

LATE CHANGES IN THE GONADS OF MALE WHITE RATS UPON COMBINED TREATMENT WITH LOW FREQUENCY VIBRATIONS AND RAMROD INTOXICATION

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One of the essential issues, related to the increasing intensification of agriculture, is the noxious effect on human health exerted by the chemical preparations made use of. It has become an urgent problem worldwide (2, 5, 15). Of particular interest is the combined effect of noxae, and their late sequelae in the organism (18, 25). For the time being, researches along this line are rather scarce, and concern mainly some internal organs of test animals (4, 30).

Proceeding from the fact that the reproductive system is particularly sensitive to a variety of harmful agents (3, 9, 14, 20), and also that its damage has an essential bearing on normogenesis and inheritance, we set out to study the changes in the gonads of male white rats, developing under the effect of low-frequency vibrations against the background of previous ramrod intoxication (6). It appears that the combination of such noxious agents augments the hazard of their untoward effect. In the testes the occurrence of circulatory disorders, destructive-dystrophic changes, inhibition of cell division and atrophy of spermatopoietic epithelium is observed (6). In this work the evolution of changes and the regenerative capacity of gonads are followed up.

Material and methods

Sixty male white rats, Wistar breed, with initial weight 150 ± 10 g were used in the experiment. The animals were equally distributed in the following groups: group I — ten control animals; groups II — ten animals subjected to low frequency vibrations, at frequency 15 Hz and amplitude 1.6 mm, for 3 hrs daily over a period of 30 days; source of vibrations — ground technique for pesticide application used in the routine practice; group III — a) ten animals intoxicated over a period of 4 months with $1/20$ LD₅₀ ramrod, and b) ten animals intoxicated over a period of 4 months with $1/100$ LD₅₀, and group IV — a) ten animals intoxicated for 3 months with $1/20$ LD₅₀, and within a month thereafter, subjected to simultaneous vibration treatment and intoxication with identical dose; b) ten animals intoxicated for 3 months $1/100$ LD₅₀, and within a month subjected to simultaneous vibration exposure and intoxication with the same dose. The length of the treatment terms and the doses picked out were complied with the chronic experimentation requirements in toxicology. As LD₅₀ were accepted 1200 mg/kg, according to data furnished by the firm — producer (25a). The animals were poisoned per os, via sound, using water solution of 65 per cent moistened ramrod powder. They were raised under vivarium conditions at standard alimentary regime. Vibrator stand

VUS 500/200, made in USSR, was employed as a source of vibrations. The animals were killed within thirty days of treatment cessation. The testes were fixed in Boiun' fluid and prepared after the paraffin method. The listed below histological and histochemical methods were made use of: staining with hemalaun eosin, with azan after Krutsay, Gomori, van Gieson, Feulgen for DNA, after Brachet for RNA under ribonuclease control, and PAS reaction under alpha — arnylase control.

Results

The histological study shows that within a month of discontinuing vibration treatment, the changes in testicular structure are most variegated and

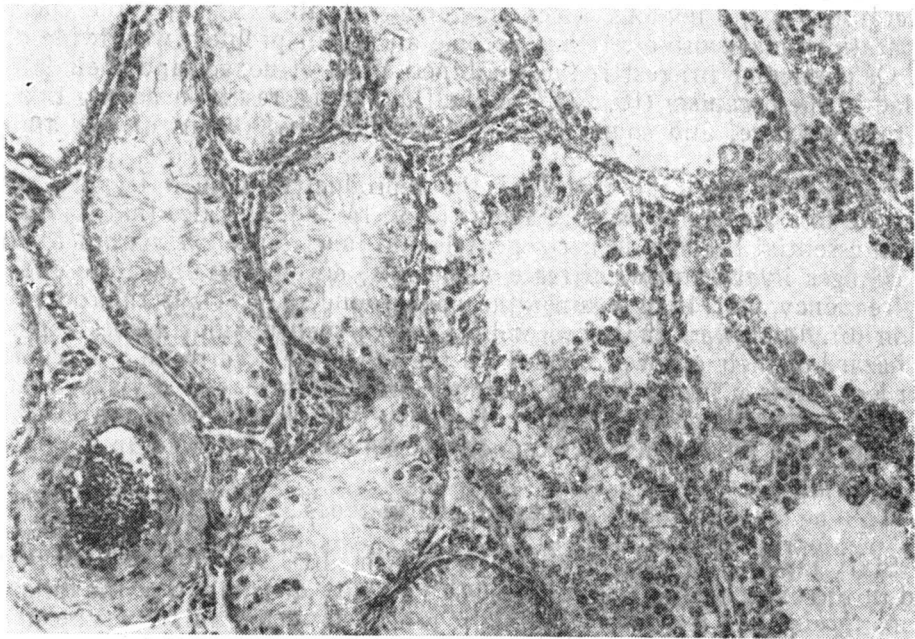


Fig. 1: Atrophic seminal tubules around a fibrotic vessel, filled with vacuolized Sertoli's cells, reticular and collagen fibers in a rat within a month of discontinuing vibration treatment. Stain. HE; magnif. x 80.

display an ununiform character, imparting a "mosaicism" pattern to the alterations. Microcirculation disorders recorded just after cessation of the vibrations (edema, plasmorrhaxis) subside, but the walls of the blood vessels are fibrotic and thickened (Fig. 1). A great number of seminal tubules, situated subcapsulary and perivasally, are atrophic and contain very few spermatogonia and vague spermatopoietic cellular forms. Some of the tubules are filled only with Sertoli's cells with heavily vacuolized cytoplasm. In other animals, similarly to those killed just after the cessation of vibrations, incompleteness of spermatopoietic epithelium is noted. However, the dystrophically altered spermatogenic cells are much less numerous, and no debris is found in the

lumen of tubules. In some tubules, situated in the central zones, signs of intense spermatogenesis, accompanied by a rise of the ribonucleoproteins' content in epithelial cells is observed. The basement membranes are thickened, with a slightly positive PAS reaction. The reticular and collagen fibers which render them denser, penetrate inside the tubules. An increase in fibrous and cellular connective tissue elements is established in the interstitium.

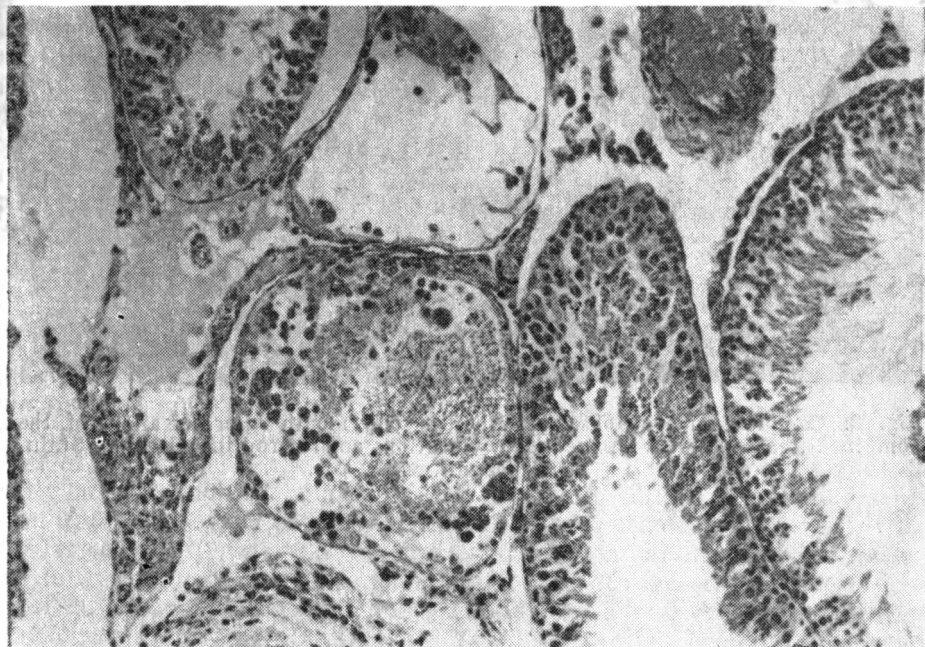


Fig. 2: Dilated, deformed seminal tubules filled with detritus material, dystrophic multinucleate epithelial cells within a month of discontinuing 4-month long intoxication with $1/20$ LD_{50} ramrod. Stain. HE; magnif. x 80.

In the animals killed within a month of discontinuing ramrod intoxication, the structural changes are similarly characterized by "mosaicism". Here the degenerative and necrotic changes show a clearcut intensification. Some of the seminal tubules are filled with granular, eosinophilic, PAS+ nonglycogenic substance and desquamated epithelial cells with pyknotic nuclei and scarce cytoplasm (Fig. 2). It is a matter of dilated tubules with irregular shape. Once again, like in the immediate post-intoxication period, multinuclear epithelial cells are observed. At many points the germinative epithelium is detached from the basement membrane. In some of the tubules an intense spermatogenesis is recorded, by no means detectable immediately after the cessation of poisoning. The vascular walls are thickened by fibrous tissue. At this particular term, the edema changes are mildly pronounced. Among the animals intoxicated with $1/100$ LD_{50} ramrod spermatogenesis disorders are rather moderate.

One month after discontinuing combined treatment with either of the factors, a virtually full subsidence of acute circulatory derangements is observ-

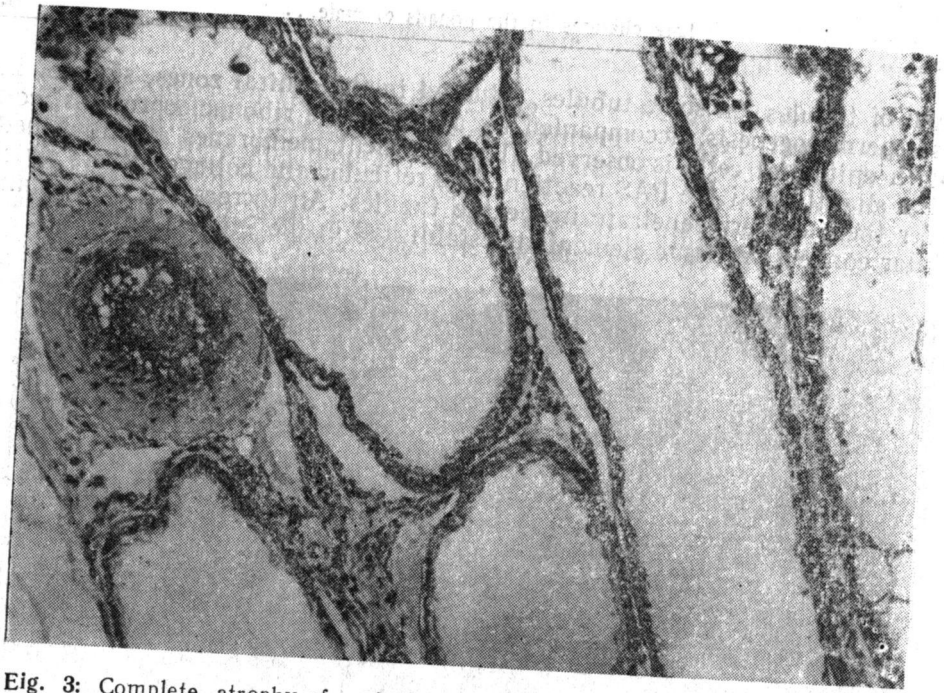


Fig. 3: Complete atrophy of seminal tubules one month after cessation of the combined treatment with low-frequency vibrations and ramrod intoxication. Stain. HE; magnif. x 80.

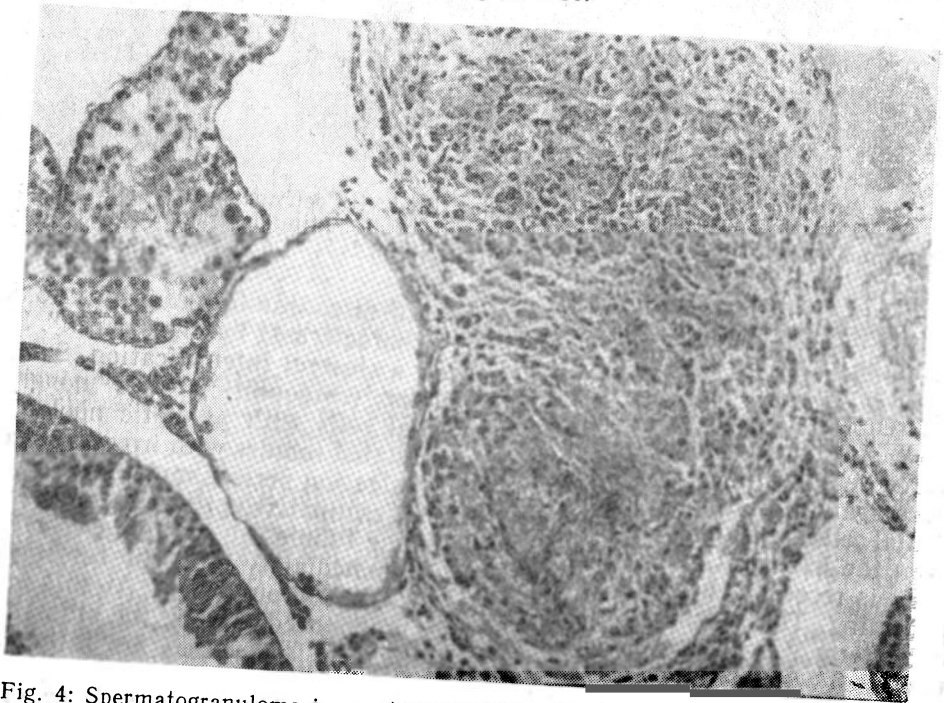


Fig. 4: Spermatogranuloma in a rat one month after cessation of the combined treatment with low-frequency vibrations and ramrod intoxication. Stain. HE; magnif. x 80.

ed. In this case too the blood vessels are fibrously thickened, whilst in the interstitium focal infiltrates of connective tissue cells are noted. The acute dystrophic and necrobiotic changes, recorded just after the cessation of combined treatment, are no longer present, but in most of the tubules, particularly those situated subcapsulary, the spermatopoietic epithelium is completely destroyed. Within the lumen of some tubules vacuolized Sertoli's cells are seen, and somewhere multinuclear cellular forms with a low content of ribonucleoproteins as well. Tubules are encountered displaying irregular form and thickened, finely undulated basement membrane, covered only by a single row of hyperchromatic epithelial cells and cells filled with fibrous tissue where calcium salts are deposited. Along with atrophic changes, an intensive spermatogenesis is recorded in some tubules, associated with increased pyroninophilia within the spermatogonia. In one animal spermatogranuloma, made up of macrophages, epitheloid cells and lymphocytes enwrapped by connective tissue fibers, is observed (Fig. 4).

Discussion

The study shows that regardless of discontinuing the isolated and combined effect of vibrations and ramrod herbicide, the testicular structure of the animals experimented upon remains up to a great extent changed. It is a matter of morphological changes dependent in a varying degree on the specific factor of treatment. While within a month of stopping vibration stimuli, practically all dystrophic changes in the spermatopoietic epithelium disappear, in ramrod intoxicated animals degenerative and necrotic processes in the sexual tissue persist. This may be explained by eventual differences in the pathogenetic mechanism of the damaging effect of both factors. Most likely, upon treatment with vibrations, a primary role is played by hypoxia resulting from derangements in microcirculation. Such a vibration effect has been comprehensively described for limbs and parenchymatous organs (1, 13, 19, 26), as well as for the testes (6). Along with that, by no means less important role is played by the direct aftereffect of mechanical fluctuations on the germinative epithelium (9, 12, 29). In ramrod intoxication an important point, apart from the direct effect on sexual tissues, similar to that of other pesticides (3, 14, 16, 24, 27), is the general toxic action leading in turn to secondary spermatogenesis inhibition. The preparation whose basic toxic mechanism consists in the derangement of polypeptide synthesis in the cells (21), disturbs also the protein metabolism in gonads — a fact corroborated by total protein reduction and changes in amino acid content in the testes of the same animals (7, 8).

Moreover, a clearcut difference is detectable in the degree and forms of regeneration, dependent on the character of the noxious agent. In rats killed after stopping the vibration stimuli, along with the tubules wherein dyscomplexation and nondifferentiation of cellular forms is observed, also cells with active spermatogenesis are established. In ramrod poisoned animals, and in those subjected to combined treatment, both normal and pathological spermatogenesis with formation of multinuclear giant cells is noted. This is an indication that as the result of systemic and local lesion, disturbances in metabolic processes, and first and foremost in protein synthesis (7, 8), a stormy process of regeneration takes place within the seminal tubules, partially manifested with the formation of pathological cellular forms also.

The third difference is represented by the degree of sex tissue atrophy. One month after cessation of the vibratory action, atrophy of some seminal tubules, containing few, poorly differentiated cellular forms is observed in the testes of animals, whereas among those intoxicated, owing to persisting of the dystrophic and necrobiotic processes, the stationary stage of sex tissue atrophy is not yet reached. However, in animals subjected to combined treatment, practically complete destruction of spermatopoietic epithelium is seen in part of the tubules. This points to the fact that as the result of the combined effect of both noxae, the lesion of gonads in the animals has reached such a degree that discontinuation of their action is not in a position to account for normal spermatogenesis restoration. It points to the fact also that the combined effect of both factors inhibits the division of spermatopoietic epithelium in its earlier phases, and therefore its recovery in isolated tubules proves to be impossible. The persistence of changes in sexual tissues, despite stopping the combined action of the damaging factors, should be interpreted as an expression of a profound derangement of metabolic processes. Also, it is noteworthy that under the effect of vibrations the cells become more sensitive to other noxae (10) with ensuing increase in the toxic effect of ramrod. Although the preparation is estimated as a moderately dangerous herbicide (5), our data demonstrate that it interferes with the processes of biosynthesis in the gonads.

The lacking complete restoration of testicular structure is also due to vascular lesions. The "mosaicism" of the changes observed is in favour of the latter assumption. The most illustrative is the atrophy, and the weakest is the recovery in the vicinity of the major subcapsular arteries which prove to be the most heavily involved ones.

Finally, attention should be called to the granulomatous reaction established in an animal testis. Probably, the formation of granuloma results from the stimulating action of toxic products penetrating from the injured epithelial cells across the damaged basement membranes. They play the role of antigens leading to the granulomatous reaction observed, a finding described by other authors too (17, 22). It is well known that the basement membrane of tubules, which is essential for the hemato-testicular barrier (11, 23, 28), undergoes lesion both during isolated, and during combined effect of the factors applied (6).

Conclusions

1. After cessation of isolated and combined treatment with the noxious factors, over a period of 30 days, the testicular structure of the test animals remains to a high degree pathologically altered.

2. The forms and the degree of regeneration depend on the character of the harmful effect.

3. As the result of combined application of the two noxae, the damage of gonads has reached such a level that cessation of their action fails to bring about a normalization of spermatogenesis.

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**ПОЗДНИЕ ИЗМЕНЕНИЯ В ГОНАДАХ БЕЛЫХ КРЫС
МУЖСКОГО ПОЛА ПРИ КОМБИНИРОВАННОМ ВОЗДЕЙСТВИИ
НИЗКОЧАСТОТНЫХ ВИБРАЦИЙ И ИНТОКСИКАЦИИ РАМРОДОМ**

М. Златева Е. Малева

РЕЗЮМЕ

Исходя из новых проблем гигиены труда в аграрном производстве, авторы исследовали поздние изменения в структуре тестисов опытных животных, подвергнутых комбинированному хроническому воздействию низкочастотными вибрациями и гербицидом Рамрод.

Обнаружено, что через 30 дней после прекращения самостоятельного и комбинированного влияния обоих факторов, гонады остаются до большой степени патологически измененными. Формы регенерации и ее степень зависят от характера вредного воздействия. При сочетанном применении обоих вредностей, в тестисах наступают прочные атрофические изменения, показывающие, что поражение сперматопоэтического эпителия охватило его ранние фазы деления.