HISTOLOGICAL INVESTIGATION OF TESTIS IN INFERTILE MAN

PART II. PATHOLOGICAL PROBLEMS

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ABSTRACT

Histological investigation of the testes of 214 patients with azoospermia, 56 patients with oligozoospermia and 11 men with known fertility was made. In 24 of these cases, 17-ketosteroids and pituitary gonadotropin in urine were determined. Based upon the results of these studies, pathological problems of functional infertility in human male were discussed.

1) No qualitative difference in the histological findings of testes was observed between azoospermia and oligozoospermia.

2) All atrophic testes, smaller than thumb-head by scrotal palpation, showed highly damaged spermatogenesis.

3) In 27% of the patients with oligozoospermia, with less than $20 \times 10^6$ per ml in sperm concentration, no spermatozoa were found in the seminiferous tubules of the biopsied specimen. The evidence shows that possibility of histological misdiagnosis is inevitable, though the artefact due to technical procedure has to be taken into account.

4) In 19% of non-obstructive azoospermia, active spermatogenesis was recognized in the majority of the seminiferous tubules. The epididymo-vasal secretion of them did not contain any spermatozoa. The evidence calls for the necessity of examination of the rete testis and the distal end of the seminiferous tubules.

5) In normals, the mean thickness of the tubular walls ranged from 4.5 to 5.5 $\mu$. In hypofunctional testes, the distribution could be grossly divided into two groups, one group with the tubular walls as thin as the normals and the other the group with tubular walls thicker than 6.5 $\mu$.

6) In normals, the mean diameter of the seminiferous tubules ranged closely around 150 $\mu$. In oligozoospermia, the distribution was grossly divided into two groups, one with the tubular lumina as wide as the normals and the other with narrow tubular lumina, less than 110 $\mu$ in diameter.

7) Generally, the development of germinal cells was well maintained in tubules with thin walls and wide lumina, and Leydig cells were distributed normally around these tubules. On the contrary, germinal maturation was lowered in tubules with fibrously thickened walls and narrow lumina, and Leydig cells were hyperplastic to various degrees.

8) The histological picture of testis in infertile human male closely resembles to experimentally allergic lesion of testis in guinea-pig or rat.

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INTRODUCTION

Male infertility is very common, perhaps even more than female infertility. Causes of male infertility due to passage disturbance of the seminal tract have decreased and in recent years most cases are due to maturation defects of the germinal cells. But in the vast majority of cases, the cause of infertility cannot be explained. The history of patients reveals no cause, puberal development has been normal and they are normally virile and are perfect in general health. Clinical examinations show no abnormal findings, except for testicular biopsy. This pathological condition is almost permanent and resists most medical treatments available today.

The causal factors of male infertility without obstructive lesions are as follows; 1) Maldevelopment of the gonadal ridge, 2) Abnormality of sex chromosome, 3) Abnormality of pituitary function, 4) Acquired causes such as irradiation, heat, malnutrition and/or toxic factors and 5) Unknown etiology (so called essential male sterility). It is difficult to differentiate them, and occasionally these causal factors can not be found. Therefore, practically, a classification of male infertility is made by histological findings of testes that are the results of the disordered condition.

Many reports have been published concerning testicular histology in the consideration of male gonadal dysfunction. Furthermore, histochemical study including enzymological investigations has been developed, though little is yet known about the pathogenesis of male infertility.

During the past five years testicular biopsy was performed on 270 patients with male infertility, and the pituitary gonadotropin and 17-ketosteroids in urine were examined in some. Based on this clinical experiences, the pathology of male infertility was discussed in the present paper.

MATERIALS AND METHODS

The results were obtained based on an evaluation of testicular specimens presented in the previous paper.

Hormone determinations in urine were made by Zimmermann-Miyake's modification for 17-ketosteroids and by mouse-uterine-weight method for pituitary gonadotropin.

Histological observations were made as follows.

(1) Diameter of seminiferous tubule

The internal diameter of the seminiferous tubules was measured with an ocular micrometer on 20 tubules arbitrarily taken from each sample, using H-E or PAS stained specimens. In cases of tubule with an egg-like form, the shorter diameter was recorded.
TESTICULAR HISTOLOGY IN MALE INFERTILITY

(2) Thickness of tubular wall
On all of 20 tubules examined for their diameters, the thickness of the tubular wall was measured for each sample under oil immersion, using H-E or PAS stained specimens.

(3) Intratubular cell component
By observation of all 20 tubules examined for their diameters, the samples were classified into the following five groups by the grades of germinal maturation, using H-E stained specimens:

I a: The specimens showing normal appearance in intratubular cell distribution, and showing disturbance of the normally ordered sequence of spermatogenesis or sloughing of immature cells were included in this category.

I b: Though sperm cells or spermatids are seen in a few tubules, the majority of the tubules is lacking in these mature cells.

II a: This is a state where spermatogenic process fails to progress beyond one of the generation of germinal cells. When the most mature cells are secondary or primary spermatocyte, the specimens were included in this category.

II b: When there is no mature germinal cell beyond the spermatogonium, the specimens were included in this category.

III: The tubules are populated exclusively by the sustentacular cell of Sertoli and lack the germinal cells completely.

IV: Peritubular fibrosis is very wide-spread and devastating. When all or almost all tubules are either completely obliterated or are so seriously damaged that the spermatogenic process is halted by peritubular fibrosis, the specimens were classified into this category. The intratubular areas usually contain unusual amounts of hyalinous material and fibroblasts.

Occasionally, variable appearances are seen in one section of a specimen. In such a case, its histological classification was expressed by the most mature grade of germinal cells.

(4) Leydig cell
By observation of the interstitial cells, the testes were classified into the following four groups, using H-E or Heidenhain stained specimens:

(-): Several Leydig cells are distributed in the intertubular spaces such as in normal testis.

(+): Slight increase of Leydig cells.

(++): Moderate Leydig cell hyperplasia with clumping of 10 to 20 cells.

(++): Further advanced hyperplasia of Leydig cells, looking like Leydig cell tumor.

(5) Interstitial connective tissue
By observation of the interstitial connective tissue, the testes were classi-
fied into the following four groups, using H-E or Heidenhain stained specimens: Normal (−), Slightly hyperplastic (+), Moderately hyperplastic (++) and Highly hyperplastic (+++).

(6) Thickening of interstitial capillary wall

By observation of the interstitial capillary, the testes were classified into the following four groups, using H-E or PAS stained specimens:

Not thickened as normal (−), Slightly thickened (+), Moderately thickened (++) and Highly thickened (+++).

RESULTS

The most important point in the histological classification of testis is that variable appearances are found even in a single specimen. There are clumps of tubules that look normal among advancedly hyalinous tubules, like flower gardens in a desert. The results described in this paper are based on the recognition that it is dangerous to suggest the character of the whole testis, from a finding of only one piece of tissue section.

I. Germinal cell

In the finding of infertile testis, various degrees of pathological changes were seen, from almost normal to completely hyaline. On 270 biopsied testes, 214 with azoospermia and 56 with oligozoospermia, the frequency of the various histological changes is shown in Fig. 1. The classification was made by the criteria described above.

![Diagram of germinal cell development]

FIG. 1. Distribution by type of germinal cell development.
Azoospermia (214 cases)

The most frequent type, 39% (83 cases), was Sertoli-cell-only syndrome with seminiferous tubules populated only by Sertoli cells, while germinal cells were absent (Type III). This evidence indicated that germinal cells may be damaged completely, if a pathological condition once happens. In some patients of this type, the tubular walls were thin and Leydig cell components were normal, however in some patients there were variable degrees of peritubular thickness with Leydig cell hyperplasia.

The second types were, ironically in azoospermia, specimens showing almost a normal picture or sloughing in the intratubular cell order (Type Ia) and the specimens with a few tubules containing normal maturation of spermatozoa among a field of damaged tubules (Type Ib). The former accounted for 19% (40 cases) and latter 18% (39 cases). The ejaculates contained no spermatozoa, in spite of the testes maintaining spermatogenesis, more or less, in 37% of non-obstructive azoospermia. These results present an important problem in the consideration of pathogenesis of male infertility.

The third group, 11% (24 cases), was the highly damaged testis type (Type IV). Some cases lost entirely the testicular structure, and the germinal epithelium was replaced totally by hyaline substances.

The rarest pictures were those of developmental arrest at the stage of secondary or primary spermatocyte (Type IIa) and spermatogonium (Type IIb). They totalled 13% (28 cases).

In 44 cases out of 214 with azoospermia, the testes were smaller than thumb-head and had no normal consistency by scrotal palpation. The intratubular appearance of them is shown at the bottom of Fig. 1. 63% (28 cases) of them were Sertoli-cell-only syndrome or were complete hyalinization of the seminiferous tubules (Type III or IV), and in 9% (4 cases) tubules containing spermatogonium-like cells were observed (Type IIb). Although in 28% (12 cases), spermatogenesis had been maintained in a few tubules (Type Ib), in general, they were also associated with advanced histological degeneration, such as peritubular thickening, Leydig cell hyperplasia and increase of interstitial connective tissues.

Oligozoospermia (56 cases)

In 56 patients with oligozoospermia, the appearance of the germinal situation is also shown in Fig. 1. Generally the maturation of germinal cells has progressed when compared with azoospermia, however no characteristic difference in quality was seen between oligozoospermia and azoospermia.

Most of the cases were Type Ia, 61% (34 cases). The majority of them could be hardly differentiated from the normal testis, and Type Ib was encountered in 12% (7 cases).

As an important finding, Type IIa (7%, 4 cases), Type IIb (4%, 2 cases)
and Type III (16%, 9 cases) containing no sperm cells in their tubules were encountered in oligozoospermia. They totalled 27% of the cases. However, the evidence that the ejaculates contained sperm cells even if reduced in concentration, in spite of maturation defect or aplasia of the germinal cells in their testicular histology, indicated that there must be undamaged tubules elsewhere in the testis. In other words, it suggested the possibility that the pathological change in these testes is not diffuse but regional or localized.

On the other hand, there was seen almost normal histology in 19% of the testes with azoospermia. Epididymo-vasal secretions were collected, by the technique described in Part I \(^{47} \), from six patients having normal histology, in order to study where the spermatozoa once produced in the germinal tubules disappeared. But no spermatozoa could be seen microscopically in all six. This result indicated that the spermatozoa in these patients might have been digested and absorbed before transversing to the epididymis.

**II. Tubular wall**

In order to observe the correlation between tubular wall and the developmental feature of germinal cells and the distribution of Leydig cells, the
thickness of the tubular wall was measured.

Comparison of thickness of tubular wall

The frequencies of the mean thickness of the tubular wall in each specimen is shown in Fig. 2.

In the normals, the thickness of the tubular wall fell in the narrow range of 4.5 to 5.5 μ.

In the testes with infertility, the most frequent distribution was in the normal range of thickness, but an additional peak was formed, as an important finding, in the range of 7.0 to 7.5 μ. In the atrophic testes of smaller size than thumb-head, shown by black-shading in Fig. 2, all the tubular walls were fibrous and thicker than 6.0 μ. A half of the atrophic testes had completely hyaline tubules, and the border between tubular wall and hyaline substances in the tubular lumen could not be determined.

Relationship between tubular wall and germinal cell

The relationship between the thickness of tubular wall and maturation stage is shown in Table 1. As far as the mean value of thickness is concerned, it seemed that the tubular wall shows no well correlation with germinal maturation. Table 1 shows the germinal maturation to be damaged even in the tubule with non-fibrous wall, while on the other hand, it seems that the spermatogenesis is maintained also in the tubule with highly thickened wall. However by observation of individual tubules, there were no thickened walls in the seminiferous tubules with sperm production. This finding was seen in specimens of Type I b, where the healthy tubules occasionally remained like flower gardens in a desert of highly damaged tubules.

Tubular wall and Leydig cell

In Table 2 also, no appreciable relationship could be found between the thickness of tubular wall and the appearance of Leydig cell hyperplasia, as

<table>
<thead>
<tr>
<th>Thickness of tubular wall</th>
<th>Oligozoospermia</th>
<th>Azoospermia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I a</td>
<td>I b</td>
</tr>
<tr>
<td>4.0-4.5</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>4.5-5.0</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>5.0-5.5</td>
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<td>19</td>
</tr>
<tr>
<td>6.0-6.5</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>6.5-7.0</td>
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<td>3</td>
</tr>
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<td>7.0-7.5</td>
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<td>2</td>
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<tr>
<td>7.5-8.0</td>
<td>2</td>
<td>2</td>
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<tr>
<td>8.0-8.5</td>
<td>2</td>
<td>2</td>
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<tr>
<td>8.5-</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 1. Relationship between Thickness of Tubular Wall and Germinal Cell Development.
TABLE 2. Relationship between Thickness of Tubular Wall and Leydig Cell Distribution

<table>
<thead>
<tr>
<th>Thickness of tubular wall</th>
<th>Proven fertility</th>
<th>Oligozoospermia</th>
<th>Azoospermia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(−)</td>
<td>(+)</td>
<td>(†††)</td>
</tr>
<tr>
<td>4.0-4.5</td>
<td>5</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>4.5-5.0</td>
<td>1</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>5.0-5.5</td>
<td>2</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>5.5-6.0</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6.0-6.5</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>6.5-7.0</td>
<td>7</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>7.0-7.5</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>7.5-8.0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Complete hyalin.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

far as the mean value of wall thickness is concerned.

In men with proven fertility, Leydig cell hyperplasia and fibrosis of tubular wall were not seen.

It seemed that a little thickening of the tubular wall exists in the groups of oligozoospermia with Leydig cell hyperplasia, (+) to (†††).

No correlation between thickness of tubular wall and Leydig cell distribution was observed in oligozoospermia, as they had very variable distribution. Occasionally in the testes without clumping of Leydig cells, a high mean value of membranous thickness was found, but on the contrary, even in the testes with increase of Leydig cells, the mean value of the thickness of tubular wall was low.

As was described regarding the correlation with germinal maturation, however, the pathological finding was not always diffuse but regional or nodular. Although clumping of Leydig cells was observed here and there, this was not always seen over the entire field in specimens of Type I a, II a, II b or III. The tubular wall was sclerotic near the hyperplastic Leydig cells, more or less, and on the contrary it was almost normal in thickness near the normally distributed Leydig cells.

III. Diameter of seminiferous tubule

The mean values of the tubular diameters in the three clinical categories depending on the semen character are summerized in Fig. 3.

They fell in the narrow range of about 150 μ in the normal testes.

In oligozoospermia, the distribution resembled the picture of the normals, but were slightly deviated to the range of narrow diameter.

In azoospermia, they showed characteristically a biphasic distribution. One showed the same appearance as the normals and the other ranged in the narrow tubular lumen. This biphasic distribution of tubular diame-
FIG. 3. Distribution of mean diameter of seminiferous tubule.

FIG. 4. Relationship between diameter and wall thickness of seminiferous tubule in azoospermia.
ters resembled the histogram of membrane thickness in azoospermia (Fig. 2). Now Fig. 4 showed the relationship between diameter and thickness of tubular wall in azoospermia. They are definitely directly proportional, and from Figs. 3 and 4 the germinal tubules of azoospermia are seen to be grossly divided into the tubules with wide lumen and thin wall, and the tubules with narrow lumen and thickened wall.

IV. Gonadotropin and 17-ketosteroids

Table 3 shows the pituitary gonadotropin and 17-ketosteroids levels in urine of three males with proven fertility, six with oligozoospermia and eighteen with azoospermia. In the vast majority of patients, the levels of these hormones were within normal limits, and no significant correlation was observed between the hormone level and the situation of germinal cells, Leydig cell development or the thickness of tubular walls.

| Table 3. Gonadotropin and 17-ketosteroids in Men with and without Fertility |
|-------------------|-----------------|-----------------|------------------|-----------------|
| Germinal development | Thickness of tubular wall (μ) | Leydig cell distribution | Gonadotropin (m.u.u./day) | 17-KS (mg/day) |
| Normals            |                               |                    |                               |                 |
| 1                  | Ia                            | 5.5                | (−)                           | 8               | 18.0          |
| 2                  | Ia                            | 5.0                | (−)                           | 12              | 12.4          |
| 3                  | Ia                            | 4.8                | (−)                           | 8               | 6.6           |
| Oligozoospermia    |                               |                    |                               |                 |
| 4                  | Ia                            | 5.6                | (−)                           | 14              | 3.8           |
| 5                  | Ia                            | 6.2                | (+)                           | 8               | 10.8          |
| 6                  | Ia                            | 5.0                | (−)                           | 8               | 13.7          |
| 7                  | Ib                            | 5.2                | (−)                           | 14              | 7.0           |
| 8                  | Ib                            | 7.4                | (+)                           | 12              | 18.8          |
| 9                  | Ib                            | 6.0                | (++)                          | 8               | 11.1          |
| Azoospermia        |                               |                    |                               |                 |
| 10                 | Ia                            | 5.1                | (−)                           | 8               | 25.0          |
| 11                 | Ia                            | 5.3                | (−)                           | 24              | 9.3           |
| 12                 | II a                          | 5.5                | (+)                           | 6               | 8.8           |
| 13                 | II b                          | 5.5                | (−)                           | 16              | 12.3          |
| 14                 | II b                          | 5.3                | (−)                           | 18              | 9.5           |
| 15                 | III                           | 8.0                | (++)                          | 28              | 10.5          |
| 16                 | III                           | 4.8                | (−)                           | 8               | 13.2          |
| 17                 | III                           | 5.2                | (−)                           | less than 3     | 13.0          |
| 18                 | III                           | 5.4                | (−)                           | 12              | 12.5          |
| 19                 | III                           | 7.0                | (+)                           | 18              | 7.7           |
| 20                 | III                           | 6.5                | (++)                          | 6               | 6.8           |
| 21                 | III                           | 7.9                | (++)                          | 18              | 9.1           |
| 22                 | III                           | 6.7                | (+)                           | 18              | 6.2           |
| 23                 | III                           | 6.7                | (++)                          | less than 3     | 9.4           |
| 24                 | IV                            | hyal.              | (+)                           | 8               | 15.5          |
| 25                 | IV                            | hyal.              | (++)                          | 6               | 10.1          |
| 26                 | IV                            | hyal.              | (+)                           | 8               | 8.6           |
| 27                 | IV                            | hyal.              | (++)                          | 18              | 14.4          |
DISCUSSION

I. Changes of germinal cell

The chief histological changes in infertile testis are maturation defects of germinal cells, hyperplasia of Leydig cells and fibrosis of testicular walls. In the literatures\(^1\)\textsuperscript{1} - \(^1\)\textsuperscript{12}, histological classification of low-functioning testis has been presented by these factors, as follows:

(1) Spermatogenesis is in progress but is failing to proceed beyond one of the immature phases of the process with decrease of total number of germinal cells. They are called hypospermatogenesis, germinal cell arrest, spermatogenic arrest, germinal cell hypoplasia and/or degeneration. Nelson\(^6\) reported a state without a cell order in germinal tubules, while the sperm maturation exists, and insisted on its clinical importance. It was called sloughing or disorganization.

(2) The condition where the germinal walls are completely absent and Sertoli cells are only seen in the tubules, is called aspermatogenesis, germinal cell aplasia, Sertoli tubules or Sertoli-cell-only syndrome. The tubules are usually slightly smaller than normal, with or without thickening of the tubular wall. Occasionally cases are encountered in which a few tubules contain younger germinal cells.

(3) In the more advanced damage, even Sertoli cells completely disappeared in the tubules and fibrous or hyaline substances fill the germinal tubules in place of the intratubular cell components. They are called fibrosis, sclerosis or hyalinization.

The problem arises when these changes occur. It is a question whether germinal cells have been arrested in spite of spermatogenesis having been once completely active, or not; whether the prepuberal testicular failure began during male development, or not. It is easily recognized that toxic or thermal factors cause the former and that lack of testicular development is dependent on congenital factors such as the cryptorchidism. Since prepuberal histology of the testis cannot be known in all cases of infertility, it is inevitable to investigate the histology only at the terminal stage of this disease.

The concept that the histological picture shows a point in the slow moving process in testicular disease (normal histology → germinal hypoplasia → germinal aplasia → hyalinization) can be proven only by repeated biopsies during the life-term of the patient, but this is nearly impossible in practice. Though Kumamoto\(^5\) supported the concept by the view point that various histological findings were mixed in a single section, the difference in this experiment was not so varied. Two or three kinds of histological change were only seen in one section, e.g. islets-like normal tubules in the field of highly damaged tubules, and there was no specimen that showed all kinds of histological
appearance in a single section. In experimentally damaged testis produced by irradiation, heat and metal ions in animals and birds, the pathological changes were not regional nor nodular. The author believes that the variety of histological picture may be related with the change of capillary.

On the other hand, the studies of Voisin et al., Freund et al. and Furuta showed that a specifically allergic lesion of the testis was produced in guinea-pig and rat following injection of homologous testicular material with Freund adjuvant. The histological findings of the allergically hypoproliferative testis resemble closely some pictures of infertile human testis. In the allergic testis, according to Waksman, the germinal epithelium is destroyed in varying degree, though Sertoli cells maintain a normal appearance in relatively advanced stages, and occasionally Leydig cell hyperplasia is seen. The most interesting sign is that these changes appeared variably in parts of the section. Although the invasion of inflammatory cells is the most distinct sign in allergic lesion of the testis, it is never observed in human infertile testis. If the mechanism of the disease is explained by auto-immunity, the unknown allergic factor must affect the testis slowly, and its antigenicity may be very weak. Therefore, it is not surprising that the testis of infertile man does not contain inflammatory infiltrates. In addition, the sequence of histological changes in allergic orchitis has several points of similarity to the development of the lesion due to mumps orchitis in man reported by Gall.

This similarity in histopathological picture encourages research on male infertility from the view point of auto-immunization, though serological examinations have not clearly shown an antigen-antibody reaction.

Many cases of azoospermia were encountered which had the quite normal histological appearance, in spite of no obstructive lesion. On the question, "How and where the spermatozoa, once produced in the seminiferous tubules, are digested and absorbed?", no satisfactory explanation is now available. Although Nelson and Girgis et al. stated that there should be obstructive lesion elsewhere in the male tracts, the fact must not be so simple. Kumamoto explained that this phenomenon manifests an initial phase of a continuous pathological process (normal→oligozoospermic→azoospermic). The result, in our experiment, that no spermatozoa exist in the epididymo-vasal secretion shows that sperm cells do not transverse at least to the epididymis or the vas (see Part I). They are probably digested and absorbed in the seminiferous tubules. Therefore, the following schema is suggested.

\[
\text{Obstruction of rete testis or distal end of tubules} \downarrow \quad \text{Spermatozoa as foreign body} \uparrow \\
\quad \text{Damage of interstitial capillary} \quad \quad \quad \text{Formation of antispermatzoal antibody}
\]
This hypothesis can be supported by the recent report of Johnson\textsuperscript{22} that the immunological damage induced by homologous immunization of guinea-pig is more frequent in the rete testis and is less present in the testicular parenchyme.

\textit{II. Peritubular fibrosis}

In normal tubules, the tubular wall is 4.5 to 5.5 $\mu$ in thickness, composed of the following four layers: 1) the most inner is about 1 $\mu$ thick and PAS-positive, 2) the next hyaline layer is 2 to 3 $\mu$ thick and almost PAS-negative, 3) the third is a PAS-positive thin layer, about 1 $\mu$, which is formed of long cells and thin fibers, and 4) the most outer layer is, occasionally defective, PAS-positive elastic fiber\textsuperscript{9}.

A thickening of the wall is often observed in infertile testis and it is said that thickening is due chiefly to the increase of elastic fiber of the second layer. Kondo\textsuperscript{23} stated in his electron-microscopical study that this phenomenon is due to endothelioid cell hyperplasia under the basement membrane and the presence of collagen fiber. The etiology of the thickening of tubular wall is unknown and the suspected causal factors are very variable. The phenomenon was reported by Heller \textit{et al.}\textsuperscript{24} after administration of testosterone to normal men, by Maddock and Nelson\textsuperscript{25} after the injection of chorionic gonadotropin to normal males and by De la Balze \textit{et al.}\textsuperscript{26} after estrogen treatment of patients with prostatic cancer. The direct effect of these hormones or their roundabout effect through pituitary and adrenal, may thicken the tubular wall. De la Balze \textit{et al.}\textsuperscript{27} stated that the causal factors were not only endocrinological but thermal and chromosomal, as they found the phenomenon in the testis of cryptorchidism, Klinefelter syndrome and senile male. In addition, according to a recent report by Shishito\textsuperscript{29}, the phenomenon with interstitial cell hyperplasia can be observed in dog testis when the testicular nerve is cut away.

Nutritional supply to the germinal cells and Sertoli cells by water soluble substances, \textit{i.e.} mucopolysaccharides, is transmitted through the tubular wall\textsuperscript{26,28,30}. It is understandable that the exchange of substances is inhibited by thickened tubular wall, and under-nutrition of intratubular cells disturbs spermatogenic function. On the contrary, the wall of the hypospermatogenic tubules is not always thickened. In 43% of Sertoli-cell-only syndrome in this experiment, the tubular wall was not thickened, and ranged from 4.0 to 6.0 $\mu$ thick.

The phenomenon seems much more related to the degree of Leydig cell hyperplasia than to the degree of spermatogenic function. Johnsen\textsuperscript{8} has described that hyperplasia and dysfunction of Leydig cell is one of the causes of tubular wall thickening from the evidence that peritubular fibrosis in Klinefelter syndrome develops at puberty and is influenced by Leydig cells.

But the fact that some cases of hypospermatogenic testes are encountered
without peritubular fibrosis, suggests the complexity of the pathological mechanism of male infertility.

III. Tubular diameter

It has been said that the diameter of seminiferous tubules increases gradually with maturation of the individual. However, it is widely held currently that the tubular lumen is about 50 μ in diameter and stable till puberty, but a rapid increase begins suddenly at puberty.

The histograms of tubular diameter show a biphasic distribution in dysfunctional testes and the specimens can be divided into testes with narrow tubular lumen and testes with normally spaced tubular lumen. If the tubular lumen becomes suddenly and rapidly larger at puberty, male infertility may be divisible into two gross groups where the onset of disease is post-puberal and where the tubular condition remains in the prepuberal stage. This concept is also supported by the finding that narrowing of tubular lumen is not seen in various experimentally induced testicular damage, even in testicular necrosis.

But in the other hand, according to Matousek, the narrowing of tubular space is definitely observed in immunologically sterilized testis produced by the administration of male tract secretion obtained from heterogenous animals.

IV. Interstitial cell

An attempt has been made to determine androgenic activity by means of morphological classification and calibration of interstitial cell volume, but it is generally accepted that this attempt has little significance at present. And it is said that the determination of gonadotropin and total 17-ketosteroids in urine shows no significant difference between infertile and fertile men, and the results of this experiment are entirely accorded with these works. The levels of these hormones are poorly correlated with the number of Leydig cells.

The results show that an increase in number of Leydig cell does not always cause a rise in androgenic secretion, and this is in agreement with the opinion that the cells can be classified into an active-form which is in good secretory condition and into an inactive-form which is degenerated, atrophied, young and low-functional. And the level of testicular androgen dependent, therefore, on the amount of active-form of Leydig cells.

Johnsen stated that urinary gonadotropin levels of infertile men were distributed in higher ranges than normals, and Mikawa supported this only in the cases with Leydig cell hyperplasia. They considered the high gonadotropic condition to be due to decreased inhibition of FSH secretion in the pituitary by the low spermatogenesis. Johnsen possessed the idea of the following circulus vitiosus.
According to him, the germinal damage leads to increased gonadotropin release from the pituitary and presumably to a change in FSH/LH ratio; Leydig cells are stimulated, or rather abnormally stimulated, with their hyperplasia and dysfunction; and hyalinization process is induced by dysfunctional Leydig cells, since it appears at puberty with the beginning of abnormal Leydig cell function in Klinefelter syndrome.

V. Intertubular capillary and connective tissue

The major change in the blood vessel is a thickening of intima and media, and the degree of change is generally proportional to the degree of tubular damage.

Ichikawa\textsuperscript{45}, Kumamoto\textsuperscript{5} and De la Balze \textit{et al.}\textsuperscript{26} described that the hypofunctional seminiferous tubules resulting from the low intratubular nutrition, is caused by capillary sclerosis, and Ochiai further suggested that the regional fibrosis of seminiferous tubules is due to advanced sclerosis of the arterioles. This concept is in agreement with the results of this experiments where regionally damaged tubules were associate with capillary or arteriolar sclerosis and thickened vessels were not seen near the area of normal looking seminiferous tubules.

Hyperplasia of interstitial connective tissue is often observed in testicular damage. Nelson\textsuperscript{6} presumed chronic inflammation as the cause of hyperplasia of connective tissue. However, an inflammatory picture was never observed histologically in this study. Takeda\textsuperscript{46} stated that the fibrosis with hyperplasia of capillary and connective tissue might be produced when the causal agent without antigenicity permeated feebly and slowly into the organ. But the causal agent has remained obscure, although this is a very important matter.

Since there is ample evidence that testicular tissue and spermatozoa are antigenic, auto-immunity may have been initiated. But capillary sclerosis and hyperplasia of connective tissue in the interstitium are greater in the testes in which maturation arrest seems to be initiated prepuberally. Also, it should be considered that germinal cells are never produced in these testes at any time. From the above reason, the auto-immune theory can explain little about capillary sclerosis and fibrous hyperplasia.

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TESTICULAR HISTOLOGY IN MALE INFERTILITY


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