rats between one and twenty-one weeks after the onset of manifest diabetes. Histological examinations revealed tubular alterations of diverse degrees, characterized by a delay in spermatogenesis. The final development of spermatides and spermatozoa was especially affected (Fig. 8). These changes were progressive and mainly degenerative in character. The amount of germinal tissue was considerably decreased and the Sertoli cells were prominent. The basement membrane was thickened. Interstitial cells and connective tissue were remarkably increased in some cases (Fig. 9).

The ovaries in human diabetics: Almost all the human autopsy cases which were histologically examined for ovaries fell under the age group after the menopause, and senile atrophy of the organs was remarkable. Even in a 38-year-old female, however, mature follicles disappeared almost completely, accompanied by fibrosis, hyperemia and bleeding (Fig. 10).
2. Histochemical observations of the testes in diabetes

The cytochemical distribution and behavior of polysaccharides and mucopolysaccharides of the testes of the experimental diabetic animals and human diabetics were examined. The basement membrane of diabetic testes was stained stronger than that of the normal testes of control animals with PAS technique (Fig. 11). Spermatogenic cells, especially those near the basement membrane, exhibited strong affinity to Alcian blue (Fig. 12). The metachromasia with toluidine blue on tissue sections was distinctly demonstrated at pH 1.5 or 3.0 (Fig. 13). The reactions were concerned with the enzyme activities of Embden-Meyerhof shunt, fructose monophosphate shunt and TCA cycle of the testes in diabetic conditions. However, no definite tendency in the reactions was found in relation to the disease. The findings as previously described of human diabetic testes were also confirmed.

3. Biochemical observations of the testes

The results of biochemical investigations are summarized in Table 2.

The values of $Q_{o2}$ showed a slight and transient decrease in the initial stage of
TABLE 2. Biochemical activities of the testicular tissue in diabetics
(µl/100 mg d.wt./hr.)

<table>
<thead>
<tr>
<th>Duration</th>
<th>R.Q.</th>
<th>QO₂</th>
<th>QCO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated controls</td>
<td>0.55</td>
<td>173.5</td>
<td>94.4</td>
</tr>
<tr>
<td>1 week (Average of 3 cases)</td>
<td>1.95</td>
<td>78.9</td>
<td>183.9</td>
</tr>
<tr>
<td>3 weeks (Average of 3 cases)</td>
<td>0.85</td>
<td>452.2</td>
<td>383.8</td>
</tr>
<tr>
<td>13 weeks (Average of 3 cases)</td>
<td>0.51</td>
<td>367.2</td>
<td>186.9</td>
</tr>
</tbody>
</table>

the disease, but after 3 weeks they were significantly elevated, while those of QCO₂ were significantly increased throughout the experimental periods. The values of R.Q. were significantly higher than that of the untreated controls in the beginning, but they gradually returned to the normal level.

4. Determinations of urinary gonadotropin and urinary steroids

The average daily excretion of gonadotropin in human adults was from 6 to 12 M.U.U. (mouse-uterus-unit). Determinations of gonadotropin in 15 diabetics revealed that gonadotropin values over 12 M.U.U. were found in only 2 patients and the other 13 showed values under 6 M.U.U. (Fig. 14).

The results on urinary steroids were shown in Fig. 15. The average value of daily 17-KS excretion of healthy men of all ages was 8±3 mg, and that of 17-OHCS was 6±3 mg. Determinations of 17-KS in 19 diabetics revealed values over the normal limit in only 3 patients. In 10 patients, 17-KS excretion was within the
Sexual Disturbances in Diabetes

Fig. 15. Urinary excretion of 17-KS and 17-OHCS in diabetics.

normal range and in 6 it was subnormal. Urinary excretion of 17-OHCS was enhanced over the normal limit in only 4 of 19 patients. It was in the normal range in 12 and subnormal in 3.

DISCUSSION

Histological changes of the adenohypophysis in diabetes have hitherto been reported by many investigators. Kraus observed decreased weight of the pituitary and significant decrease, atrophy and atypism of eosinophil cells, stating that many of the nuclei of eosinophils were pyknotic in the pituitaries of human diabetics. He also recognized the changes previously described for experimental diabetic cats. Experimental observations of the adenohypophysis of diabetic dogs were made by Nagayo, Iwata, Nishimura, Honjo and many others. They noted a decrease of basophil cells as well as acidophil cells. On the other hand, Jersov said that no remarkable change was recognized in the adenohypophysis of diabetic dogs. Bassi reported that in the early stage eosinophil cells were increased in the adenohypophysis of alloxan-diabetic rabbits, and basophil cells were gradually increased. Nasu said that increase of eosinophil cells, and decrease of basophil cells and chromophobes were recognized in the adenohypophysis of experimental diabetic animals. In our investigation, the characteristic changes in the adenohypophysis of human subjects were a remarkable increase of eosinophil cells and a remarkable decrease of basophil cells, especially of delta cells.

The role of the pituitary gland in diabetes is not clearly demonstrated. It is generally accepted that alpha cells are the source of somatotropin. A remarkable increase of alpha cells in the hypophysis of diabetics suggests that hypersecretion
of pituitary somatotropin is important in the pathogenesis of this disease. On
the other hand, the low percentage of delta cells stands in a marked contrast to
the generally high proportion of alpha cells in diabetics. It is generally believed
that delta cells are one of the sources of gonadotropin. Clinically, diabetic
patients showed hyposcretion of the hormone. These results suggest that
pituitary gonadotropin deficiency will play an important part in the pathogenesis
of sexual disturbances in diabetics.

Warren and LeCompte noted that in rats with alloxan diabetes remarkable
changes suggesting hypothyrodism were observed, but similar changes were not
confirmed in the thyroid of human diabetics in their study. We observed,
however, a remarkable change suggesting reduced thyroid function in human
diabetic subjects.

Nagahama, Weller, Nishimura et al., Kraus, Nagayo and many others
reported that reduced lipoid, hyperemia, bleeding and hyperplasia of the zona
fasciculata with vacuolated cells and atrophy of the zona reticularis in the adrenal
cortex were found in human diabetics, in alloxan-diabetic rabbits and rats and
in subtotally panreatectomized dogs. Both Nasu and Hasegawa observed
hyperemia, hyperplasia of cells containing many magenta vesicles, disturbances of
cell arrangement and many giant cells in the zona fasciculata in allxoan-diabetic
rabbits. These authors also found hyperemia, hyperplasia of the cells of the zona
reticularis intermingled with many vesiculated cells. On the other hand, they
noted that there was no remarkable change in the zona glomerulosa and medulla of
the adrenal gland. In our investigation, characteristic features of the adrenal
cortex in diabetes were hyperplasia of the zona fasciculata. No remarkable
change except hyperemia, in the zona glomerulosa, zona reticularis and medulla of
the gland was recognized. It is generally accepted that the zona fasciculata
is associated with secretion of glucocorticoid. In this respect, it is interesting that
the adrenal gland of diabetics shows a remarkable change in the zona fasciculata.
But the mechanism of impairment of this layer is still unknown. Botella thought that the zona reticularis is associated with secretion of androgenic
hormone. Nishimura et al., Nasu and Hasegawa observed a remarkable
change in the zona reticularis in experimental diabetic animals. We suppose that
the lesion of reticular zone may be one of the causes of sexual disturbances in
diabetics.

Warren and LeCompte, Schöffling et al. and Ishigami observed thickening
of the tubular basement membrane, defective spermatogenesis and a reduced
number of the Leydig cells. Hasegawa reported that defective spermatogenesis,
thickening of tubular basement membrane and giant cells in the tubules were
observed in diabetics, but he also said that the change in the interstitial tissue was
not so remarkable. In our investigation, the characteristic change in the testicular
tissue of diabetics was represented by thickening of tubular basement membrane,
defective spermatogenesis, edema and fibrosis of the interstitial tissue. The
Leydig cells were not increased in number except in a small number of cases.
Schöffling thought that impotency and infertility in male diabetics were
frequently due to hypogonadotropic hypogonadism. We suppose that testicular
hypofunction in diabetics may result from pituitary gonadotropin deficiency on
account of reduced delta cells. In addition, both Saito\textsuperscript{25} and Kurihara\textsuperscript{26} in our
Department observed degenerations of nerve fibers innervating the gonads in
human diabetics and experimental diabetic dogs (Fig. 16). These results suggest
that testicular atrophy of diabetics are not only due to hormonal, but also to
neurogenic factors.

The activities of the ovary in diabetes are poorly understood. The ovaries
of our female diabetics before climacterium were distinctly atrophied just like the
testes in male diabetics. We believe that these noticeable changes are exerted
by hormonal and neurogenic factors responsible for diabetic disturbances.

Arzac,\textsuperscript{27} Montagna and Hamilton,\textsuperscript{28} Mancini \textit{et al.}\textsuperscript{29} and many others applied
some histochemical techniques to normal and atrophic human testes and made
a detailed description on the localization of various lipid types, glycogen,
mucoprotein, phosphatase, ascorbic acid and so on in examined gonads. However,
a report of histochemical investigations of the testes in diabetes is not known up to
the present time. In our investigation, the basement membrane of the testes in
diabetics was stained stronger than the structure in the normal testes with PAS
technique. Spermatogenic cells, especially those in the vicinity of the basement
membrane, were intensively stained with Alcian blue. Distinct metachromasia
with toluidine blue was recognized at pH 1.5 and 3.0 in the testicular tissue of
diabetics. These histochemical changes would be associated with the disturbance
in carbohydrate metabolism in the course of diabetes.

Subsequent to testicular injuries, $Q_{O_2}$ of the testicular tissue of adult rats
rises as shown for the cryptorchid testis by Tepperman \textit{et al.}\textsuperscript{30} and for the nitrofen-treated or X-ray irradiated testis by Steinberger and Wagner.\textsuperscript{31} Featherstone \textit{et al.}\textsuperscript{32} concluded that the elevation of $Q_{O_2}$ was related to the absence of the more
mature types of the germinal epithelial cells in the damaged organ. Tepperman, on
the other hand, suggested that the altered ratio of germinal epithelial cells to
the Leydig cells is responsible for the changes in $Q_{O_2}$. Steinberger and Wagner\textsuperscript{31}
also considered that increased $Q_{O_2}$ was related to the presence of the Leydig
cells, and that the ability of the cells to change $Q_{O_2}$ was apparently controlled by
gonadotropin. In our experimental investigations, $Q_{O_2}$ and $Q_{CO_2}$, especially the
latter of the testicular tissue was generally found increased.

Miller and Mason,\textsuperscript{33} Horstmann\textsuperscript{34} and Schöffling \textit{et al.}\textsuperscript{2} observed that urinary
excretion of 17-ketosteroids in diabetic patients with impotency was increased.
Schöffling also said that chromatographic fractionation of 17-ketosteroids
revealed that their augmented excretion reflected an increase in metabolites of
adrenal steroids of low androgenic potency, while metabolites of testosterone were decreased. On the other hand, Ishigami reported that urinary excretion of 17-ketosteroids in diabetics was decreased. Mori noted that chromatographic fractionation of 17-ketosteroids revealed that the ratio of androsterone+etiocholanolone to dehydroisoandrosterone+11-oxy-17-ketosteroid strikingly decreased. He also stated that the ratio of these 17-KS fractions was not influenced by increase or decrease of total urinary 17-KS excretion. In our diabetic patients it was demonstrated that urinary excretion of 17-KS and 17-OHCS was normal in most of the patients. We assume that these results are related to relatively intact Leydig cells and well preserved zona reticularis in our diabetic patients.

As mentioned above, histological, histochemical and biochemical changes in the internal secretory organs in diabetics were noticed. The mechanism of impairment of these organs is not yet obvious. But the results of our present investigations make it probable, that these changes are due to deficiency of TSH, ACTH and gonadotropin resulting from the degenerative changes of the adenohypophysis in diabetics. Moreover, sexual disturbances in diabetics are not only attributable to deficient pituitary gonadotropin, but also to degeneration of nerve fibers innervating the gonads.

Acknowledgment

We wish to thank Dr. Nobuaki Sasano, Professor of Pathology, Tohoku University School of Medicine, for his kind help in the histological examinations.

References

10) Porter, C.C. & Silber, R.H. A quantitative color reaction for cortisone and related


Explanations for Figs. 1-13 and 16

Fig. 1. Anterior pituitary in a case of diabetic coma.  
Female, aged 52; 195/63.  
A large number of alpha cells are observed. Beta cells and chromophobes are also present, but no delta cells are seen. Iron-PAS stain, 400 ×.

Fig. 2. Anterior pituitary in a control case.  
Female, aged 57; pyelonephritis; 148/61.  
Alpha, beta and delta cells and chromophobes are seen. Iron-PAS stain, 400 ×.

Fig. 3. Thyroid in a case of diabetes.  
Male, aged 26; 192/64.  
Follicles are large and epithelial cells are low and flat. Colloid is increased in amount and thick. H-E stain, 100 ×.

Fig. 4. Thyroid in a control case.  
Male, aged 43; malignant nephrosclerosis; 387/63.  
Follicles are small and epithelial cells are tall and columnar. Colloid is decreased in amount, thin, pale and vacuolated or scalloped around the edges. H-E stain, 100 ×.

Fig. 5. Adrenal cortex in a case with diabetes.  
Male, aged 26; 132/64.  
Hyperplasia of zona fasciculata composed of compact cells with small vacuoles and disturbances of cell arrangement are seen. H-E stain, 250 ×.

Fig. 6. Adrenal cortex in a case of diabetes.  
Female, aged 60; 404/63.  
Small nodular hyperplasia, composed of compact cells, demonstrated. H-E stain, 250 ×.

Fig. 7. Testis in a case of diabetes.  
Male, aged 26; 132/64.  
Thickening of the basement membrane, cessation of spermatogenesis and edema of the interstitial tissue are seen. H-E stain, 250 ×.

Fig. 8. Testis from a dog sacrificed 3 weeks after the onset of manifest diabetes.  
Blocked maturation in the testicular epithelial cells and desquamation of immature cells are seen. H-E stain, 250 ×.

Fig. 9. Testis from a dog sacrificed 21 weeks after the onset of manifest diabetes.  
Thickening of the basement membrane, reduction of the testicular epithelial cells with blocked maturation and increase of interstitial tissue are seen. H-E stain, 250 ×.

Fig. 10. Ovary in a case of diabetes.  
Female, aged 38; 112/63.  
Ceased maturation in the ovarial follicles and fibrosis are seen. H-E stain, 250 ×.

Fig. 11. Testis from a diabetic dog.  
Note intensely PAS-positive substances in the basement membrane. PAS technique, 100 ×.

Fig. 12. Testis from a diabetic dog. Note strong affinity of spermatogenic cells, especially those in the vicinity of the basement membrane to Alcian blue. Alcian blue technique, 100 ×.

Fig. 13. Testis from a diabetic dog.  
Note remarkable metachromasia with toluidine blue in tissue section at pH 3.0. Toluidine blue technique, 100 ×.

Fig. 16. Nerve fibers innervating the gonad in human diabetes. Degeneration of nerve fibers is seen. Osmic acid stain, 100 ×.