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TOLERANCE OF ISCHEMIA BY TESTIS OF RATS - AN EXPERIMENTAL STUDY

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*SUMMARY : **An experimental study was undertaken by subjecting the testis of rats to total ischemia and studying the biopsy specimens obtained serially upto 135 minutes of ischemia. The specimens were examined under light microscope for histopathological changes. The aim was to obtain a guideline of tolerable ischemia time of the various tissues of the testis with maintenance of structural and functional integrity. It was observed that the first signs of irreversible damage i.e., marginal clumping of chromatin appeared in some of the germinal epithelial cells 60 minutes after induction of ischemia, whereas these changes occured much later in Sertoli and Leydig cells. It is recommended that ischemia time of testis in clinical situation be restricted to lesser than this period to maintain the structural and functional integrity.***

INTRODUCTION

The process of revascularisation of an ischemic organ, if performed well within the limits of its own tolerance, should preserve its structure and function. Different viscera have different time limits of tolerance of ischemia and effects are also determined by the rapidity and mode of occlusion of its vascular supply, availability of collateral circulation and rate of metabolism. In literature, there is no specific time limit mentioned for testis. There are situations where revascularisation of testis is needed, for example in surgery of difficult cases of high undescended testis and injury to the organ. With a view to find out the time by which irreversible histopathological changes set in, following induction of ischemia of testis, an experimental study was undertaken in rats. The results may serve as a guideline in clinical situations.

MATERIALS AND METHODS

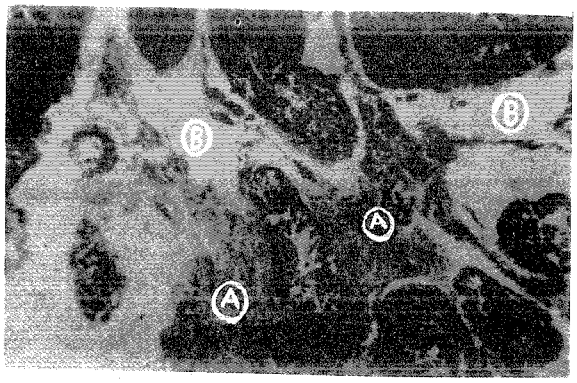
Ten albino rats weighing about 300-350 gms. were selected for the study. The rats were anaesthetised by intraperitoneal injection of a mixture of Ketamine and Diazepam (0.5 ml each). Further small doses (0.1 - 0.2 ml) of the same mixture were sometimes necessary to maintain anaesthesia during the operation. After preparation of scrotum and groin region, the testes were exposed through a vertical incision on the scrotum and a control specimen was taken for biopsy. Thereafter the spermatic cords were ligated about 1.5 to 2cm proximal to the upper pole of the testis to induce total ischemia. Subsequent specimens for biopsy were taken every 15 minutes for up to 135 minutes. For each specimen including the control, 2 pieces were made and one was preserved in 10% formaline saline, the other in Bouin's fluid. They were then stored in the refrigerator at 4 deg. centigrade before transportation to the pathology laboratory. Finally an orchiectomy was performed and the rats returned to the cage. Each specimen including the control was subjected to routine paraffin block preparation and hematoxylin-eosine staining and examined under a light microscope.

RESULTS AND ANALYSIS

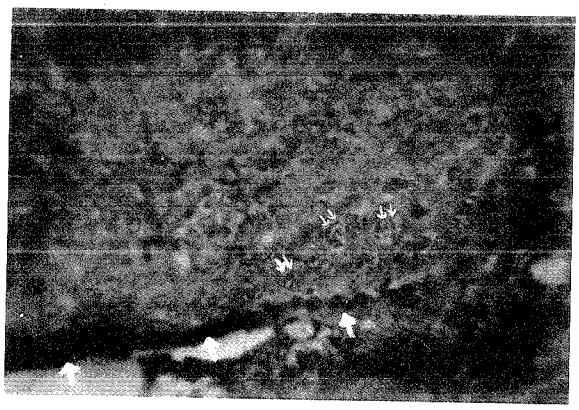
In the initial stages of the study, 2 rats expired and were excluded from consideration. Out of those 10 surviving rats which were included, only 2 had early onset of irreversible damage to seminiferous tubules. Otherwise, the findings were remarkably consistent and are given in a tabular form below:

Period of ischemia (in minutes)	Histological findings
Nil (Control)	Seminiferous tubules with normal architecture (Fig.1).
15	Mild edema present in intertubular space. Seminiferous tubules pale, swelling and vacuolar changes in some of the germinal epithelial cells.

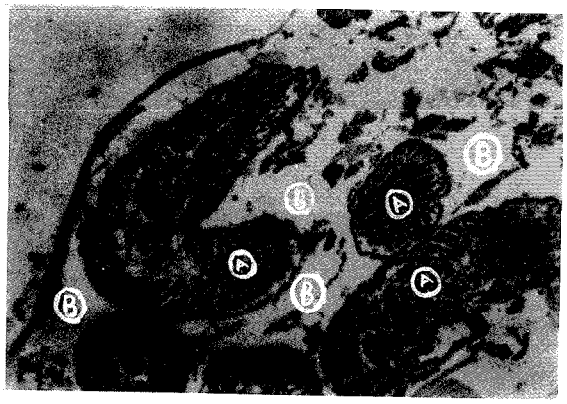
Period of ischemia (in minutes)	Histological findings
30	Increase of existing edema and focal areas of haemorrhage. The vacuolar change of germinal epithelial cells are more marked at this stage.
45	Some of the blood vessels show ruptured walls with haemorrhage being more marked and appearance of few polymorphs in the intertubular space (Fig 2).
60	In some of the tubules, hydropic changes of the germinal epithelial cells along with swollen, vacuolar, ground-glass like nuclei and marginal clumping of chromatin occur marking the onset of irreversible changes. The unaltered Sertoli cells are seen standing out prominently in a row along the basement membrane of the tubules (Fig 3).
75	Changes are more marked than in the previous stage.
90	A good number of Sertoli cells show cloudy swelling. The Leydig cells appear scattered in the edematous & haemorrhagic background and many of them show cloudy swelling as well.
105	Hydropic changes have appeared in some of the Sertoli cells. Most show swollen, vacuolated, ground-glass like nuclei with marginal clumping of chromatin in some of the nuclei. Leydig cells also show vacuolar and hydropic changes and a few of them have marginal clumping of chromatin in their nuclei as well.
120	Some of the seminiferous tubules appear broken. Some of the germinal epithelial cells show pyknotic nuclei while some others show nuclear fragmentation. Few germinal epithelial cells appear as disintegrated cells with dark, large irregular nuclei and scanty, indistinct cytoplasm. There is invasion of blood clot by a fair number of polymorphs and macrophages. The Leydig and Sertoli cells have disintegrated.
135	The seminiferous tubules are disorganised. Cellular outline of most of the germinal epithelial cells is lost and their nuclei pyknotic. In some of the tubules, there are patchy areas of eosinophilic mass without any nuclei representing ghost cells (Fig 4).



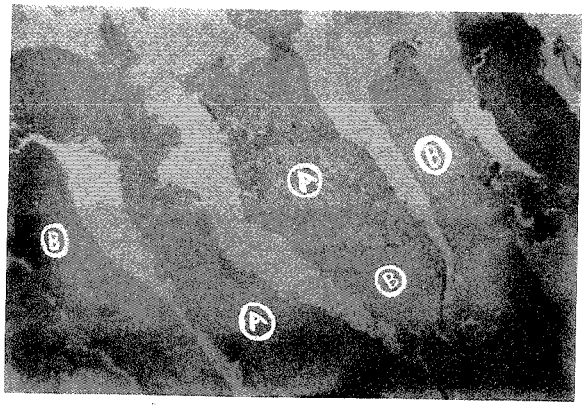
(Fig-2) Photomicrograph at 45 minutes showing haemorrhage with polymorphonuclear infiltration (A) and oedema (B) of the intertubular spaces.(H & E x 60)



(Fig-3) Photomicrograph at 60 minutes showing vesicular nuclei with clumping of chromatin (double arrow) hallmark of irreversible change in germinal epithelial cells. A prominent row of unaltered Sertoli cells (thick arrow) is also present.(H & E x 240)



(Fig-1) Photomicrograph of control specimen showing intact seminiferous tubules (A) and clear intertubular spaces (B) (H & E x 60)



(Fig-4) Photomicrograph at 135 minutes showing total destruction of seminiferous tubules (A) with patchy, eosinophilic areas devoid of nuclei (B). (H & E x 60).

From the table above, it can be seen that from 60 minutes onwards, hydropic changes occur in germinal epithelial cells along with swollen, vacuolar, ground-glass like nuclei and marginal clumping of chromatin in some of the cells indicating onset of irreversible stage of degeneration. Sertoli cells are still unaltered at this stage. By 120 minutes, pyknotic nuclei have appeared in germinal epithelial cells along with disintegration of Sertoli and Leydig cells. A little later (135 minutes), appearance of eosinophilic mass and ghost cells indicates a stage of total destruction. Even Sertoli and Leydig cells, which tolerate ischemia for a much longer period than germinal epithelium, are destroyed at this stage. Since the first signs of irreversible ischemic damage appear in seminiferous tubules after 60 minutes of ischemia, it is reasonable to expect reversal of histopathological and functional damage if revascularisation can be accomplished before this period.

DISCUSSION

The effect of ischemia on rat testicular function has recently been studied by magnetic resonance spectroscopy utilising ATP signals[1]. It begins to fall immediately after clamping of feeding vessels and disappear within 60 minutes. After 4 hours of ischemia, on releasing the vessels, ATP did not recover within 2 hours and the testes became necrotic after one week. In another study on the effect of ischemia on rat spermatogenesis[2], it was shown that 8 and 16 weeks after 1 hour period of testicular ischemia in rats, weights significantly decreased in comparison with controls. The return to almost normal values was accomplished by 24 weeks. Both these studies go on to show the importance of one hour period of ischemia in preserving rat testicular function. However, morphological characteristics have not been studied in detail in any of the above studies. In our experiment, it has been further proved that earliest signs of irreversible damage appear at one hour after the onset of testicular ischemia in rats and therefore attempts at revascularisation should be complete in about 45 minutes in order to preserve function of this vital organ. Sertoli cells which act as blood-testes barrier preventing auto immune destruction of testes are still intact after one hour of ischemia thereby ensuring success of the procedure of revascularisation.

In human beings, testes are subjected to periods of ischemia during some of the operative procedures for high undescended testes and cases of trauma. With the advent of Fowler-Steven's technique[3] of high ligation of spermatic vessels, it was possible to do away with testicular vessels altogether. However, in their long term follow up, half of them were found to have atrophied. Even the successful cases could still be subfertile because of the reduced blood supply in the testis that appeared viable in the post operative period. Before the variable presence of collaterals were demonstrated, results of ligation of spermatic vessels were disastrous[4]. However, subsequent modifications consisting of ligation of spermatic vessels in the first stage and orchiopexy in the second resulted in improvement of final outcome of the operative treatment. Kogan[3], who has a series of 38 selected cases, claims 89% success whereas others with smaller number of patients are not so lucky[4]. Snyder and Duckett[5], who have a comparable number of cases with that of Kogan, in a review of 10 years experience, showed atrophy in 12 out of 41 cases following ligation of spermatic vessels. Even in Kogan's series, the author has cautioned on using this technique in cases where previous dissection had been done. Lastly, to prove that the last word on the problem of difficult high undescended testis has not yet been said, Kogan himself has undertaken a prospective trial in patients with bilateral intraabdominal testes comparing microvascular anastomosis on one side and orchiopexy with transection of testicular vessels on the other.[3] Silber and Kelly[4] are credited with first autotransplantation of testis by microvascular technique. Subsequent work also proved the necessity of preserving testicular vascular supply and the fact that collateral supply may not be sufficient for preserving its structure and function[6]. Even with the use of microsurgical techniques, cases of atrophy have occured underscoring the importance of testicular vessels. It is only natural that the best form of blood supply to any organ is through the vessels that were originally meant for that particular organ.

A case of bilateral testicular replantation after traumatic funicular severance with an ischemia period of 6 hours has been reported and functional recovery was claimed to be complete[7]. Similar isolated cases of replantation have been heard of, but functional assessment is yet to be reported.[8,9]

Though species variation occurs, this experiment gives a guideline regarding the time a surgeon can avail for a revascularisation procedure of the testis.

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