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EFFECT OF HYPERTHERMIA ON SPERMATOGENESIS IN MICE AND THE ROLE OF HEAT TRAINING IN ADAPTATION OF THE SEX CELLS TO HIGH TEMPERATURES

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Keeping sexually mature albino mice in a hot and humid chamber at a temperature of 43°C and a relative humidity of 65% for a single exposure causes destruction of the spermatogenic epithelium, as reflected in degeneration and desquamation of the sex cells in 55% of the seminiferous tubules. Preliminary heat training for 10 days increased the physiological degeneration of the spermatogenic cells in only 16% of seminiferous tubules. This could be evidence of adaptation of the sex cells to the action of high temperatures.

KEY WORDS: adaptation; hyperthermia; spermatogenesis.

Hyperthermia in man and animals is known to cause damage to the cells of the spermatogenic epithelium. The degree of this damage depends on the height of the ambient temperature and the duration of exposure to it [2, 5, 6, 8, 9].

The problem of whether adaptation of sex cells to the action of heat takes place during daily exposures of increasing duration to high temperatures has not been discussed in the literature. The investigation described below was carried out for this purpose.

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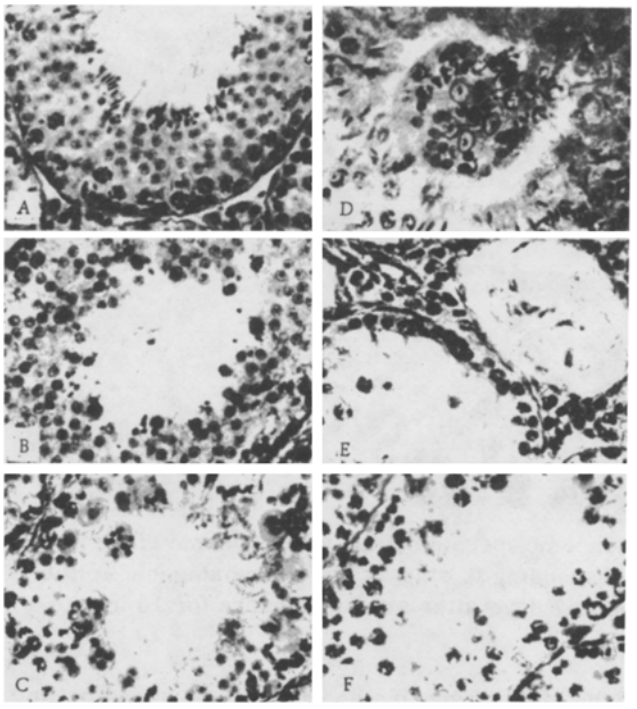

Fig. 1. Testes of mice 7 days after a single exposure for 10 min to a temperature of 43°C: A) seminiferous tubule with normal structure; B) mild degree of damage to spermatogenesis: karyopycnosis and karyorrhexis of individual spermatogenic cells; C) severe degree of damage to spermatogenesis: many degenerating and multinucleate spermatids; D) severe degree of damage to spermatogenesis: desquamation of spermatogenic cells and their separation into lumen of seminiferous tubule as a cast of degenerating tissue; E) depopulation of seminiferous tubules; F) typical picture of intratubular regeneration. Here and in Fig. 2: hematoxylin–eosin, 400×.

EXPERIMENTAL METHOD

Two series of experiments were performed on 45 sexually mature noninbred male albino mice weighing 20-30 g. The mice of series I (15) were exposed once for 10 min to hyperthermia in a well-ventilated, hot, and humid chamber with an air temperature of 43°C and a relative humidity of 65%. The animals of series II (15) were kept in the same chamber at the same air temperature and relative humidity for 10 days: for 1 min on the first day and increasing by 1 min the exposure of the animals in the chamber on each subsequent day. Fifteen intact mice, not exposed to hyperthermia, acted as the control. The experimental animals of series I were killed by decapitation 7 days after the single exposure to hyperthermia and the animals of series II were killed 7 days after the last exposure (which was for 10 min).

The testes of the control and experimental animals were weighed on torsion scales and then fixed in Bouin's fluid. Paraffin sections were stained with hematoxylin–eosin and, to reveal the acrosomal system of the developing spermatids, which is a marker of the stages of the spermatogenic epithelial cell cycle (SECC), the sections were treated with PAS reagent and counterstained with Ehrlich's hematoxylin [4, 7].

The degree of disturbance of spermatogenesis was judged from the number of seminiferous tubules belonging to each of the following five types: 1) seminiferous tubules with normal structure, sex cells of different degrees of differentiation arranged in them in concentric layers, in full agreement with the stages of SECC (Fig. 1A); 2) a mild degree of disturbance of spermatogenesis, characterized by degenerative changes in individual cells (karyopycnosis, karyorrhexis, hyperchromasia of the cytoplasm) present in the seminiferous tubules (Fig. 1B); 3) a severe degree of injury to spermatogenesis found in the seminiferous tubules: many degenerating and multinucleate giant cells (Fig. 1C), desquamation of spermatogenic cells, and their separation as a cast of degenerating tissue in the lumen of the convoluted tubule (Fig. 1D); 4) seminiferous tubules partly depopulated, with only Sertoli cells, spermatogonia, and a few primary spermatocytes still remaining in them, adjacent to their walls (Fig. 1E); 5) seminiferous tubules with incomplete spermatogenesis but with no sign of degeneration of the sex cells [the typical picture of intratubular regeneration (Fig. 1F)].

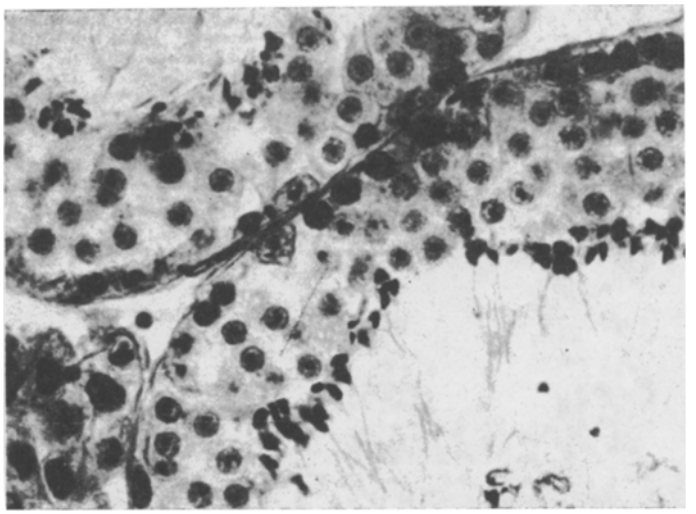

Fig. 2. Absence of spermatocytes at pachyneme stage in seminiferous tubules corresponding to stage VII of spermatogenic epithelial cell cycle in mouse testes 7 days after single exposure for 10 min to a temperature of 43°C.

Quantitative analysis of the state of spermatogenesis was carried out in the testes of 7 animals from each group, by counting 1300-1500 transversely cut seminiferous tubules in each case.

EXPERIMENTAL RESULTS

Exposure to heat for 10 min led to a decrease in the relative weight of the testes in the experimental mice of series I ($5.8 \pm 0.4‰$ compared with $6.7 \pm 0.2‰$ in the control), but had no effect on the corresponding indices in the animals of series II ($6.5 \pm 0.4‰$).

Microscopic examination of the control mice revealed active spermatogenesis in all seminiferous tubules, but 5.9% of the tubules belonged to type 2, for a picture of mild damage, assessible as physiological degeneration of the spermatogenic epithelium, was observed in them.

In the mice in the experiments of series I only 44.7% of seminiferous tubules were found to be of type 1, with normal structure; and 11.4% of the seminiferous tubules belonged to type 5, for spermatogenesis in them was incomplete, with no sign of degeneration of the sex cells. In the other seminiferous tubules the pattern was one of varied destructive changes. A mild degree of damage was found in 20.1% of seminiferous tubules, which contained single spermatocytes with pycnotic nuclei and with hyperchromasia of their cytoplasm, mainly in stage XII of SECC, together with a few desquamated cells. A severe degree of damage to the sex cells, as described in the section "Experimental Method," was found in 23.3% of seminiferous tubules. In many seminiferous tubules, corresponding to stages VI, VII, and VIII of SECC, no spermatocytes at the pachyneme stage were present, whereas the other sex cells, forming the cell clusters characteristic of these stages (Fig. 2), were still intact; 0.5% of the seminiferous tubules were partly depopulated and contained only Sertoli cells, type A spermatogonia, and solitary primary spermatocytes.

In the animals in the experiments of series II the microscopic structure of the testes was almost indistinguishable from that of the intact controls. In 84.4% of seminiferous tubules no pathological changes were present and these tubules could be regarded as belonging to type 1; in 15.6% of seminiferous tubules there was a mild degree of damage. In the experimental mice of series II, exposure to heat for 10 min thus caused only some increase in the degree of physiological degeneration of the spermatogenic epithelium.

The results of this investigation are in agreement with those of many others which have shown that an increase in the temperature of the testes caused by elevation of the ambient temperature, by fever, or by experimental cryptorchidism, leads to rapid destruction of the spermatogenic epithelium [8-10]. As several workers have observed, the structures most sensitive to the harmful action of a high temperature are the spermatocytes [3, 6]. Degenerative changes found in the spermatocytes in stage XII of SECC and the absence of spermatocytes in the pachyneme stage in stages VI, VII, and VIII of SECC in many seminiferous tubules are in agreement with results obtained by other workers and confirm their conclusion that spermatocytes in the

early stages of meiosis are most sensitive to the action of high temperatures. In fact, if it is recalled that the seminiferous tubules corresponding to stages VI, VII, and VIII of SECC were exposed to heat in stages VIII, IX, and X of the preceding cycle, respectively, the cells from which the spermatocytes at the pachyneme stage must have developed 7 days later were primary spermatocytes at the leptoneme stage, i.e., cells just commencing the long prophase of meiosis.

No investigations into the possibility of adaptation of cells of the spermatogenic epithelium to the harmful action of heat could be found in the accessible literature. Some workers have investigated the testes after repeated exposure to heat, but only in one case [6] was evidence of intratubular regeneration of the spermatogenic epithelium observed after mice had been kept continuously for 35 days in a chamber at a temperature of 35°C, and then only in individual seminiferous tubules; in the opinion of these workers, this could indicate the possibility of adaptation of the sex cells to the action of a raised temperature.

The present investigation showed that preliminary heat training leads to adaptation of the spermatogenic epithelial cells to the harmful action of heat.

As Aleksandrov [1] rightly points out, the adaptive effect can be achieved at different levels of structures or systems in the organization of living matter, starting from the macromolecular level and ending with the whole organism. The precise nature of the temperature adaptation of the sex cells and the mechanism of its operation will become clear in the future. However, the results now obtained suggest that preliminary heat training for persons before starting work which involves exposure to heat would be desirable. It would prevent the development of permanent disturbances of spermatogenesis and the associated male sterility in such persons.

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