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EFFECTS OF IMMOBILIZATION ON SPERMIOGENESIS

E. R. Meitner

Translation of "Einfluss der Immobilisation auf die Spermiogenese,"  
Acta Anatomica, No. 95, pp 300-308, 1976

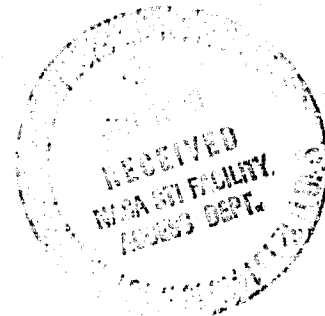
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16. Abstract The author investigated the influence of immobilization stress on spermiogenesis in rats. After 96 hour immobilization, histological changes began to manifest themselves in the form of a practically complete disappearance of cell population of the wall of seminiferous tubule as well as a markedly increased number of cells with pathologic mitoses. Enzymological investigations have shown in particular groups (24, 48 and 96 hour immobilization) various changes of activity (of acid and alkaline phosphatase, nonspecific esterase) which, after temporary negativity, became positive again in the last group.					
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## EFFECTS OF IMMOBILIZATION ON SPERMIOGENESIS

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### Introduction

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The effects of temperature, light, various infectious diseases and of cryptorchism on the germinal epithelium of the testes are well known. They involve specific morphological and functional changes. We became interested in determining whether general changes in the state of the entire organism due to immobilization stress would have any effects of spermiogenesis.

### Material and Methods

In our experiments we used nine adult Wistar rats (three other rats served as control animals) divided in groups of three animals each, which were immobilized for 24, 48 and 96 hours individually in such a manner as to prevent any outside effects on their testes. The testes removed immediately following decapitation were subjected in part to HE-staining (paraffin sections), partly to enzymatic reactions (Baker, frozen sections).

### Description of Findings

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In normal state the seminiferous tubules show the layer arrangement of spermiogenesis, from spermatospores over spermiocytes and spermatides to sperm filling the lumina of the tubules (Ill. 1). Following a 24-hour immobilization, no pronounced changes can be detected

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\*Numbers in the margin indicate pagination in the foreign text.

in the cell population of the Tubuli seminiferi: the layer sequence of germ cell generation is undisturbed. Following a 48-hour immobilization the number of spermatospores apparently decreases, spermiocytes have lost some of their size and chromatin of spermiocytes of the 2nd order have somewhat weaker coloring. Numerous spermatides die off; the volume of the seminiferous tubules contains only isolated sperms the cell nuclei of which have no coloring (Ill. 2). Filamentous crystalloids appear in varying quantities among the spermiogenetic cells. The greatest changes were detected following a 96-hour immobilization. The number of spermatospores with hyperchromic nuclei showed a considerable decrease. Regeneration of all cell types of spermiogenesis leads in numerous sections of the seminiferous tubules to complete disorder in the layer arrangement; in many cases the wall of a seminiferous tubule consists only of loosely suspended undeveloped seminal cells. In the often only isolatedly in the lumen sporadically present spermiocytes with highly prismatic cell plasma, which are sometimes mutually connected by cytoplasmic bridges, appear indications of nuclei disintegration: the cytoplasm of these cells shows a honeycomblike structure, and in all cases also small vacuoles, as signs of necrobiosis. Spermatides and sperms occur only sporadically and in very small numbers. Sperm agglutination is never detected in the lumina of the tubules, all that can be seen there are nuclei of dead cells. The overall picture gives the impression of a total decomposition of the spermatogenic cell population (Ill. 3). Numerous spermatospores are converted into small, oval, hyperchromic elements without detectable mitosis modes. It is often possible to find on the Membrana propria tightly attached ovoid nuclei poor in chromatin content with a dark plasmosome which resemble Sertoli's cells even though their

longitudinal axis is parallel to the Tunica propria. In some spots they occur in larger quantities and crowd spermatospores away from the Membr. propria (Ill. 4). Presence of eosinophilous liquid in tubule sections is not a rare occurrence (Ill. 5).

A very remarkable and frequent change consists in appearance of large, round to polyhedral cell elements with varying pathological mitosis modes and pale cytoplasm which often occur in multiple layers/304 in the entire circumference of the tubule, sometimes isolated in the lumen, only with difficulty identifiable as a type of spermiogenesis cells (Ill. 6). These isolated cells are found on the inner surface of the Membrana propria and, thus, must be interpreted as undeveloped seminal cells -- in spite of pathological mitosis. In several cases we found nuclei modifications resembling an eccentrically situated hollow nucleus (Ill. 7). In other loci we detected in the cells one or two vacuoles in addition to pathological mitoses. Between cells with pathological mitosis modes there is a frequent occurrence of plasma residues of dead cells, vacuolated cells and filamentous crystalloids (Ill. 8). Elsewhere we found spermiocyte chromatin -- as an indication of nucleus pyknosis -- agglutinated into two homogeneous dark-blue clumps.

For enzyme-histochemical studies we performed the following reactions: 1. Gomori's reaction to acid phosphatase, 2. Naphthol-AS-BI-phosphate (pH 5.5) to acid phosphatase, 3. a/naphtholphosphate (pH 5.5) to acid phosphatase, 4. a/naphtholphosphate (pH 9.2) to alkalic phosphatase, 5. Reaction to unspecific esterases and 6. Reaction to glucose-6-phosphatase.

The following findings were made on control animals: 1. Acid phosphatase according to Gomori shows in the central parts of the wall of the seminiferous tubules activity in the form of a brown precipitation (Ill. 9); 2. and 3. In both reactions the acid phosphatase showed activity in peripheral sections of the wall of the seminiferous tubules in the form of a reddish or red-brown coloring; 4. The naphtholphosphate reaction to alkalic phosphatase took the form of a fine, very thick, black, granular precipitation active in the peripheral Tubulus section (Ill. 10); 5. The non-specific esterases were active throughout the width of the wall in the form of blue grains up to 306 striate granular agglutinations radially converging on the lumen of the tubule (Ill. 11); 6. The glucose-6-phosphatase is active in the central sections of the seminiferous tubules in the form of coarsely granular brownish precipitation (Ill. 12).

The enzymes studied in the walls of seminiferous tubules showed the following changes after a 24-hour immobilization: Reaction sub 1 was more intensive in most of the tubuli (Ill. 13). Reactions sub 2 and 3 were for all practical purposes negative, and the reaction sub 4 was completely negative. The reaction listed sub 5 showed a very greatly diminished activity (Ill. 14), and reaction sub 6 was also diminished. The 24-hour immobilization led thus to an increased activity of acid phosphatase (according to Gomori), the reactions to acid phosphatase (naphthol-AS-BI, a-naphthol) and the reaction to alkalic phosphatase (a-naphthol) were negative, reaction to non-specific esterases was strongly diminished, as was the activity of glucose-6-phosphatase.

Following a 48-hour immobilization, the reaction sub 1 was strongly positive (Ill. 15), reactions sub 2, 3, 5 (Ill. 16) and 6 (Ill. 17) were positive, while the reaction sub 4 was negative.

Following a 96-hour immobilization, activity of acid phosphatase (according to Gomori) was considerably stronger than in the control animals (Ill. 18). Reactions sub 2, 3, 4 (Ill. 19) and 5 (Ill. 20) were positive, while glucose-6-phosphatase showed no activity.

In summary it can be stated that immobilization has varying effects on the activity of the studied enzymes. With the exception of acid phosphatase (according to Gomori) and the glucose-6-phosphatase which show activity after 24 and 48 hours, activity in the case of acid and alkalic phosphatase and of non-specific esterases is negative. It is noteworthy that following a 96-hour immobilization the activity of acid phosphatase (according to Gomori) exceeds that of the control animals and that other enzymes, with the exception of glucose-6-phosphatase, show signs of activity. Even taking into account the individual immobilization groups (24, 48 and 96 hours), the behavior of enzyme activity varies. After initial negativity it again becomes positive.

### Discussion

The described changes in the cell population of seminiferous tubules are most pronounced following a 96-hour immobilization. We find two morphological states. In part there occurs a complete restoration/307 of the spermiogenic layers of the tubules with a practically empty lumen, in part there occurs the formation of numerous irregularly distributed large cell elements with pathological mitoses. All that



can be identified in the surfaces of tubule walls built up of such cells are hyperchromic spermatospores without mitosis modes. Between the large pale cells are crystalloids that occur also during seminiferous tubule changes due to other etiology; plasma residue of dead cells is found in the tubule's lumen. Both changes in the cell population, regressive as well as proliferative, during which there occurs a complete suspension of spermiogenesis, we choose to interpret as the result of negative effects on spermiogenesis by the 96-hour immobilization, in all layers of the cell population of the seminiferous tubules, whereby formation of cells with pathological mitoses can be viewed as the effort of the organism to keep up even under a spermiogenic stress situation -- even though in a roundabout way and without attaining the final goal, i. e., without -- formation of good ripe sperm.

We refrain from making any statements regarding the specific type and manner in which the described changes occur in seminiferous tubules through immobilization. We just generally assume that the complex effects of the immobilization state which adversely influence metabolism, the hormonal balance, blood circulation, the neurovegetative and acidobasic balance, are responsible for the micromorphological and enzyme-histological changes. The fact that germinal epithelium reacts to negative effects with regenerative, and in our experimental arrangement also with cell proliferation changes, points to the easy vulnerability (also through nonmechanical effects) of the testes.

The regenerative changes occurring in 96-hour immobilization resemble those occurring in diphtheria and in cryptorchism. Comparable or similar changes can be caused in the testes by serious psychical

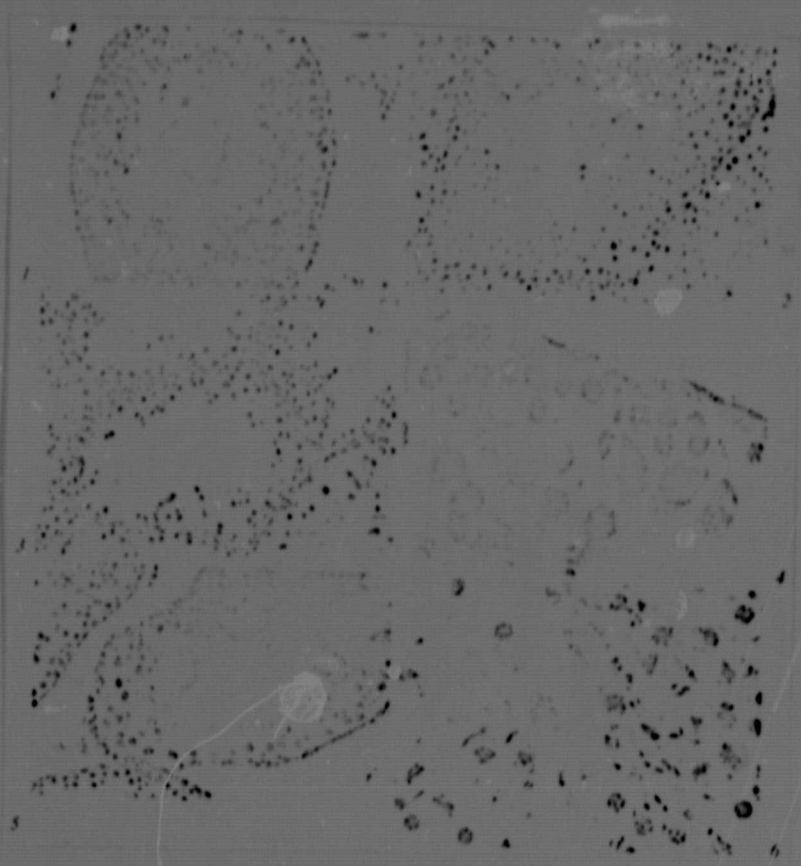
traumata (STIEVE, 1925, 1930), as well as by pharmaceutical preparations with antiandrogenic effects (Cyproteron acetate = Androcur: NEUMANN, 1973). Chemical and hormonal effects (Lysergamid or Cortison, thyroxine, among others) on spermiogenesis were also described (MELLAN et al, 1968; GO et al, 1971). Basophilic crystalloids are interpreted by STIEVE (1930) as regenerative phenomena in the testes of older men. As shown by our experiments, they can also occur in general stress situations. In connection with our experiments, we found no references in available literature to formation of cells with pathological mitoses. 1308

The results of micromorphological and enzymological studies confirm an adversary behavior between cytological and enzymatic changes. Even though the wall structure of seminiferous tubules is completely upset following a 96-hour immobilization, in other areas the activity of acid phosphatase exceeds that of the control animals and also that of other enzymes will become positive after a temporary negativity. The general state of the organism adversely affected by immobilization progressed differently in the individual groups in relation to enzyme activity of the seminiferous tubules' population.

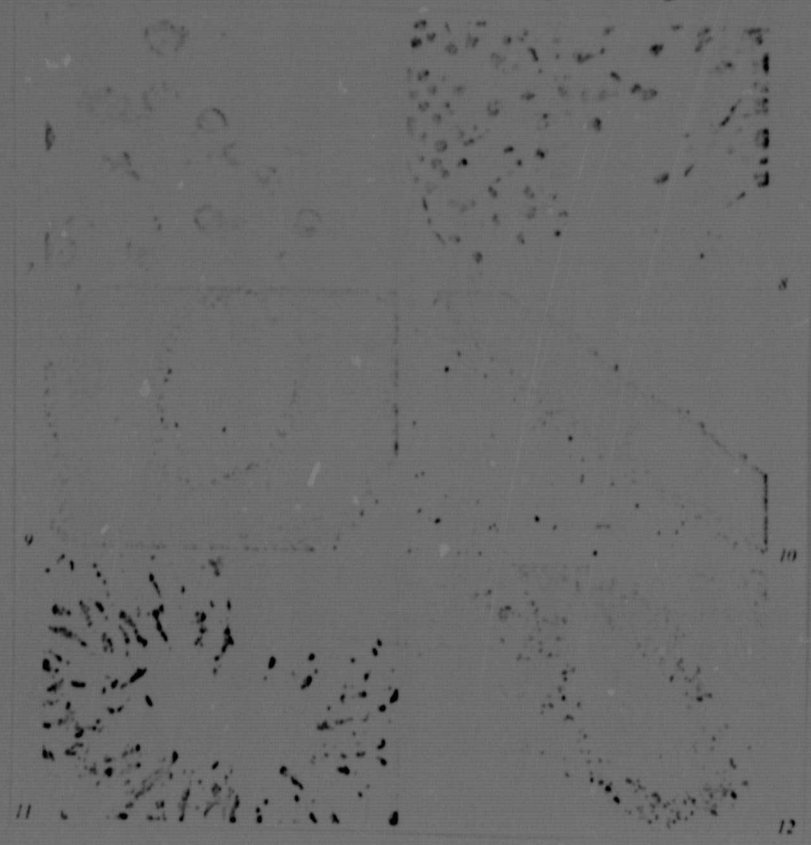
#### Summary

The author investigated the influence of immobilization stress on spermiogenesis in rats. After 96-hour immobilization, histological changes began to manifest themselves in the form of a practically complete disappearance of the cell population of the wall of seminiferous tubule as well as a markedly increased number of cells with pathologic mitoses. Enzymological investigations have shown in particular groups

(24, 48 and 96-hours immobilization) various changes of activity (of acid and alkaline phosphatase, nonspecific esterase and glucose-6-phosphatase) which, after temporary negativity, became positive again in the last group.



Effects of Immobilization on Spermiogenesis

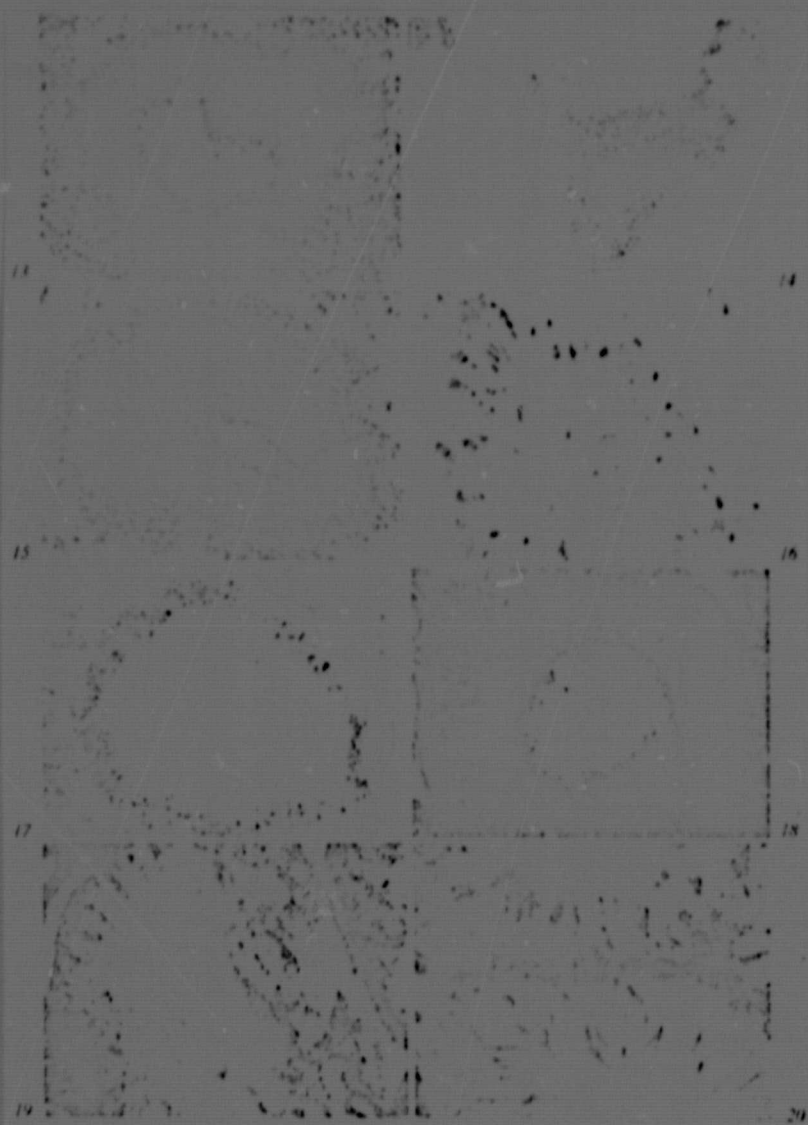


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Effects of Immobilization on Spermiogenesis

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CAPTIONS TO ILLUSTRATIONS:

- Ill. 1. Normal seminiferous tubules of an adult rat. HE. x64. 1302  
Ill. 2. Seminiferous tubules following 48-hour immobilization: reduced number of spermatospores, dead spermatides, isolated sperms without stained cell nuclei. HE. x64.  
Ill. 3. Seminiferous tubules following 96-hour immobilization: dissolution of the spermatogenic cell population. HE. x 64.  
Ill. 4. Wall of a seminiferous tubule following 96-hour immobilization: multiplied Sertoli cells with low chromatin content proliferating on the Membrana propria. HE. x 640.  
Ill. 5. Seminiferous tubules following 96-hour immobilization: eosinophilous liquid in lumen. HE. x64.  
Ill. 6. Seminiferous tubules following 96-hour immobilization: numerous pathological mitosis modes. HE. x160.

- Ill. 7. Hollow nucleic cell elements in seminiferous tubules following 96-hour immobilization. HE. x640. /303
- Ill. 8. Pathological mitoses, plasma residue, crystalloids in seminiferous tubules following 96-hour immobilization. HE. x640.
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- Ill. 10. Control animal: alkaline phosphatase (a-naphtholphosphate reaction), activity in peripheral tubulus section. x160.
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- Ill. 14. 24-hour immobilization, reduced activity of nonspecific esterases. x64.
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