EFFECTS OF ANTIFERTILITY SUBSTANCES ON MALE JAPANESE QUAIL

PAMELA JONES, EVA KOMINKOVA AND H. JACKSON

Unit of Reproductive Pharmacology, University of Manchester

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Summary. The effects of several compounds of established antifertility action in male rodents have been investigated in male quail.

A short period of infertility soon followed administration of oral doses of triethylenemelamine and trimethylphosphate. Triethylenemelamine, aziridinyl urea and ethylenedimethanesulphonate by the intraperitoneal route led to infertile egg production for several weeks.

Differences between rats and quail regarding effective dose and length of sterility are discussed.

INTRODUCTION

The usefulness of Japanese quail (Coturnix coturnix japonica) for many types of biological research has been repeatedly demonstrated. Its advantages include hardiness, ease of handling, short life cycle, early sexual maturity and abundant egg laying.

Over the past few years, quail have been frequently used for observations of the effect of chemosterilants upon reproductive performance. These investigations involved the use of large mixed groups (about twenty birds), and compared the breeding capacity of controls and treated birds when the chemicals were administered in the diet (Shellenberger, Skinner & Lee, 1967; McFarland & Lacy, 1969). The present study describes the effects of various compounds, which have an established antifertility action in male rodents, upon the reproductive processes of male Japanese quail.

METHODS

A small flock of quail has been reared and maintained under controlled conditions of lighting and temperature as described elsewhere (Padgett & Ivey, 1959; Miles, 1966; Lansdown, Crees & Wilder, 1970). In contrast to previous studies using insecticides, it has now been found quite convenient to use small groups of birds for each experiment. Three or four sexually mature males (aged between 3 and 6 months), housed separately, each with two females, provide a satisfactory group for treatment, with the same number as controls. During a pretreatment period of 3 to 4 weeks, birds were selected for high fertility by routine incubation and examination of the daily egg output. In a small flock, this selection necessarily limits the number of quail available for experimental work but the high
production of fertile eggs compensates to some extent for the small number of males used.

Compounds were administered orally (p.o.) or intraperitoneally (i.p.), by a routine of single doses or five doses daily, without unnecessary separation of the birds housed together. Weekly changing of females as in the serial mating tests applied to rodents (Bock & Jackson, 1957) proved both unacceptable to the male bird and unnecessary. Toxicity tests, with histological investigations of effects on the testes, were first performed in order to provide information about a practicable and effective dose level.

During and after a course of treatment, egg collection was maintained and continued for a minimum period of 5 weeks. Eggs, appropriately marked, from 2-day collections were incubated, 50% being opened after 7 days for rapid assessment of drug action, the remainder after 15 days’ incubation. In these, embryos would normally have completed organogenesis so that detection of abnormalities was facilitated. For analysis of data, eggs were classified as fertile, abnormal or non-fertile. Abnormal eggs comprised those in which development was retarded so that embryos were smaller at 7 or 15 days, or showed visible external abnormalities.

A brief antifertility effect may only be seen when egg fertility data are plotted from consecutive 2-day periods; when the effect is more prolonged, a weekly assessment is adequate. The fertile egg yield is also conveniently expressed as a percentage of the corresponding control figure. Besides the fertility test procedure, additional treated male birds were killed at intervals and a smear made from the ductus deferens to assess its sperm content and their motility. Testes were also removed, weighed and fixed in Bouin’s fluid for histological observation.

Four different types of antifertility compound have been so investigated. The first of these, trimethylphosphate (TMP), was given in five daily doses of 250 mg/kg p.o. The next two were aziridine compounds, tretamine (TEM) and N-ethyleneurea (EU). Two dose levels of TEM were used, one group receiving a single dose of 5 mg/kg p.o., and the second group, 2 mg/kg i.p. Similarly, EU was administered to two groups of birds as five daily doses of 20 mg/kg or 10 mg/kg i.p.

Finally, ethylene-1,2-dimethanesulphonate (EDS) was given as a single dose of 200 mg/kg or 150 mg/kg i.p. An additional group received testosterone propionate (TP, 2 mg/bird i.p. in arachis oil for 10 consecutive days) and one dose of EDS (200 mg/kg i.p. on Day 5). Control groups were injected with TP or arachis oil. The purpose of this experiment was to investigate a possible protective action of TP over the effect of EDS, such as occurred in the rat (Jackson, 1971).

RESULTS

Control data were very similar in each experiment, with the percentage of fertile eggs remaining at a high level throughout and relatively small numbers of abnormal eggs.

The maximum tolerated dose of TMP (5 x 250 mg/kg p.o.) produced a
rapid decline in the percentage of fertile eggs during 10 days from the first dose, followed by a short but well marked sterile phase lasting about 7 days, and a return to control fertility levels by 25 days (Text-fig. 1). No spermatozoa

![Graph](image)

**Text-fig. 1.** Brief sterilant effect on male quail of oral trimethylphosphate (five daily doses of 250 mg/kg).

were observed in the smear from the ductus deferens at autopsy on Day 11 (Table 1), which is consistent with their absence in the testis sections at this time (Pl. 1, cf. Figs. 1, 2, 3 and 4).

Oral TEM (5 mg/kg) caused a rapid, short-term sterilant effect so that between Days 3 and 8 after treatment, no fertile eggs were produced (Text-fig. 2). Testis sections were normal in appearance. The injected, lower dose (2 mg/kg) again produced a short-term sterilant effect and a second sterile phase during Weeks 3 to 5. These birds then remained subfertile until the end of the experiment (Text-fig. 2). This result illustrates the fact that aziridines are relatively ineffective by the oral route. Histologically, 7 days after injection, considerable reduction in the cell population of the testis was apparent, although spermatozoa were still present. After 4 weeks, eggs were still non-fertile but
Table 1
THE EFFECT OF VARIOUS ALKYLATING CHEMICALS ON THE TESTIS AND REPRODUCTIVE CAPACITY OF MALE QUAIL

<table>
<thead>
<tr>
<th>Compound and dose</th>
<th>Infertile period (s)</th>
<th>Days from first dose</th>
<th>Average body weight (g)</th>
<th>Average testis weight (g)</th>
<th>Sperm. motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td>127 (2)†</td>
<td>R. 1.45</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L. 1.41</td>
<td>+ +</td>
</tr>
<tr>
<td>TMP 250 mg/kg p.o.*</td>
<td>10 to 17</td>
<td>11</td>
<td>118 (4)</td>
<td>R. 1.44</td>
<td>Aspermic</td>
</tr>
<tr>
<td>TEM 5 mg/kg p.o.</td>
<td>3 to 8</td>
<td>6</td>
<td>120 (3)</td>
<td>R. 1.32</td>
<td>+ +</td>
</tr>
<tr>
<td>TEM 2 mg/kg i.p.</td>
<td>6 to 10</td>
<td>7</td>
<td>115 (6)</td>
<td>R. 1.04</td>
<td>+ +</td>
</tr>
<tr>
<td></td>
<td>20 to 38</td>
<td>28</td>
<td>122 (2)</td>
<td>R. 0.81</td>
<td>+</td>
</tr>
<tr>
<td>EU 10 mg/kg i.p.*</td>
<td>Subfertile</td>
<td>5 to 30</td>
<td>110 (4)</td>
<td>R. 0.88</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L. 0.96</td>
<td>+</td>
</tr>
<tr>
<td>EU 20 mg/kg i.p.*</td>
<td>10 to 36</td>
<td>21</td>
<td>111 (4)</td>
<td>R. 0.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L. 0.47</td>
<td>±</td>
</tr>
<tr>
<td>EDS 150 mg/kg i.p.</td>
<td>8 to 40</td>
<td>21</td>
<td>132 (3)</td>
<td>R. 0.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L. 0.38</td>
<td>–</td>
</tr>
<tr>
<td>EDS 200 mg/kg i.p.</td>
<td>8 to 47</td>
<td>21</td>
<td>113 (2)</td>
<td>R. 0.40</td>
<td>Aspermic</td>
</tr>
</tbody>
</table>

* Five consecutive daily doses.
† Number of birds given in parentheses.

EXPLANATION OF PLATES
Figures show sections of quail testis. × 50 and × 125.

PLATE 1
Figs. 1 and 2. Low and high magnification of control testis. Normal arrangement of spermatogenic epithelium; note the characteristic 'cluster' formation of spermatozoa in the lumen.
Figs. 3 and 4. Low and high magnification of testis, 11 days after the first dose of TMP (five doses of 250 mg/kg p.o.) Normal arrangement of spermatogenic cells in all tubules but the clusters of mature spermatozoa are absent.

PLATE 2
Figs. 5 and 6. Low and high magnification of testis, 7 days after injection of TEM (2 mg/kg i.p.). Spermatogenic cells greatly reduced in number. Some sperm clusters are still present.
Figs. 7 and 8. Low and high magnifications of testis, 28 days after injection of TEM (2 mg/kg i.p.). Some tubules contain only a ring of Sertoli and spermatogenic cells. Otherwise the epithelium is of normal appearance.

PLATE 3
Figs. 9 and 10. Low and high magnifications of testis, 21 days after first dose of EU (five doses of 20 mg/kg i.p.). Most tubules contain a few Sertoli cells only.
Figs. 11 and 12. Low and high magnification of testis, 21 days after injection of EDS (200 mg/kg i.p.). Many tubules contain a few Sertoli cells only, whilst the remainder have one or more layers of spermatogenic cells.
most of the tubules were fully spermatogenic (Pl. 2, Figs. 5 to 8). No empty tubules were seen at any stage.

After EU (5 x 20 mg/kg i.p.), eggs were non-fertile between Weeks 2 and 5, with subfertility on either side of this period. Again, the subfertile state persisted until the end of the experiment. After 3 weeks, a depletion of the spermatogenic epithelium was seen in most of the tubules (Pl. 3, Figs. 9 and 10). The testis weight was also reduced, being about one third that of the control (Table 1). At the lower dose of 5 x 10 mg/kg i.p., no sterile phase occurred, fertility fluctuated throughout the whole experimental period, and after 3 weeks the testis histology appeared normal.

Both dose levels of EDS (200 and 150 mg/kg i.p.) produced similar effects on fertility. A rapid decline in the number of fertile eggs occurred during the 1st week, followed by a period of infertile egg production during Weeks 2 to 6, with a gradual return to normal between Weeks 6 and 9 (Text-fig. 3). After 3 weeks, the testes were very small in both experiments (Table 1). Approximately 50% of the tubules at this time contained a nondescript population of spermatogenic cells (Pl. 3, Figs. 11 and 12).

In a comparable experiment in which EDS treatment (200 mg/kg i.p.) was given on Day 5 of a course of 10 daily injections of testosterone propionate (2 mg/bird i.p.), the pattern and length of the sterile phase was identical to the
group receiving EDS alone. In parallel control (arachis oil) and TP groups, fertility remained unaffected throughout the experiment (Text-fig. 3). The above experiments are summarized in Table 1.

**DISCUSSION**

Following treatment, the fertility patterns emerging from the present study appear to be comparable to those seen in rodent experiments (Jackson, 1964). The phases of sterility which occur at different times after treatment undoubtedly reflect drug action on different stages of the spermatogenic process. Unlike the rat, however, the overall timing of spermatogenesis and its stages have not been established in the quail, and therefore any attempt to correlate drug action with particular spermatogenic cell types is rather speculative. While quail differ from rats in having abdominal testes and less distinct epi-
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didymal development, histologically the structures of rat and quail testes are very similar.

From the experiments described, the conclusions are that antispermatogenic effects in quail qualitatively resemble those produced in rodents, but, not unexpectedly, there are some differences in susceptibility. Thus, rats given TMP (5 x 100 mg/kg p.o.) became sterile in a period of 3 and 4 weeks from commencing treatment, and in 2 to 5 weeks at a higher dose level (5 x 250 mg/kg) (Jackson & Jones, 1968; Jackson, 1970). In quail, however, this dosage only produced a short sterile phase (Days 10 to 17). A surprising finding was that in the quail the sterile phase coincided with absence of morphologically mature spermatozoa in testes which otherwise appeared normal (Pl. 1, Figs. 3 and 4) and with lack of spermatozoa in the ductus deferens. No complex epididymal system is present in this species. In the rat, the sterility induced by TMP is of the ‘functional’ type, with normal testis histology except at near-lethal doses, when wholesale destruction of the seminiferous epithelium occurs and prolonged sterility ensues (Jackson, 1970). In both quail and rat, therefore, it is evident that the most sensitive target cells are late testicular spermatids and spermatozoa.

In general, the antispermatogenic effects of the aziridines (TEM and EU) are similar in rat, mouse and quail. The spermatogenic epithelium can be severely damaged but sufficient spermatogonia may survive to repopulate the epithelium and restore fertility. As in the rat (Jackson, 1966), a biphasic antifertility effect is seen in quail after injected TEM, corresponding to a differential action on pre- and post-meiotic stages.

In rats, after EDS (100 mg/kg i.p.), sterility commences in the 2nd week and lasts for 2 to 3 months. All stages of spermatogenesis are thus involved except ‘Week 1’ spermatozoa. In quail, the above dose showed little effect histologically, although the higher doses of 150 and 200 mg/kg i.p. were effectively antispermatogenic and produced prolonged sterility. On the basis of fertility studies in rats, testosterone protects post-, though not pre-meiotic cells against the action of EDS (Jackson, 1971). No such protective action occurred in quail. It is interesting that testosterone has been reported as ineffective in supporting the seminiferous epithelium in hypophysectomized quail (Baylé, Kraus & van Tienhoven 1970), although capable of so doing in the rat (Boccabella, 1963; Clermont & Harvey, 1965).

The above comparison between rat and quail fertility illustrates species difference both in effective dose levels and period of sterility. In all experiments, the length of the sterile period in quail is short compared with the rat. A possible explanation may be that, in abdominal testes, spermatogenesis proceeds more rapidly at the higher body temperature and metabolic rate. The relative duration of the post-testicular phase in the two species has also to be considered. Assessments made following the labelling of cock spermatozoa with radioactive phosphate suggested that the transit time for spermatozoa through the epididymis and ductus deferens was 4 days (Takeda, 1969). The minimum time for the primary spermatocyte to be converted into spermatozoa was assessed at 12 days, i.e. 16 days in all, a process which may take about 6 weeks in the rat.

F
It is interesting that, in the present study, α-chlorohydrin (3-chloropropane-1, 2-diol) had no effect on quail fertility at the maximum tolerated dose (5 × 100 mg/kg p.o.); in the rat, at low doses, it is an epididymal sterilant (Ericsson & Baker, 1970). Moreover, there was no evidence in the quail of spermatocoele formation and permanent sterility such as occurs in the rat following a single oral dose of 100 mg/kg (Ericsson, 1970). This emphasizes the possibility of developing discriminative chemosterilants for rodents, birds and other vertebrate pests.

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REFERENCES


