ESTIMATION OF THE DURATION OF SPERMATOGENESIS IN JAPANESE QUAIL, *COTURNIX COTURNIX JAPONICA*, USING ANTISPERMATOGONIAL CHEMICALS

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(Received 15th May 1972, accepted 26th June 1972)

A recent publication has demonstrated the usefulness of Japanese quail in the detailed investigation of male antifertility chemicals (Jones, Kominkova & Jackson, 1972). The present study describes the effect on quail fertility of various dose levels of two compounds, busulphan (Myleran) and cyclohexane-1,4-diol dimethanesulphonate. Because of the established antispermatogonial actions of these substances in the rat, it was anticipated that an estimate of the duration of spermatogenesis in the avian species would emerge from the present work.

The fertility procedure adopted for these experiments has recently been described (Jones *et al.*, 1972). Myleran, suspended in arachis oil, was administered to groups of four male birds as a single intraperitoneal dose or daily for 5 consecutive days. Cyclohexane-1,4-diol dimethanesulphonate (Jackson, 1971) was administered orally on 5 consecutive days in the same vehicle. Following the procedure described (Jones *et al.*, 1972), eggs were collected daily from each group of mated birds (two females per male) and incubated. The fertility of the males was assessed from the results of opening the eggs on Days 7 and 15.

For both compounds, consistent effects occurred on the fertility pattern, varying in intensity according to the dose used. After single doses of Myleran (40, 30 or 20 mg/kg intraperitoneally), the fertility of the males declined to about 30% of the control value between Day 4 and Day 7 after treatment, followed by a rapid rise to levels approaching or equal to those of controls. This initial decrease was probably due to a functional sterilizing action upon spermatozoa or spermatids (Jackson, 1964). A second antifertility effect later developed with the region of minimal fertility between Days 25 and 27 (Text-fig. 1).

Following the administration of five daily doses of the compound, a similar biphasic pattern of egg fertility occurred, the higher dose level (5×10 mg/kg) leading to sterility of the eggs collected on Days 26 to 28 inclusive (Table 1). At autopsy, the testes of male birds similarly treated were of minimal size during Weeks 2 and 3 after the two higher single treatments with Myleran and in Week 3 after five daily doses (10 mg/kg), although spermatozoa were still seen in seminal smears from some males.

TEXT-FIG. 1. Effect on egg fertility of single intraperitoneal doses of Myleran to male Japanese quail (four birds per dose level): x, 40 mg/kg; o, 30 mg/kg; ●, 20 mg/kg.

TABLE 1
ANTISPERMATOGONIAL EFFECT OF SULPHONIC ESTERS IN JAPANESE QUAIL MANIFEST BY EGG-INFERTILITY

Compound and dose* (mg/kg)	Percentage of fertile eggs (Controls = 100%)															
	Days from first dose															
	1 to 2	3 to 4	5 to 7	8 to 9	10 to 11	12 to 14	15 to 16	17 to 18	19 to 21	22 to 23	24 to 25	26 to 28	29 to 30	31 to 32	33 to 35	36 to 39
Busulphan																
10	106	69	9	13	53	75	125	80	74	77	22	0	8	31	41	47
5	114	60	33	58	69	85	125	100	95	86	43	59	110	106	90	100
Cyclohexane-1,4-diol dimethane-sulphonate																
30	78	94	11	27	60	75	76	117	35	8	0	0	0	0	11	32
20	100	61	23	11	40	72	100	63	75	36	15	6	31	43	86	82

* Five daily doses in each case, four birds per experiment.

Both courses of cyclohexane-1,4-diol dimethanesulphonate induced a Myleran-type effect on quail fertility (Table 1). After the higher dose level (5×30 mg/kg), the second phase of low fertility was associated with sterility between Days 24 and 32. After the lower dose treatment, the antifertility action was again most marked between Days 26 and 28 from the first dose.

In the male rat, the sterilising effect of Myleran has been correlated with a selective lethal action on the spermatogonia (de Rooij & Kramer, 1968; Kramer & de Rooij, 1970). Over the dose range of 4 to 10 mg Myleran/kg (Jackson, Fox & Craig, 1961), the duration of spermatogenesis was assessed to be between 9 and 10 weeks as judged by the onset of sterility with aspermia. A similar estimate was obtained in the rabbit on the basis of an acute phase of aspermia in Week 10 following a single dose of 40 mg Myleran/kg (Fox, Jackson, Craig & Glover, 1963), and a later study in the rabbit using tritiated thymidine supported this view (Swierstra & Foote, 1965). Despite the initial spermatogonial damage, the remaining spermatogenic cells continue to develop into spermatozoa, which accounts for the continuation of normal fertility until the supply of cells from these sources is exhausted.

Like the rabbit, mice tolerate much larger doses of Myleran (e.g. 40 mg/kg intraperitoneally) and the main antifertility effect resembles that seen in the rat (Jackson, 1966). A brief initial sterilizing effect was evident in the mouse after this dose. The onset of sterility in the 6th week from treatment is also consistent with the estimate of 42 days for the duration of spermatogenesis from studies following radiation-induced damage or the use of tritiated thymidine (Oakberg, 1956).

In the rat and mouse, it was recently shown that cyclohexane-1,4-diol dimethanesulphonate produced a similar sterilizing effect to that of Myleran (Jackson, 1971). A brief inhibitory action on fertility occurred in both rats and mice during Week 2 from the given dose of this compound, followed by recovery and later onset of sterility in Weeks 6 and 8 in mice and rats, respectively.

The biphasic antifertility effect found in Japanese quail after both Myleran and the cyclohexane-1,4-diol dimethanesulphonate shows a consistent resemblance to that obtained in rodents, the major action of the compounds again being directed against the spermatogonia. Like the mouse, rabbit and guinea-pig, the Japanese quail is more resistant than the rat to these compounds. The present data provide an estimate of about 25 days for spermatogenesis and sperm transit time through the ductus deferens in Japanese quail. According to Mather & Wilson (1964), mature spermatozoa are being produced by Japanese quail 36 days after hatching. Spermatogonia were first observed in this species about 11 days after hatching (Purcell, 1970). This information provides an alternative estimate of 25 days (36 minus 11 days), for the duration of spermatogenesis, which is in close agreement with the findings in the current work.

This work was carried out with the support of a grant from the Ford Foundation.

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