EFFECT OF MODERATE HEAT ON THE TESTES OF RATS AND MONKEYS

P. S. VENKATACHALAM AND K. S. RAMANATHAN

Nutrition Research Laboratories,
Indian Council of Medical Research, Hyderabad-7, India

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Summary. The effect of daily immersion of the scrotum in warm water (44°±1° C) for short periods of time for 8 weeks on the structure and function of testes in albino rats and monkeys was investigated.

Both the rats and monkeys showed loss of weight of testes, atrophy of tubules and degeneration of the germinal epithelium. Judged on the basis of reproductive performance, the rats were found to be infertile. The epididymal smears of the monkeys showed no spermatozoa.

Suspension of immersion treatment for 6 weeks in the rats resulted in partial regeneration of germinal epithelium of the testes.

INTRODUCTION

The process of spermatogenesis has been found to be efficient at a temperature slightly lower than that of the interior of the body and the location of the testicles in the scrotal pouch serves this purpose efficiently. Several investigators have shown that exposure of testes to temperatures above that of the scrotum for a few to several minutes results in the degeneration of the germinal epithelium and failure of spermatogenesis in rats, guinea-pigs, rabbits, sheep, dogs and cats (Fukui, 1923 [quoted by Macleod & Hotchkiss, 1941]; Moore, 1924 [quoted by Macleod & Hotchkiss, 1941]; Moore & Oslund, 1924; Young, 1927). Fukui further showed that exposing the scrotas of rabbits for 10 min to sunlight resulted in a selective destruction of germinal epithelium. The general conclusion from all these experiments was that the germinal epithelium showed degenerative changes immediately and the effect of this damage was felt in the form of a decline in fertility after several weeks.

Clinical conditions such as cryptorchidism, varicocele and hydrocele have also been reported to interfere with the heat loss of testes and consequently spermatogenesis (Hanley, 1960). Artificially induced hyperpyrexia even for short periods is known to cause depression in sperm production which takes several weeks to return to normal (Macleod & Hotchkiss, 1941).

However, these experiments do not provide any information on the effects on the testes of repeated temperature stress for short periods of time daily for prolonged periods and the effects on the gonads after suspension of the heat treatment. This is of some significance because the constant repetition of a stress could bring about an adaptation on the part of the tissues and the
resulting reaction could be completely different from that produced by the
application of the same stimulus once. Further, from the standpoint of applica-
tibility to human subjects it is the effect of repeated exposures of short duration
that would require investigation. In the following study, the effect of immersion
of the scrotum in warm water (44° ± 1° C) daily for short periods on spermatogenesis and reproductive performance of rats and monkeys was studied.

MATERIALS AND METHODS

Healthy male albino rats and monkeys (Macaca radiata) were used in this
investigation.

ALBINO RATS

Sixteen rats of body weight 80 to 100 g were divided into two groups. The six
animals in Group 1 (Nos. 1 to 6) were subjected to an abdominal operation
to put the testes inside the abdominal cavity. Without interfering with the
blood supply, the testes on both sides were pulled up and anchored indepen-
dently to the abdominal wall to simulate the condition of cryptorchidism. Ten
animals in Group 2 (Nos. 7 to 16) had their testes, hind legs and lower third of
the abdomen immersed in warm water daily. A perforated aluminium cylinder
with two perforated lids at either end was used for this purpose. An animal was
placed inside each cylinder, the lid replaced and the cylinder was suspended
vertically in the warm bath so that the scrotum was completely under water.
There was plenty of room for the rat to breathe and relax during this period.
The animals were subjected to this treatment for 10 min twice daily, morning
and evening. The temperature of the bath was maintained at 44° ± 1° C
throughout the immersion. At the end of 10 min, the rats were removed from
the cylinder, the scrotum, lower part of the abdomen and the hind legs dried
with a towel and the animals placed in separate cages. The bath was repeated
6 days a week for 8 weeks.

At the end of 8 weeks, three rats from Group 1 (Nos. 1, 2 and 3) and four
from Group 2 (Nos. 7, 8, 9 and 10) were killed and the testes removed for histological examination. The remaining male animals in the two groups were
each mated with two female adult rats (body weight 150 to 200 g) from the
stock colony. The eighteen female rats used for mating were proved to be
fertile on the basis of their earlier reproductive performance in the stock colony.
After 7 days' mating period, the males and females were separated and the
latter kept in individual cages to determine pregnancy. All the three male
rats in Group 1 (Nos. 4, 5 and 6) and two animals from Group 2 (Nos. 11 and
12) were killed at the same time and the testes removed for histological examina-
tion. The remaining four male animals in Group 2 (Nos. 13, 14, 15 and 16)
were killed 5 weeks after mating or 6 weeks after the suspension of the im-
mersion treatment and the testes removed for histological examination.

MONKEYS

Five adult male monkeys of body weight 4·0 to 4·5 kg were used in this experi-
ment. Under anaesthesia, both the testes were removed from one of the monkeys
(M1) for histological examination, and the animal discarded. The other four monkeys (M2, M3, M4 and M5) had their testes immersed in the warm bath daily. Each monkey was placed in a special wooden cage built for this purpose and the cage was immersed in the bath so that the testes, the gluteal region, the proximal half of the thighs and the lower abdomen were immersed in the bath. The animal was free in the cage and while it could turn round in the vertical axis the arrangement did not permit the animal to climb up. The monkeys were subjected to this treatment for 20 min daily. The temperature of the bath was maintained at $44^\circ \pm 1^\circ$ C throughout the immersion. At the end of 20 min, the animal was transferred to its original cage. This treatment was given 6 days a week for a total period of 8 weeks. At the end of the period, the testes on both sides were removed, under anaesthesia, for histological study from all the animals, and the animals were sacrificed.

Throughout the investigation, the rats and the monkeys received stock colony diet and water ad libitum. The maximum shade temperature in the animal house ranged between $32^\circ$ C to $35^\circ$ C throughout the investigation. The normal mean rectal temperatures of the rat and the monkey were found to be $37.1^\circ$ and $38.5^\circ$ C, respectively.

RESULTS

GENERAL CONDITION OF THE ANIMALS AND BODY WEIGHT

The animals, both rats and monkeys, appeared healthy and active throughout the period of the study. The mean gains in body weight of the rats of Groups 1 and 2 at the end of 8 weeks were 166 g and 152 g, respectively, and they were comparable to those of stock colony male rats of a similar age group. It was concluded that the operation of orchidopexy and the warm water immersion of testes did not have any effect on the weight gain of rats. The weight of the monkeys, on the other hand, remained stationary throughout the period of the experiment. The same was also the case in a large group of stock colony adult monkeys.

SIZE OF TESTES

The testes weights at the time of killing the animal were not recorded in rats. However, it was observed that the abdominal testes of the animals subjected to orchidopexy (Group 1) were the smallest, while those of the animals of Group 2 were only half the size of those of stock colony normal males of similar body weight. The testicular size continued to remain the same in four animals (Nos. 13, 14, 15 and 16) even after discontinuing the immersion treatment for 6 weeks.

The weight of both testes of the normal control monkey (M1) killed at the commencement of the experiment was 10.6 g. The difference in weight between the two testes was negligible. The weights of the testes of the experimental monkeys (M2, M3, M4 and M5) at the end of the 8th week of immersion treatment were 3.4 g, 2.5 g, 5.1 g and 5.3 g, respectively. The maximum difference between the weights of the individual testes was observed in monkey M2; it was only 0.3 g.
REPRODUCTIVE PERFORMANCE

This was tested only in rats. All the female rats mated with animals of Group I failed to conceive. Out of the twelve female rats mated with animals of Group 2, only one became pregnant. The female animals were observed for a total period of 40 days before declaring them as 'not conceived'. At the end of this period, the seventeen test female animals were mated again with nine stock colony male adult rats to establish their fertility. It was observed that all became pregnant and gave birth to young.

EXAMINATION OF EPIDIDYMAL SMEARS FOR SPERMATOZOA

In the case of the monkeys, epididymal smears were made at the time of removal of testes and were examined for spermatozoa. Spermatozoa were absent in the smears of all the experimental monkeys (M2, M3, M4 and M5).

HISTOLOGICAL APPEARANCE OF THE TESTES

The testes of the rats were fixed in Bouin's fluid and those of the monkeys in Zenker's fluid. The sections were stained with haemotoxylin-eosin. The alterations in the histological appearance of the organs in the experimental animals were assessed by comparing them with sections of testes obtained from male controls of similar body weight.

Albino rats

Sections of testes of all experimental animals except Nos 13, 14, 15 and 16 showed generalized atrophy of the tubular epithelium with complete absence of spermatogenic activity. There was marked depletion of germ cells in several tubules and in a few even Sertoli cells were absent. The interstitial cells were preserved.

Sections of testes of animals Nos. 13, 14, 15 and 16, which were killed 6 weeks after suspension of the immersion treatment, showed a nearly normal tubular pattern. There was evidence of regeneration of germinal epithelium in the form of layers of cells in several tubules. The interstitial cells were preserved.

Monkeys

As in the case of the rats, the tubular epithelium showed generalized atrophy with total absence of sperm-cell formation. The spermatogonia were atrophic in several tubules and in some had completely disappeared. The tubules showed only Sertoli cells close to the basement membrane. The atrophic changes were most marked in monkey M2. No relationship between weight of testes and histological appearance was observed. The interstitial cells were preserved. The histological examination of the testes indicated that the atrophic changes observed in the rats were of greater severity than those seen in the monkeys.

DISCUSSION

The results of the present investigation have shown that immersion of the scrotum of albino rats and monkeys in a warm bath (44° ± 1° C) even for short periods of time daily for several days produced severe atrophy of the
testes. The atrophy seemed to affect only the seminiferous tubules leading to sterility of the animal. This treatment, however, did not affect the interstitial cells which probably continued to function adequately. Glover (1956) reported that in large animals no loss of libido occurred as a result of the elevation of scrotal temperature. The greater severity of lesions observed in the rat testes might be due to the longer period of exposure given to these animals in relation to their life span. It is also possible that in the case of rats the exposure was carried out twice a day even though the total duration of exposure was the same in rats and monkeys. It is also likely that the difference observed in the degree of testicular damage between the rat and monkey in this study might be a species difference. If it is so, then it raises the important question of species variation, an observation that has not received attention in the earlier studies.

The mechanism of the tubular atrophy is not clear at present. If the atrophic changes are mediated through changes in blood supply, it is difficult to explain why the degenerative changes are exclusively confined to the tubular epithelium. The direct injury to the tubular epithelial cells due to transference of heat across the different layers of scrotum and testes is another possible mechanism that may have to be considered. The rise in environmental temperature of the scrotum and the creation of conditions preventing the organ from reacting to this increased temperature by compensatory mechanisms appeared to be the cause of this atrophy.

The significance of the observation made on rats and monkeys for human testicular function and reproductive performance remains to be assessed. It was stated earlier that certain clinical conditions affecting the testes result in low sperm count and decreased spermatogenesis in man. Akira Wantabe (1959) studied the sperm count in semen in unmarried healthy volunteers after repeatedly immersing the scrotum in a water bath at 43° C to 47° C. It was not possible to ascertain the duration of immersion on each occasion. Exposure daily for 6 to 12 days was ‘followed by a steep rise in sperm count 2 to 3 weeks later, followed by a precipitous decrease in count which persisted up to 9 weeks’. This, in turn, was followed by a ‘sharp rise well above pre-treatment level with a gradual return to normal level’. He also found that such treatment given for 3 consecutive days at 3- or 4-weekly intervals kept the sperm count at a very low level. In all the subjects, the count returned to a normal level ultimately. It appeared, therefore, that the human testes, if immersed for short periods in warm water as in the present experiment, would probably behave similarly to those of the experimental rats and monkeys. The improvement in the histological appearance of the testes of the rats 6 weeks after suspension of the immersion treatment indicated that the atrophic changes induced in the testes were probably reversible. It is possible, however, that prolongation of the immersion treatment for several days more might induce irreversible changes in the tubules. The significance of these observations on the larger question of male subfertility and sterility in man remains to be assessed.

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