THE SPERMATOGENETIC ACTIVITY TEST IN MALE STERILITY INVESTIGATION

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Summary. Phosphorus, being one of the basic compounds of the nucleic acids, is actively concentrated in tissues in which there is an abundance of cells capable of frequent mitosis. The degree of spermatogenesis can be roughly estimated by the Spermatogenetic Activity Test (SAT) by measuring the amount of radioactivity in the testes after giving the patient a very small quantity of radioactive $^{32}$P. In fifty-two patients with oligospermia, azoospermia due to obstruction, dysgenesis or atrophy, necrospermia and impotence, the SAT was investigated in parallel with seminal analysis and testicular biopsy. By comparing the results a close correlation was found between the SAT and the histological results. It was possible, in many cases, to predict the histological pattern from the SAT curve without operative discomfort to the patient and at less cost. The clinical value of SAT was most significant in cases of azoospermia due to obstruction and in cases where seminal analysis was not available.

INTRODUCTION

Since Charny (1940) and later Hotchkiss (1953) first published their studies on human testicular biopsy, this procedure has steadily gained in popularity. Today, next to semen examination, biopsy of the testis is considered by many investigators to be the basic procedure in the evaluation and diagnosis of male sterility and of hormonal disorders and in the screening out of those patients who cannot be expected to respond to any available treatment. Nevertheless, there are some investigators who are less enthusiastic and more reserved in regard to the indication for testicular biopsy.

According to Getzoff (1955), quite a few specialists in various fertility centres do not feel that this procedure is justified in most cases. Others admitted that they had become discouraged after having made several trials. Various reasons were mentioned in his inquiries:

(1) In many cases the histological examination failed to make any significant contribution to the final solution of the patient's infertility problems.

(2) The histological pattern does not always agree with the clinical finding. Microscopic changes occur with each phase of activity and the slides are difficult to evaluate with an accepted degree of standardization.
A high percentage of males were willing to provide a seminal specimen but refused to have a biopsy done, for obvious reasons.

Nelson (1953) published an outstanding classification and interpretation of the histological changes in the testis. Others have tried to apply them in various clinical syndromes. Simmons (1952), Mannion & Cotrell (1961), Ragab, Tarkhan, Ibridi & Girgis (1961), and Sant & Bhatt (1963) have tried to establish the correlation between the histological examination and the sperm counts in cases of oligospermia and azoospermia.

Simmons (1952) stated that in cases of oligospermia it is impossible to predict from studying the testicular biopsies what the seminal analyses will show, except in cases of complete tubular degeneration and atrophy. Normal testicular tissue is often found despite deficiencies of the semen, and vice versa. In cases of azoospermia, testicular biopsy reveals either complete failure of the germinal epithelium or a variable histological picture when obstruction of the ejaculatory canals is the cause of absence of spermatozoa.

Sant & Bhatt (1963) presented cases of oligospermia and azoospermia in 50% of which there was a normal testicular histology. In these cases, there was no correlation between the anatomic state and the functional capacity of the gonad. The authors observed, however, that while many of those with normal tissue or only slight histological changes had a good prognosis, the prognosis was hopeless in cases in which biopsy revealed aplasia or degeneration. They therefore concluded that the anatomic state is valuable for determining the future prognosis, but is often not accurate enough for the evaluation of the present functional state of the gonads.

Some authors have stressed the need for new tests which would be more acceptable to the patient and which at the same time would provide more information about the functional state of testicular tissue and the spermatogenetic capacity as well as about the prognosis (Paynee & Skeel, 1955). In 1959 Czerniak & Gross-Avigad described a new in-vivo test for spermatogenesis, the Spermatogenetic Activity Test (SAT) using a tracer dose of radioactive phosphorus ($^{32}$P) injected intravenously. The concentration of phosphorus in any tissue indicates a high degree of mitotic activity; thus the injected $^{32}$P is taken up only by a testicle with active spermatogenesis. The $^{32}$P emits beta radiation which passes through the scrotum. A radiation detector (a Geiger–Müller tube) applied on the scrotum measures this beta radiation 1 hr and 1, 2, 3, 4, 6 and 8 days after the injection, and a curve is traced. In the presence of spermatogenetic activity a plateau appears in the $^{32}$P uptake curve. This plateau appears 3 to 5 days after the injection in normal adults (8 to 10 days in children) (Czerniak & Rotem, 1961) (Text-fig. 1). The authors compared the histological picture and the results of the SAT in twenty-five cases and found that in all but three cases the results concurred (Czerniak, 1962).

In the present studies, the SAT was measured in parallel with seminal analyses and testicular biopsies. The results obtained have been correlated in an attempt to establish the clinical value of SAT in the diagnosis and prognosis of male sterility, as well as its usefulness as an aid in clarifying any discrepancies occurring in the seminal analysis and the histological examination.
METHODS

The sat was investigated in fifty-two male patients attending the Beilinson Hospital Fertility Clinic. Their ages ranged from 26 to 40 years. Their chief complaint was sterility of at least 3 years' duration. The sat was performed in the Department of Radiotherapy and Isotopes, Tel-Hashomer Hospital. The testicular biopsy was done in the Department of Urology, Beilinson Hospital. The clinical diagnosis based upon the seminal analyses of these patients is given in Table 1.

TABLE 1

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>No. of cases</th>
</tr>
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<tbody>
<tr>
<td>Oligospermia</td>
<td>19</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>16</td>
</tr>
<tr>
<td>Necrospermia</td>
<td>6</td>
</tr>
<tr>
<td>Impotentio coeundi</td>
<td>5</td>
</tr>
<tr>
<td>Retrograde ejaculation</td>
<td>1</td>
</tr>
<tr>
<td>Obstruction of ducts</td>
<td>5</td>
</tr>
</tbody>
</table>

RESULTS

The results are summarized in Table 2.

(1) The examination of nineteen cases of severe oligospermia gave the following results: The sperm count was from 3 to 12 millions/ml. Histological examination demonstrated various degrees of atrophy in fourteen cases and germinal cell hypoplasia in two cases. In these sixteen patients the sat was either
negative or weakly positive in one or both testes. By weakly positive is implied a phosphorus concentration curve with a very low plateau, an early short peak, or a peak which was too late, with an infantile pattern (Text-fig. 2 and Pl. 1, Fig. 1).

### Table 2

**RESULTS OF SAT, HISTOLOGIC EXAMINATION AND SPERM COUNT**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cases</th>
<th>Sperm count ($10^6$/ml)</th>
<th>Histologic result SAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P  WP  N</td>
</tr>
<tr>
<td>Oligospermia</td>
<td>19</td>
<td>3 to 12</td>
<td>-</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>16</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Obstruction of ducts</td>
<td>5</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Necrospermia</td>
<td>6</td>
<td>10 to 100</td>
<td>-</td>
</tr>
<tr>
<td>Impotence</td>
<td>5</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Retrograde ejaculation</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

P, Positive; WP, weakly positive; N, negative.

**Text-fig. 2.** A case of oligospermia: 35 years old, married for 2 years. Seminal analysis: quantity 3 ml, $6 \times 10^6$ spermatozoa/ml, with 80% motility, 10% pathological forms. SAT weakly positive, with an early peak on the left side, negative on the right, ○, Right testis; ●, left testis; ×, ethmoid.

In another two cases, arrest of maturation was found on histological examination and the SAT was negative. The SAT was also negative in the nineteenth case, in which the histological pattern was normal but the sperm count low, the patient having been sterile for 4 years.
Fig. 1. A case of oligospermia (see Text-fig. 2). Biopsy of testis: few normal tubules, much peritubular connective tissue, germinal cells fewer than normal.

Fig. 2. A case of azoospermia (see Text-fig. 3). Biopsy of testis: atrophic tubules, no germinal cells, thickened basement membrane.

Fig. 3. A case of azoospermia due to obstruction (see Text-fig. 4). Biopsy of testis: normal.

Fig. 4. A case of necropermia (see Text-fig. 5). Biopsy of testis: disorganization.
(2) In eight of the sixteen cases of azoospermia complete atrophy of the germinal tissue was found in the biopsy material. In six of these patients the sat was negative and in two it was weakly positive (Text-fig. 3 and Pl. 1, Fig. 2). In the other eight cases the histological examination revealed weak spermatogenic activity: few mitoses, thickened basement membranes, peri-tubular fibrosis and various stages of atrophy. In all these patients the sat was weakly positive.

(3) In five other cases of azoospermia, obstruction of the ducts was suspected. We were especially impressed by the normal size and consistency of the testes which was unusual for patients with azoospermia. On surgical exploration obstruction was found in three patients and congenital agenesis of the vasa deferentia in two. In the first group of three, a history of gonorrhoea was given in two cases, while the third had had tuberculosis in adolescence. Histological examination of the biopsy material was normal and the sat was positive (Text-fig. 4 and Pl. 1, Fig. 3). In two cases of agenesis the biopsy revealed a disturbed spermatogenic pattern—some proliferation of Sertoli and interstitial cells and thickening of the basement membranes. The sat showed a late, small, infantile plateau.

(4) In six patients, necrospermia was found. In four of these cases, histological examination showed various stages of atrophy of the germinal tissue. The sat was negative. In the other two cases histological examination of the biopsy material was almost normal. The sat was positive, but an early plateau appeared on the 2nd or 3rd day (Text-fig. 5 and Pl. 1, Fig. 4).

(5) Five patients suffering from impotentio coeundi were included in this series. No specimen was available for seminal analysis. In two cases biopsy
was normal and the sat was positive. In the other three cases, atrophic changes were found on histological examination and the sat was negative.

One patient was unable to ejaculate seminal fluid on repeated trial. Retrograde ejaculation was diagnosed. The sat was positive and the biopsy normal.

DISCUSSION

In most cases of oligospermia and azoospermia there was full correlation between the sat and the histological results. The test was weakly positive in cases
where there were histological signs of partial atrophy of the germinal tissue, or where the tissue was histologically normal but there were signs of functional inferiority. In patients with azoospermia the sat was negative in cases in which the histological examination revealed complete atrophy and weakly positive in cases with a lesser degree of, or incomplete, atrophy. In the latter cases there were probably still some isolated centres of weak, incomplete spermatogenic activity without production of mature cells. It was not possible to diagnose cases of complete or incomplete maturation arrest using only the sat curve which was either negative or weakly positive. Only after biopsy could a correct diagnosis be made and the proper treatment started.

In cases of azoospermia with complete atrophy, the size of the gonads is usually somewhat smaller than normal. Thus, in cases with normal gonadal size and with a positive sat, obstruction or agenesis of the ducts may be suspected.

It was observed that in cases of acquired obstruction the sat and biopsy were normal, whereas in cases of congenital agenesis the histological pattern was disturbed and the $^{32}$P uptake curve was infantile.

The sat may differ from one examination to another, probably in relation to higher or lower spermatogenic activity at that time and parallel to changes which were noticed in sperm counts. Our impression is that, in most cases, the sat shows a higher correlation to the histological results than does the seminal analysis. This observation is in accord with the findings of previous authors (Simmonds, 1952).

In one case of oligospermia a normal histological pattern was found but the sat was negative. It is possible that in such a case there is a disturbance in the metabolism of DNA and that phosphorus was not concentrated in the nuclei. Could this possibly be a cause of sterility? Is it an inborn or acquired error of metabolism? The sat, but not the microscope, is able to detect this feature.

Only 0.02% of the ingested $^{32}$P is taken up by the testes. This $^{32}$P undergoes disintegration and the testicular tissue is exposed to the ionizing radiation. Careful evaluation determines this exposure to be equal to 80 to 110 m-rad. This is a small amount, similar to the natural, yearly exposure of all inhabitants: it is a 1/300 part of the doubling mutation dose and is smaller than the roentgen dose during any pelvic examination. It may therefore be stated that from a radiological point of view the sat is safe (Larsson, 1958; Salinger, 1959; Sternberg, 1960).

CONCLUSION

The sat described by Czerniak & Gross-Avigad (1959) could play an important role in the investigation of male sterility and has some advantages over biopsy as a diagnostic method. It is a dynamic-functional evaluation of the state of the whole gonad at the time of examination. The histological pattern of the biopsy material represents the static anatomic state of only a small part of the gonad from which we deduce the most probable state of the other parts.

In many cases of oligospermia and azoospermia there is a close correlation between the sat and the histological results and it is possible to predict the histological pattern from the sat curve. In these cases almost as much information is
obtained from the SAT as from the biopsy, without the operative trauma and discomfort to the patient, and at less cost. In cases in which arrest of maturation is suspected, a correct diagnosis can be reached only through biopsy and not by the SAT.

In cases of azoospermia due to obstruction, a positive SAT is an important diagnostic and prognostic tool indicating that surgical exploration with a view to anastomosis may be undertaken.

The SAT is a most valuable means of diagnosis in cases in which no specimen can be obtained because of a pathological state such as in cases of impotence and retrograde ejaculation, or because of religious scruples.

It may be concluded that SAT is an important additional tool in the diagnosis of male sterility. In some cases it may take the place of testicular biopsy and in all cases it offers additional information on the functional state of the gonad.

REFERENCES


