THE INFLUENCE OF OVERCROWDING ON SPERMATOGENESIS, SIZE OF LEYDIG-CELL NUCLEI (HISTOMETRICAL INVESTIGATION), AND THE ADRENAL CORTICOSTERONE CONTENTS IN MICE

By

K. Gärtner, H. Reznik-Schüller and G. Reznik

ABSTRACT

Adult male NMRI-mice were kept alone or in groups of 5, 10, 20, 30, 40 and 60 animals for 28 days and were then sacrificed. The corticosterone levels of their adrenals were determined and their testes were examined histometrically. The corticosterone levels of the adrenals increased two-fold with increasing population size. Furthermore the increased population size caused a suppression of some testicular functions: the seminiferous tubules decreased up to 20% and the relative frequency of the spermatids up to 10% whereas the numbers of spermatocytes and spermatogonias increased correspondingly. A decrease of about 25% of the testosterone dependent postmeiotic stages of spermatogenesis is assumed. Since the diameters of the Leydig-cell nuclei decreased about 20%, a reduction of the testicular testosterone secretion can be projected. All measurements proved the group with 5 animals to be the most favourable group size.

Many contradictory results concerning the influence of stress on gonadal function have been reported (literature review, Giuliani 1969). The literature indicates that social stressors may cause effects different from those induced by physical or pharmacological ones. Therefore, the interpretation, which is
Table 1.
Experimental conditions and histometrical results of the testes in NMRI-mice that were kept for 28 days in groups of different sizes.

<table>
<thead>
<tr>
<th>Animals per cage</th>
<th>Square dimension of the cages (cm)</th>
<th>Number of animals</th>
<th>Body weight at end of experiment (g)</th>
<th>Weight of both testes absolute (g)</th>
<th>Weight of both testes relative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Start</td>
<td>End</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>21 × 10</td>
<td>20</td>
<td>20</td>
<td>35.9 ± 2.5°</td>
<td>0.19</td>
</tr>
<tr>
<td>5</td>
<td>21 × 10</td>
<td>30</td>
<td>29</td>
<td>35.8 ± 2.7</td>
<td>0.21</td>
</tr>
<tr>
<td>10</td>
<td>23 × 17</td>
<td>40</td>
<td>37</td>
<td>36.2 ± 2.7</td>
<td>0.23</td>
</tr>
<tr>
<td>20</td>
<td>39 × 23</td>
<td>60</td>
<td>51</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>30</td>
<td>55 × 33</td>
<td>60</td>
<td>48</td>
<td>35.3 ± 2.3</td>
<td>0.23</td>
</tr>
<tr>
<td>40</td>
<td>55 × 33</td>
<td>40</td>
<td>29</td>
<td>35.2 ± 2.4</td>
<td>0.21</td>
</tr>
<tr>
<td>60</td>
<td>55 × 33</td>
<td>60</td>
<td>30</td>
<td>32.8 ± 2.5</td>
<td>0.20</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation.

often given for the effects of stress has to be modified in order to explain accurately the consequences of social stress. The present investigations contribute to this explanation by giving an analysis of organs susceptible to the corticotrophic and gonadotrophic hormones.

It is known that with an increase of population density, the concentration of corticosterone in serum and adrenals of rats and mice is augmented while fertility decreases (Calhoun 1962, 1965; Davis 1951; Christian 1959; Dickson 1964). Maternal influences on the pre- and post-natal developments have been detected earlier to cause these changes. A damage to male fertility, by increased population size was also suggested by the decreased weights of the testes, glandulae praeputiales and glandulae vesiculares (Brain & Novell 1971; Christian et al. 1965). Snyder (1967) proved by means of breeding experiments that the reproductive capacity of male animals is diminished when living in groups.

In the present investigations, the influence of increasing social stress on the germinative and endocrine functions of testes was examined by quantitative histometrical analysis of the germinative epithelium and the Leydig-cells. In addition, the changes of the adrenal corticosterone levels were determined.
Diameter of tubules (µm) | Tubular tissue (%) | Interstitial tissue (%) | Diameter of round Leydig-cell nuclei (µm) | Spermatides: Spermatocytes
---|---|---|---|---
179.6 ± 15.4 | 64.5 ± 15* | 10.5 ± 2.5* | 5.73 ± 0.45* | 1.40 ± 0.15*
N = 125
187.7 ± 15.4 | 73.5 ± 10 | 11.5 ± 5.0 | 5.78 ± 0.29 | 1.57 ± 0.16
N = 125
173.5 ± 11.9 | 64.0 ± 9 | 12.7 ± 5.0 | 5.55 ± 0.31 | 1.35 ± 0.13
N = 125
164.4 ± 10.8 | 62.0 ± 15 | 10.6 ± 3 | 5.09 ± 0.32 | 1.37 ± 0.15
N = 125
169.5 ± 9.9 | 74.0 ± 13 | 9.5 ± 1.5 | 5.36 ± 0.29 | 1.23 ± 0.16
N = 125
171.7 ± 10.2 | 75.5 ± 15 | 10.0 ± 3.0 | 5.29 ± 0.32 | 1.50 ± 0.12
N = 125

**Material and Methods**

Mature male NMRI mice (NMRI Orig/Kisslegg) were divided into different groups and caged in different Makrolon® cages (Table 1, columns 1 and 2) for 28 days. They were kept under the following conditions: light period from 9 p.m. to 9 a.m. 22 ± 2°C, 50–60% relative humidity, pelleted diet (RMH, Hope Farms®) and water *ad libitum*. On the 28th day, the animals were killed with chloroform at 9 a.m. The corticosterone content of the adrenals was determined according to the method described by Gärtner & Bonath (1971). Immediately after death, body weights and weights of the testes were determined; the testes were fixed in Bouin’s solution and embedded in paraffin. Five testes of each population were selected randomly for the investigations. Six histological sections (5 µm) were taken from the middle part of each testes. Hellbach (1970) reported that quantitative histometrical results do not differ in central and marginal sections of testes. The sections were stained by haematoxylin and eosin.

Determination of the ratio between the volume of the seminiferous tubules and the volume of interstitial tissue was as follows: with the integration plate II (100 points) of the integration counter (Zeiss) 300 points per testis were determined from 3 different fields of view (magnification 63 x). The 25% of points that fell upon areas without interstitial tissue (caused by fixation) were counted as “free areas”.

Determination of the volumes of Leydig-cell nuclei was accomplished by placing on one ocular tube of the microscope an integration counter (with integration plate II)
and on the other a screw-ocular micrometer (K8 Zeiss). The diameter of Leydig-cells that were determined by the points were measured with the micrometer; in round cells, one diameter and in oval cells, two perpendicular diameters (magnification 800 ×). The volumes of the cells were calculated with \( V = \frac{4}{3} \pi a b^2 \) (a = longer radius, b = shorter radius). Twenty-five diameters of round seminiferous tubules/testis were measured from tunica propria to tunica propria with the screw-ocular micrometer (magnification 180 ×).

Determination of the frequency distribution of the different types of germ cells was according to the method described by Krause (1972). 4 × 250 germ cells/testis were counted with the integration plate I (25 points and magnification 1000 ×) and classified according to Leblond & Clermont (1952a,b). The resting spermatocytes which hardly differ from the spermatogonias B were counted as spermatogonias B. Detailed descriptions for all methods are reported by Reznik (1972) and Schüller (1972).

**RESULTS**

The mortality increased with increasing population size (Table 1). In the groups with 40 and 60 animals, 28 % and 50 %, respectively, died before the end of the experiment. Autopsy did not reveal infections as a cause of death. A comparison between these two groups and the group of smaller population, can therefore be regarded only with mental reservation.

The solitary-living animals gained 0.33 g body weight/day which was similar to the weight gain of mice in population of 5, 10, 30 and 40 animals. This weight gain differed significantly \( (P < 0.05) \) from the group of 60 animals with 0.17 g body weight/day. The absolute and relative weights of the testes are demonstrated in Table 1, column 5. Only the relative weights of the testes in the solitary-living mice showed significant \( (P < 0.02) \) differences as compared to the group with 30 animals.

As indicator of the testicular endocrine function, the percentages for the interstitial tissue and the diameters and volumes of Leydig-cell nuclei were determined. The percentage of the interstitial tissue did not change with increasing populations (Table 1, column 8). Table 1, column 9, compares the diameters of round Leydig-cell nuclei (150/group) of the examined populations. The diameters decreased with increasing population size. The one way analysis of variance demonstrated a significant difference \( (P < 0.01) \) between the group with 30 mice and those with 5, 10 and 1 animal. The diameters of the round Leydig-cell nuclei did not differ significantly among the groups with 5, 10 and 1 animal; but a certain trend can be seen in Fig. 1a. In order to elucidate this trend, the volumes of all types of Leydig-cell nuclei were determined. The frequency distribution of these volumes (Fig. 2a–2f) demonstrated that the population with 5 animals had the biggest Leydig-cells and that with increasing size of population, small Leydig-cells became more frequent. An integration of the frequency distribution graphs shows a decrease of the cell
The influence of population size upon: a The diameters of the Leydig-cell nuclei (μm).
b The square dimensions of the seminiferous tubules as a measure for their volumes (μm² × 10³). c The relative numbers of the spermatocytes (empty columns) and of the spermatids (striped columns in per cent). d The relative numbers of the spermatogonias A and of the spermatogonias B (plus resting spermatocytes) in per cent.

e The adrenal corticosterone levels (μg/animal).

Fig. 1.
Fig. 2.
Volumes of the Leydig-cell nuclei in the various populations. Abscissa: Volumes of nuclei in $\mu m^3$. Ordinate: Number of cells. a Separately caged mice. b Populations of 5 mice. c Populations of 10 mice. d Population of 30 mice. e Population of 40 mice. f Population of 60 mice.

volume of more than 20% in groups with 30 animals as compared to those with 5 animals.

The influence of an increasing size of population on the germinative functions of the testes was determined by the diameters of the seminiferous tubules, the percentage of the tubular tissue and by the frequency distribution of the various types of germ cells. The diameters of the seminiferous tubules
decreased with increasing size of population (Table 1, column 6). All differences between the groups with one, 5, 10 and 30 mice were significant \((P<0.01)\). From the present values one can calculate that the square dimension of the tubules has decreased about 25\% in the groups of 30 mice as compared to those of 5 animals (Fig. 1b).

A marked influence of overcrowding on the germinative function of the testes was found in their percentage of spermatids and spermatocytes. It caused a decrease in the number of spermatids and therefore a relative augmentation of the spermatocytes (Fig. 1c). The one way analysis of variance demonstrated significant differences \((P < 0.01)\) of the spermatids/spermatocytes quotients (Table 1) between the groups with 1, 5, 10 and 30 animals. The slight increase in the number of spermatogonias A and spermatogonias B (including resting spermatocytes) however, which occurred with increasing populations, could not be statistically proven (Fig. 1d).

The function of the adrenal cortex was determined through measurement of the corticosterone level of the adrenals. With increasing population size the corticosterone levels showed a significant increase \((P < 0.001)\). These results were described in detail by Gärtner & Bonath (1971).

**DISCUSSION**

An integration of the present values should be made by comparing only the groups with 5, 10, 20 and 30 animals. The solitary mice suffered a change of the entire endocrine system caused by "isolation stress". According to Sigg et al. (1966) such stress becomes evident in mice after only 20 days of "tactile isolation". This thesis is supported by the high standard deviations of the corticosterone levels in the adrenals of the solitary mice. In the groups with 40 and 60 animals only 50–70\% survived the experiment. Possibly mice low in rank died from social stress.

The present investigations have demonstrated an injury to the endocrine and germinative functions of the testes as a result of increasing population size and consequently, increasing social stress. Similar observations were reported by Christian et al. (1965) and Brain & Novell (1971), who found a decrease in the weights of testes and accessory glands with augmenting population size.

To a certain extent, the present findings are contrary to those of Neill (1970), Dunn et al. (1972) and Ajika et al. (1972) who reported an increase in the secretion of ACTH as well as LH and prolactin from hypophysis after a stress caused by narcosis. The change of the testes that was found in the present investigations is definitely the consequence of a decreased supply with gonadotrophins.

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The present investigations demonstrate a decrease of spermatogenesis in socially stressed animals; but the number of all types of germ was not affected in the same way. The quantitative observations demonstrate an increase of spermatogonias (spermatogonias A and B + resting spermatocytes) from 6 to 9% in the group with 30 mice as compared to those with 5 animals \( (P < 0.05) \), whereas the spermatids decreased by one tenth. On the other hand, the seminiferous tubules were 20% smaller in the population with 30 mice than in those with 5 animals; the decrease of tubules appears to be the result of a loss of spermatids and consequently of spermatozoas, too; whereas the earlier stages of spermatogenesis remained stable and seemed to increase relatively only in number because of the loss of spermatides. The decrease by one tenth in the number of spermatids must be considered in relation to the marked decrease in size of the seminiferous tubules. The relative decrease of 7% in the number of spermatids, therefore, indicates an absolute decrease of about 15–30% and would quantitatively agree with the findings of Snyder (1967) in electro-ejaculate experiments.

In this review of the hormonal regulation of spermatogenesis, Steinberger (1971) found that the reductive division (late spermatocytes) takes place only when stimulated by testosterone. Since in the present investigations all stages of spermatogenesis beyond the meiotic division decreased, a diminished production of testosterone may be assumed. However, from these experiments one cannot speculate that the maturation of spermatids from stage 16–19 (Leblond & Clermont 1952a,b), which is regulated by FSH, was also reduced. Several investigators (Christian et al. 1965; Brain & Novell 1971) have proposed that a suppression of the peripheral supply with androgens in socially stressed animals causes a decrease in weight of the accessory glands. But it is still unknown whether the synthesis of this hormone is reduced in the adrenal glands or in the Leydig-cells or both (Kniewald et al. 1971). The present investigations demonstrate a decrease of the volumes of the Leydig-cell nuclei up to 20% with increasing population size. According to several investigations, the volumes of the Leydig-cell nuclei indicate the grade of their secretory activity (Murakami & Tonutti 1966; Sajonski & Smollich 1968). The present findings demonstrate a decrease of the endocrine function of the testes in the larger populations even before the weights of the testes are diminished (Fig. 2). The inhibition of the maturation of spermatids seems to be caused by insufficient androgenic activity. The reasons for the depression of testicular androgenic synthesis are not clear, different opinions are discussed. Christian et al. (1965) or Tisell & Angervall (1969) supposed that the adrenal secretion of testosterone is stimulated by ACTH. So increased adrenal testosterone secretion may cause depression of ICSH-release and testicular synthesis of androgens.

On the other hand Kniewald et al. (1971) discussed that activation of the adrenals possibly causes a lack of testosterone precursors which are also syn-
thesized in the adrenals, resulting in a depressed synthesis of androgens in the testes.

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