THE EFFECTS OF EXOGENOUS GONADOTROPHINS AND EGG REMOVAL ON CLUTCH SIZE IN THE DOMESTICATED CANARY, *SERINUS CANARIUS*

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IT is well known that not all species of birds react to egg removal in the same manner. "The term 'indeterminate layer' is commonly used to indicate species in which the number of eggs laid will vary according to the conditions in the nest, while the term 'determinate layer' indicates those species in which the number of eggs laid appears to be a more rigid and constant product of internal processes in the bird" (Lehrman, 1959).

An extreme example of indeterminate laying is the Flicker (*Colaptes auratus*) observed by Phillips (1887), which laid 72 eggs in 73 days in response to egg removal from the nest. (See Lehrman, 1959, for a review with other examples.) Brant (*Branta bernicla hrota*) and Snow Geese (*Chen caerulescens*) are determinate in normally laying four–five eggs. This clutch size decreases if environmental factors cause egg laying to begin later than the optimal time. It is known (Barry, 1960) that only a certain number of ovarian follicles develop in these geese and that follicles will atrophy under unfavorable breeding conditions. In the African parrot genus *Agapornis* only three–eight eggs are laid, although the ovary shows a graded series of follicles (Dilger, 1960), a condition that would seem to give the genus a potential for indeterminate clutches. It is clear that very different factors are affecting determinism in these several species.

The increasing use of the Canary (*Serinus canarius*) in physiological and behavioral research (*e.g.*, Hinde, 1958; Hinde and Warren, 1959; Vaugien, 1948; Warren and Hinde, 1959, 1961) made it seem pertinent to obtain some information on clutch size and the effect of egg removal on clutch size in this species. If clutch size is wholly or in part determined at the pituitary level by regulation of gonadotrophin production, one would expect gonadotrophin injections to affect clutch size in some way. Therefore supplementary information on the effects of exogenous gonadotrophin on clutch size also seemed relevant.

METHODS

Canaries were obtained from commercial breeders and laparotomized to determine sex. Pairs were then formed randomly (by drawing numbered slips of paper from a box), and each pair was housed in a perched cage constructed of ½-inch hardward cloth and measuring 25 × 40 × 48 cm (10 × 16 × 19 inches). Water, Canary seed mixture, conditioning food, cuttlebone, and gravel were given *ad libitum*. The cage floors were covered

with paper to permit easy cleaning. All birds were given eight hours of light per day until 27 February, when the photoperiod was lengthened to 14 hours. On 14 March 10 × 10 × 5 cm (4 × 4 × 2 inches) nesting cups made of ¼-inch hardward cloth were provided; sharp edges were covered with masking tape. Cotton and horse hair were furnished as nesting material. The birds could see and hear other pairs at all times.

The birds were divided randomly into six groups of five pairs each. Two experiments were conducted; and in each case two variables—hormone injections and egg removal—were investigated. Pregnant mare serum (PMS; GONADOGEN, Upjohn Company) was used as a source of gonadotrophins. During the second experiment hormone injections were reversed, so that a bird receiving PMS in the first experiment would receive normal saline in the second and vice versa, allowing those birds that laid eggs in both experiments to serve as their own controls. Injections were made in the pectoralis muscles of the females only. In those groups that were not allowed to keep full clutches, the eggs were removed on the day of laying.

In experiment A the treatment was begun on the day the first egg was laid. All pairs were treated at the same time because of the assumption, based on laparotomy, that they were in approximately the same physiological state. The initial injection was 250 International Units (I.U.) PMS, followed by injections of 100 I.U. PMS every three days until several days after the last egg was laid by any given pair. Controls were injected with an equal volume of saline on the same schedule, which extended from 14 March through 22 April. Since the first female to lay (marked with a plus sign in Table 1) responded differently than all the other females in the PMS groups, a second experiment was designed.

In experiment B the treatment was begun for each female on the day her first egg was laid. Doses were 35 I.U. PMS every day (or an equivalent volume of saline) until several days after the last egg was laid. Inclusive dates are 23 April through 25 May.

RESULTS AND DISCUSSION

The data are presented as clutch sizes in Table 1. Although the breeding success was small (40 per cent of the females laid in experiment A and 20 per cent in experiment B), the results were rather uniform, and we therefore feel justified in drawing the following conclusions:

1. Egg removal had no effect on clutch size. This concurs with the results of field studies on the Tricolored Red-wing, *Agelaius tricolor* (Emlen, 1941), in which clutch size was not increased by removal of eggs during the laying period. Field observations (Estiot, 1929; Beraut, 1932; Dupont, 1944) indicate that clutch size in wild Canaries may vary from three to

TABLE 1

EFFECTS OF EGG REMOVAL AND PMS INJECTIONS ON CLUTCH SIZES IN CANARIES

Experiment	*Treatment*	*Clutch size*
A	All eggs left + PMS	1, 1, –, –, –
	No eggs left + PMS	1, 3†, –, –, –
	One egg left + PMS	1, 1, 1, –, –
	All eggs left + saline	3, –, –, –, –
	No eggs left + saline	3, –, –, –, –
	One egg left + saline	3, 2, 4, –, –
B	All eggs left + PMS	3, –, –, –, –
	No eggs left + PMS	3*, –, –, –, –
	One egg left + PMS	–, –, –, –, –
	All eggs left + saline	2, 3*, –, –, –
	No eggs left + saline	–, –, –, –, –
	One egg left + saline	2, 3*, –, –, –

Each treatment group consisted of five pairs.

* A second clutch.

† This was the first female to lay, initiating the injection treatment for experiment A. Since she was injected only after her first egg, her treatment is comparable to that of the females in experiment B and her response is identical to that of the latter (see text).

five eggs. Vaugien (1948) reports that domesticated Canaries normally lay three clutches of three to four eggs in a season. Our clutch sizes generally fall within this range, except, of course, where PMS decreased clutch size (see below). Vaugien (1948) obtained eight clutches and a total of 25 eggs from one female by removing completed clutches, but he did not experiment with removal of eggs within a clutch period.

2. PMS decreased clutch size when given before the first egg was laid. In chickens (*Gallus gallus*) PMS causes follicular growth but inhibits ovulation (see Fraps, 1955, for a review). Ovaries were not examined during the laying period because laparotomy would probably have interrupted the laying sequence, but results in terms of clutch size indicate inhibition of ovulation. It would be worthwhile to repeat this experiment and record follicular size at different points in the cycle. Vaugien (1955) reports induction of egg laying in the fall and/or winter in *Passer domesticus, Carduelis carduelis, Chloris chloris,* Canaries, and buntings (species not given) through PMS injections. The ovary may be particularly sensitive to gonadotrophin level before the first egg is laid, and excess hormone could be inhibitory. The smallest total dosage with which Vaugien produced egg laying in winter House Sparrows was 1,200 I.U. PMS compared with our dosage of about 750 I.U. PMS. However, the background level of endogenous gonadotrophins in winter is much lower than in the breeding season so that our smaller dosage may have actually brought the total gonadotrophin level beyond that of Vaugien's birds in winter. No attempt was made to measure the endogenous gonadotrophin level.

3. Does PMS affect clutch size when the treatment is started on the day the first egg is laid? The PMS birds in experiment B and the first bird to lay in experiment A had normal clutch sizes. These are, however, only three birds, and the two in experiment B received PMS on a different injection schedule than the bird in experiment A (see above). It is possible that the inhibitory effect of our dosage of PMS was lost after the first egg was laid due to some change at the ovary and/or pituitary level, but it is also possible that the difference in injection treatment effected a disinhibition, despite the reaction of the first bird to lay in experiment A.

The fact that we were not able to increase clutch size with PMS indicates that at least some of the factors controlling clutch size involve peripheral and/or central nervous mechanisms that need not act only through the anterior pituitary gland.

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SUMMARY

Breeding Canaries were subjected to several controlled treatments involving removal of eggs from the nest and gonadotrophin (PMS) injections.

1. Egg removal had no effect on clutch size.
2. PMS decreased clutch size when given before the first egg was laid.

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