HISTOLOGICAL AND HISTOMETRICAL STUDIES OF TESTICULAR HYPERTROPHY FOLLOWING HEMICAstration OF ALBINO RATS

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Summary: This project was designed to study the effects of hemicastration in male albino rats of different ages and weights. Significant compensatory hypertrophy was seen in young rats (5 and 20 days old). The concentration of seminiferous tubules was profoundly reduced while the tubular diameter was increased in young hemicastrated rats. The weights of the kidneys were increased while the weights of the adrenal glands were not different in hemicastrated young rats. These histological and histometrical changes may be associated with specific endocrine activity.

Key words: hemicastration sham-operated immature rats testicular hypertrophy seminiferous tubules

INTRODUCTION

It has been demonstrated that unilateral castration of immature rats results in hypertrophy of the remaining testes suggesting an alteration of a possible dynamic relationship which exists within the hypothalamo-pituitary-testicular axis (2). The presence of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) in the serum and urine of immature bovine and human species has been reported (3). Cunningham and co-workers (1) reported that a dynamic relationship exists within the hypothalamo-pituitary-testicular axis. Hemicastration has been shown to raise the concentration of L.H. and F.S.H. in immature male rats (4). In this paper, the effects of hemicastration on both histological and histometrical parameters of testicular hypertrophy in the remaining gonad of male albino rats are reported.
MATERIAL AND METHODS

Male Wistar rats were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The animals were maintained on rat feed and water ad libitum. Rats were arranged into six groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Operation</th>
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<tbody>
<tr>
<td>I</td>
<td>Sham-operated at 5 days</td>
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<tr>
<td>II</td>
<td>Hemicastrated at 5 days</td>
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<tr>
<td>III</td>
<td>Sham-operated at 20 days</td>
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<td>IV</td>
<td>Hemicastrated at 20 days</td>
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<tr>
<td>V</td>
<td>Sham-operated at 180 days</td>
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<td>VI</td>
<td>Hemicastrated at 180 days</td>
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Under halothane (Fluorothane; May and Baker) anaesthesia, removal of the left testes was performed through an abdominal incision in 5 days old rats and under phenobarbitone sodium (30 mg/kg, ip) a scrotal incision in both the 20 days and 180 days was made. Rats were then sacrificed 10 days after unilateral castration. To determine the critical age for performing hemicastration, some rats were also hemicastrated at varying ages (at 10, 30 and 120 days) and killed 30 days after surgery. In some experiments the right testes were removed instead of the left testes but the results were the same in each case.

Testicular weights:

Testes were weighed on a meter electrical balance. Testicular dry weights were obtained by placing the testes in an oven containing calcium carbonate. Oven temperature was maintained at 60°C until the weights were constant.

Histology:

Testes in each group were processed for histological and micrometric studies. Testes were fixed in Zenker-formol solution. These were then processed and embedded in paraffin. Paraffin sections were stained with haematoxylin and eosin. Six sections from the central portion of each testis was studied histologically. The studies were coded so that histometrical interpretation was made without the knowledge of the group from which the sections were made.

Statistical analysis:

Micrometric analysis and micrometric scale, for the number of tubules per unit area, the seminiferous tubules were classified depending on the histometrical study:

- (1) High sperm concentration
- (2) Medium sperm concentration
- (3) Low sperm concentration

A comparison was made in adult rats between the groups.

The weight of the remaining testes of 180 days was significantly greater (P<0.001) when compared to the weight of the remaining testes of 20 days of age. Hemicastrated testes had a significant increase in diameter compared to the remaining testes of 20 days of age. Hemicastrated testes had a significant increase in diameter compared to the remaining testes of 20 days of age. Hemicastrated testes had a significant increase in diameter compared to the remaining testes of 20 days of age.
Hemicastration in Male Albino Rats

Hemicastration was performed using micrometric grid; micrometric eye piece and micrometric scale, for measuring the diameters of seminiferous tubules; and counting the number of tubules per unit area (mm²).

In adult rats besides measuring the diameters of tubules and their numbers per unit area, the seminiferous tubules were divided into three categories (on a double blind study) depending on the population of spermatozoa as:

1. High sperm concentration seminiferous tubules (>200 per tubule)
2. Medium sperm concentration seminiferous tubules (100 - 200 per tubule)
3. Low sperm concentration seminiferous tubules (<100 per tubule)

A comparison was made between sham-operated and hemicastrated rats.

Statistical analysis:

Statistical analysis was performed using the student - t - test for two means. In each age group, the sham-operated rats were used as controls for comparison.

RESULTS

The diameters of seminiferous tubules of the remaining testes of hemicastrated rats were significantly (P<0.001) increased when compared to those of control rats (Fig. 1). The increase in diameter (124-146%) was only observed in rats hemicastrated at 5 and 20 days of age. Hemicastration of rats at 180 days did not influence the diameter of the tubules.

The concentration of tubules per unit area was profoundly reduced (P<0.001) in the remaining testes of hemicastrated rats (Fig. 2). The drastic reduction in the number of tubules per unit area (44-51%) was only demonstrated in rats hemicastrated at 5 and 20 days of age. Hemicastration of rats at the age of 180 days had no effect on tubular concentration.

The weight of the remaining testes in the hemicastrated rats was significantly increased (P<0.001) when compared to testicular weight of control rats (Fig. 3 a and b). The weight increase (46-122%) was only observed in rats hemicastrated at 5 and 20 days of age. Hemicastration at the age of 180 days had no influence on testicular weight. On the other hand, the weight of seminal vesicles of such hemicastrated rats was drastically decreased when compared to the controls.
Influence of hemicastration on seminiferous tubular diameter using albino rats. \( p < 0.001 \), student - t - test, \( n = 8 \) per histogram. Vertical bars represent standard errors of mean.

Table I summarises the results obtained in this study, and histological section of tubules of six groups (I to VI) are shown in Plate I. Hemicastration had no effect on the weight of adrenal gland but insignificantly increased the weight of kidney. However, tubular diameter, tubular concentration and testicular weight (germinal cells, Sertoli cells and interstitial cells), were affected profoundly by hemicastration in group II and IV.

The rats which were hemicastrated at 10 days and 30 days showed compensatory testicular hypertrophy similar to those hemicastrated at 5 and 20 days of age. On the other hand, rats hemicastrated at 120 and 180 days showed no compensatory testicular hypertrophy.

**DISCUSSION**

The results indicate that compensatory changes occur in the remaining testes of hemicastrated male rats, over and above the normal development at this maturational phase.

Fig. 1: Influence of hemicastration on seminiferous tubular diameter using albino rats.

Fig. 2: Influence of hemicastration on number of tubules per unit area (mm²) using albino rats.
The data also show that the compensatory changes, namely increase in weight of testes, occur mainly during the period of active testicular development when the animals were still young, and undergoing maturational changes. Such data imply that the age is critical.

In the remaining tests of hemicastration at this maturational phase.

Fig. 2: Influence of hemicastration on the number of seminiferous tubules per unit area using albino rats.

* = $P < 0.001$, student - $t$ - test.

** = $P < 0.05$, student - $t$ - test, $n = 8$ per histogram. Vertical bars represent standard errors of mean.
Fig. 3a: Effect of hemicastration on the weight of the remaining testes using 5 days old albino rats.

* = $P < 0.001$, student - t - test

$n = 8$ per histogram. Vertical bars represents standard errors of mean.

Fig. 3b: Effects of hemicastration on the weight of remaining testes using 20 days and 180 days old albino rats.

* = $P < 0.001$, student - t - test, $n = 8$ per histogram. Vertical bars represents standard errors of mean.
Plate 1: Testes fixed in Zenker-formol and stained with haematoxylin-eosin. Magnification of all the sections in plate was X312. Cross sections of seminiferous tubules from:

1 : 1 5-days old sham-operated rats.
1 : 2 5-days old hemicastrated rats.
1 : 3 20-days old sham-operated rats.
1 : 4 20-days old hemicastrated rats.
1 : 5 180-days old sham-operated rats.
1 : 6 180-days old hemicastrated rats.

Vertical bars represent

(b) testes using 5 days old albino rats.
TABLE I: Body and organ weights of sham-operated (S.O.) and hemicastrated (H.C.) rats. The values quoted are arithmetic means of eight animals ± standard error of mean. Groups I & II, III & IV, and V & VI represent 5 days, 20 days and 180 days old rats respectively being their ages prior to either sham-operation or hemicastration.

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<td>Weights</td>
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<td></td>
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<td></td>
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<tr>
<td>Body (g)</td>
<td>12.88</td>
<td>± 0.30</td>
<td>13.30</td>
<td>± 0.30</td>
<td>68.00</td>
<td>± 1.00</td>
<td>68.40</td>
<td>± 1.00</td>
<td>305.00</td>
<td>± 16.00</td>
</tr>
<tr>
<td>Testes (g)</td>
<td>0.009</td>
<td>± 0.001</td>
<td>0.02</td>
<td>± 0.001*</td>
<td>0.37</td>
<td>± 0.10</td>
<td>0.540</td>
<td>± 0.10*</td>
<td>1.21</td>
<td>± 0.80</td>
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<tr>
<td>Tubular diameter (μ)</td>
<td>63.30</td>
<td>± 0.80</td>
<td>156.00</td>
<td>± 1.30*</td>
<td>131.00</td>
<td>± 1.00</td>
<td>293.00</td>
<td>± 1.80*</td>
<td>7.60</td>
<td>± 0.10</td>
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<tr>
<td>Tubules per unit area (mm²)</td>
<td>258.00</td>
<td>± 11.60</td>
<td>145.00</td>
<td>± 1.00*</td>
<td>85.00</td>
<td>± 0.50</td>
<td>42.00</td>
<td>± 0.70**</td>
<td>25.00</td>
<td>± 0.50</td>
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<tr>
<td>Adrenal gland (mg)</td>
<td>1.60</td>
<td>± 0.10</td>
<td>1.40</td>
<td>± 0.10</td>
<td>10.00</td>
<td>± 0.10</td>
<td>10.00</td>
<td>± 0.10</td>
<td>25.00</td>
<td>± 1.00</td>
</tr>
<tr>
<td>Kidney</td>
<td>59.00</td>
<td>± 0.10</td>
<td>62.00</td>
<td>± 0.10</td>
<td>380.00</td>
<td>± 10.00</td>
<td>370.00</td>
<td>± 7.00</td>
<td>930.00</td>
<td>± 30.00</td>
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* = P < 0.001, student t-test
** = P < 0.05, student t-test
in the reaction of the remaining testes to hemicastration. These results conform with
the findings of Cunningham and his co-workers (3). Our proposal is further strengthened
by the graded effects observed with increase in age until the 180 day old rats in which no
compensatory effects were observed in the remaining testes after hemicastration.

In agreement with the increased diameter of seminiferous tubules in hemicastrated
rats, tubular concentration per unit area decreased significantly (<0.001). This result
was not unexpected since increased tubular diameter would consequently lead to a decrease
in the number of tubules that can be accommodated per unit area. Figure 2 shows
that hemicastration at 5 and 20 days of age produced a profound reduction in tubular
concentration while hemicastration at 180 days of age had no effect on tubular concent-
ration.

The observation that hemicastrated rats were heavier than control rats is of interest.
This observation was however, non-significant statistically. Such an increase in body
weight may be related indirectly to the activity of growth hormone resulting from hemi-
castration.

There was no observable change in the weight of the adrenal glands of sham-
operated rats as compared to hemicastrated rats. This observation conforms with the
results of Shallerbarger (5). Nonetheless, such an observation does not rule out the
possibility of a profound functional alteration in the activity of adrenocortical hormones.

In addition, the kidneys of hemicastrated rats were heavier than those of sham-
operated rats. The result may be attributable to the effect of growth hormone released
as a result of hemicastration. However, the observation may also be due to a possible
trophic functional connection between the testes and the kidneys.

Studies are in progress to elucidate any relationship between the histological
observations and hormonal activities in the rat and this will be the subject of a further
communication.

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REFERENCES


Summary: Lung studies on forty-five healthy and non-smoking, economically and nutritionally healthy North Indian males were worked out by using 30 healthy subjects in the age group of 20-30 years with a mean body weight of 49.1 kg, height of 170.5 cm. Mean of the TI of 30.8±3.2 ml/kg weight was more stable and did not show any regional difference. As compared to North Indian males, the published results were quite comparable.

The present study on the systematic variations of lung expiratory volumes (TV, RV and FRC) and peak expiratory flow rates (PEFR) when compared with those of the lungs (TI) were quite comparable, more stable and dependable. As compared to North Indians, the results of the lung indices such as TI, TV, RV, PEFR and FRC should serve as reference norms for cardiopulmonary investigations in other different socio-economic groups.