PAIRING RESPONSES OF FREE-LIVING VALLEY QUAIL TO SEX-HORMONE PELLET IMPLANTS[1]

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GREAT progress has been made in recent years in analyzing the complex behavioral changes which occur during the normal breeding cycle of wild birds. The function of the endocrine system in this cycle has been investigated in certain domesticated species (Berthold, 1849; Hamilton and Golden, 1939; Carpenter, 1932; Shoemaker, 1939b; and others) and in at least one wild species held in captivity (Noble and Wurm, 1940), but obstacles have heretofore stood in the way of an experimental approach to the study of wild birds in the field.

Until recently, hormone-injection studies have required frequent treatments throughout the experimental period. This has been an insurmountable handicap to students of wild-bird behavior, as the repeated trapping and handling of the subjects, aside from practical difficulties, introduces a disturbance variable of considerable magnitude. The development of the hormone-pellet technique (Deansely and Parkes, 1937; Mark and Biskind, 1940) should prove of great value to studies with wild birds. Small pellets of compressed crystalline hormones, implanted subcutaneously, have been shown to absorb slowly and supply hormone to the blood stream over a period of a month or more. The behavioral responses of wild birds to introduced hormones may thus be studied in the field for an extended period with a minimum of disturbance to the subjects.

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The California Valley Quail (*Lophortyx californica vallicola*) is a sedentary species well adapted to individual study. Quail on the University of California Farm at Davis have been followed closely since 1936 with the use of colored markers applied to legs and tail, and a technique for tracing individual life histories through field observation has been developed (Emlen, 1939). Certain of these birds were used as the subjects for the present experimental study during the winter of 1940–41.

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Normal pairing behavior.—During the winter months Valley Quail at Davis are sexually inactive and are grouped into flocks or coveys of from 5 to 35 individuals. The first traces of sexual activity appear in the latter part of February when, by sustained and repeated watching of color-marked birds, the beginning of pair formation may be detected. The bonds are loose at first but become more definite early in March. Both members of a pair seem to share in the formation and maintenance of the relationship. The male, however, is generally the more attentive and alert of the two, holding his head higher, feeding less and letting the female lead the way (Emlen, unpublished data). As pairing behavior progresses, fighting between the males increases and the 'peck order' of social dominance becomes more definite (Emlen and W. E. Howard, MS). Copulation may be observed in an abortive form during any month of the year, but does not become frequent and definite until the latter part of March.

The experiments described in this paper were designed to test the behavorial response of free-living quail to sex hormones administered in pellet form during the winter season of sexual inactivity.

EXPERIMENTAL

Two experiments were carried out on a single covey of marked birds. In each a few individuals were treated with an androgen (testosterone) and a few with an estrogen (stilbestrol); the remaining birds in the covey were left undisturbed as controls. Sustained observations were made at frequent intervals during the ensuing six weeks.

Experiment 1 (Table 1) was started on November 29, and continued through January 10. Testosterone pellets were implanted subcutaneously in two males and one female, and stilbestrol pellets in three females on the first day. Four males and two females were left untreated as controls. The first unusual behavior appeared in the morning of the third day, approximately 45 hours after treatment, when each of the two testosterone-treated males (401 and 412) became pugnacious toward other males and started aggressive advances toward females 452 and 455, respectively. These females, the former untreated, the latter treated with stilbestrol, were passive except for occasional attempts to escape. Other females remained passive and unaffected. Untreated males reacted to the increased pugnacity in the stimulated males by retiring a short distance; thus the covey was somewhat scattered during the day. Aggressive behavior subsided rapidly on succeeding days as the attended females submitted

and began to follow rather than evade their mates. On the sixth day, however, one of these females (455) suddenly left her mate and the flock and flew to some brush about 200 yards distant. The attending male (412) started in pursuit but failed to overtake her. He then isolated himself from the covey and after the ninth day assumed the crowing habit typical of unmated males in breeding condition.

The testosterone-treated female (466) showed no unusual behavior until the sixth day, when she was observed pursuing an untreated male (442). During the next few days this female was absent from the covey and alone, but on the tenth day she was back and paired with male 442. The female seemed to be the more attentive member of the pair (i. e., assumed the male role) but this was not definitely ascertained. The term "male role" as used in this paper refers to the typical male pairing activity described above in the paragraph on normal pairing behavior. Pairing behavior was noted in two untreated 'control' males (441 and 403) on the thirteenth and fifteenth days, respectively.

No further developments of general importance appeared during the observation period. Unusual behavior waned after the sixth week and had practically disappeared by the beginning of the ninth week, when the second experiment was initiated.

Experiment 2 was started on January 22, and continued through March 4. Testosterone pellets were given to one male and two females, and stilbestrol pellets to one male and one female on the first and second days of the experiment (see Table 2). Six males and two females were left untreated as controls. The first unusual behavior appeared in the evening of the second day, about 30 hours after treatment, when the testosterone-treated male (413) became pugnacious toward other males. The latter, however, maintained their dominance and drove him to the edge of the covey. This male showed interest in a female on the third day but did not secure a mate until the eighth day. The mate (♀ 466), also a testosterone-treated bird, was then absent until the eleventh day, during which time the male exhibited the 'crowing' habit. On her return the female was paired with another bird (♂ 408) until he was artificially removed. She then returned to her previous mate (♂ 413).

The other female to receive testosterone (454) was a young bird that never paired in the first experiment. She was observed near male 416 (treated with stilbestrol) on the third, seventh and eighth days and was definitely paired with this male after the tenth day,

TABLE 1

EFFECT OF TREATMENTS WITH TESTOSTERONE AND STILBESTROL ON BEHAVIOR OF VALLEY QUAIL, EXPERIMENT 1

Numbers in italics refer to days after start of experiment;
T = testosterone; S = stilbestrol.
All implants were made on day *1* (Nov. 29, 1940)

Bird	*Age in months*	*Position in peck order*	*Treatment*	*First evidence of response*	*Modification of Behavior*
♂401	17	medium	T	*3*	*3*, actively pursued ♀ 452, aggressive toward other ♂♂; *4–8*, paired with ♀ 452; *8*, mate disappeared; *11*, last seen (alone).
♂412	30	high	T	*3*	*3*, actively pursued ♀ 455, aggressive toward other ♂♂; *4–6*, paired with ♀ 455; *6*, mate abruptly left, covey—gave unsuccessful chase; *6–23*, apart from covey, crowed after *9*; *32–41*, loosely paired with ♀ 466.
♂403	30+	medium	none	*15*	*14*, aggressive toward mated ♂ 441; *15*, paired with ♀ 455; *16–32*, absent with mate; *32*, returned alone.
♂411	30	low	none		No signs of unusual behavior.
♂442		medium	none	*10*	*6*, pursued by ♀ 466 and pursued ♀ 453; *10–17*, paired with ♀ 466 (female role), pugnacious towards other ♂♂; *17*, disappeared.
♂441		low	none	*13*	*13–32*, paired with ♀ 453.
♀466	2		T	*6*	*6*, chased ♂ 442; *7–9*, absent from covey; *10–17*, paired with ♂ 422 (male role); *21–41*, paired with ♂ 412.
♀455	40		S		*3*, passive to ♂ 412's attentions; *6*, leaves mate (♂412) abruptly on wing and evades him; *6–13*, alone; *15*, paired with ♂ 403; absent after *15*.
♀453	4		S		*6*, chased by ♂ 442; *7–13*, absent; *13–32*, paired with ♂ 441.
♀451	2		S		No unusual behavior; disappeared after *9*.
♀452	17+		none		*3*, passive to ♂ 401's attentions; *4–8*, paired with ♂ 401; absent after *8*.
♀454	2		none		No unusual behavior.

taking the pairing initiative (male rôle) at first. On the twelfth day she submitted to copulation and thereafter her 'maleness' seemed to wane.

Pairing behavior was noted in five of the six untreated 'control' males and in the stilbestrol-treated male between the tenth and

TABLE 2

EFFECT OF TREATMENTS WITH TESTOSTERONE AND STILBESTROL ON BEHAVIOR OF VALLEY QUAIL, EXPERIMENT 2

Numbers in italics refer to days after start of experiment;
T = testosterone; S = stilbestrol.
All implants were made on days *1* and *2* (January 22 and 23, 1941)

Bird	*Age in months*	*Position in peck order*	*Treatment*	*First evidence of response*	*Modification of Behavior*
♂413	6	low	T(1)	*2*	*2*, more pugnacious; *3*, retired from covey; 4–7, alone, calling; *8*, paired with ♀ 466; *9–10*, alone, crowing (mate not seen); *11–19*, absent; *20–43*, back, again paired with ♀ 466. Position in peck order not changed.
♂416	6	medium	S(*1*)	*12*	*3*, *7*, *8*, with ♀ 454, pairing not definite; *10*, definitely paired with ♀ 454 (female role); *12*, assumes initiative, copulates once; remains paired through *43*.
♂412	32	high	none (castrated 15)	*10*	*10*, paired with ♀ 462 (*15*, castrated, *16*, released); *17*, with mate but inattentive; *19*, at edge of covey, ostracized; slightly apart from covey (unpaired) through *43*.
♂411	32	low	none	*16*	*16*, paired with ♀ 462, incomplete copulation; *22*, again paired, and *25*, definite copulation; still paired *43*.
♂403	32+	low	none	*16*	*7*, possibly paired with ♀ 455; *16–43*, loosely paired with ♀ 455.
♂407	6	lowest (alien ♂)	none		*10*, joins covey; unpaired and unaccepted through *28*; absent after *28*,
♂408	6	medium	none (castrated 15)	*12*	*8*, possibly paired with ♀ 457; *12*, paired with ♀ 466 (*15*, castrated, *16*, released); *17–43*, at edge of covey, ostracized.
♂ Unm.		medium	none	*10*	*10–43*, loosely paired with ♀ 457; *10*, copulates.
♀454	4		T(*1*)	*10(3?)*	Paired with ♂ 416 (male role temporarily) (see ♂ 416 for details); *22–43*, lame, still paired.
♀466	4		T(*2*)	*8*	*8*, paired with ♂ 413; *9–11*, absent, alone; *12–14*, paired with ♂ 408; *20–43*, again paired with ♂ 413.
♀457	6		S(2)		*8*, possibly paired with ♂ 408; *10*, accepts copulation with ♂ Unm; remains loosely paired with ♂ Unm through *43*.
♀462	6		none		*10*, paired with ♂ 412; *16*, paired with ♂ 411; *17*, back with ♂ 412; *19–43*, again paired with ♂ 411.
♀455	42		none		See ♂ 403.

sixteenth days of the experiment. One of these birds, unpaired until the fifteenth day, immediately secured the female made available at that time by the artificial removal of a paired male. Single instances of copulation were observed in two of the controls and in the stilbestrol-treated bird.

DISCUSSION

Effect of testosterone.—Injections of male sex hormone have been shown to produce an advance in social status in low-ranking hens (Allee, Collias, and Lutherman, 1939), canaries (Shoemaker, 1939b), doves (Bennett, 1940), and night herons (Noble and Wurm, 1940) in captivity. Valley Quail males treated with testosterone in the present study, showed a marked increase in pugnacity and in pairing behavior, but apparently did not change their position in the peck order of the covey. Preliminary observations were not extensive enough to give a complete picture of the social hierarchy of the covey at the onset of the experiments; they were, however, adequate to give the approximate position of each bird in the peck order (peck right). One bird that ranked at or near the top before treatment maintained this dominant position throughout the experimental period. Another which had at least one untreated male above him failed to advance. A third, the only testosterone-treated male in experiment 2, stood near the bottom of the peck order. The marked aggressiveness that followed treatment produced no change in the status of this bird; each display of pugnacity only induced threats from dominant males with the result that he was driven to the edge of the covey. This bird remained in the same subordinate position throughout the six weeks of observation.

A peck-order response to androgen injections is probably a secondary reaction to the sexual stimulation produced. Furthermore, experiments with captive birds (various authors) and with the present, free-living quail suggest that artificial sexual stimulation activates the natural pairing and breeding behavior characteristic of the species treated. It therefore seems appropriate to examine the natural procedure of pair formation (sexual awakening) in the various species under consideration for a possible explanation of this discrepancy in social response to androgen injections. In many birds, including the night heron, dove and canary, males start the sexual (breeding) cycle by isolating themselves from the rest of the flock on mating territories where they become socially dominant; pair formation follows. In others, including the Valley Quail (cf. Lack, 1940), sexual activity starts with pair formation some time before the flock breaks up for

nesting. It is not difficult to see how the reaction to artificial sexual stimulation would be more disruptive to a flock of birds of the former category than to one of the latter.

Pairing activity appeared in testosterone-treated male quail coincident with the rise in pugnacity on the second day after treatment. With both cases in experiment 1 the male singled out a female in a manner not determined and suddenly assumed an aggressive behavior toward her, comparable in nature and intensity with that of unmated males toward mated females at the height of the breeding season. This aggressiveness subsided quickly and did not reappear so long as the birds remained paired. The change to a quiet behavior was apparently due to an acceptance of the male by the female, for after the initial aggressive phase had ended, females were at least partly responsible for keeping the pairs intact. Except as accidents interceded (male 412 in Table 1), this behavior continued without significant change until the effects of the implants disappeared about six weeks later.

This pattern of pairing behavior in the testosterone-treated males was similar to that observed in normal males during previous years with the exception of the initial aggressiveness. The sudden rise of sex-hormone activity induced by implants (Lorenz, Chaikoff and Entenman, MS.) is undoubtedly quite different from the gradual build-up of such substances at the beginning of the breeding season in Nature. It may well account for the intensity of initial reaction in the experimental birds.

Courtship (epigamic) and copulation did not appear in any of the testosterone-treated birds. This is interesting in view of the fact that copulatory behavior has been noted in wild undisturbed quail at Davis during every month of the year and was observed in one stilbestrol-treated male and two untreated males during the course of the present experiments. Shoemaker (1939b) showed that female canaries are capable of male copulatory behavior and yet, under the influence of testosterone propionate, may pair, court and attend their mates without ever showing it. Some further releasing factor, either internal or external, is apparently needed for the complete response in these birds.

The females reacted definitely to androgen implants, but their reaction was slower and apparently somewhat weaker than in males. Pugnacious behavior did not appear at all, and pairing behavior was slightly delayed. These females, however, took the pairing initiative that is normally assumed by the male. The treated female

in experiment 1 was observed on the sixth day, following or pursuing a male that was, in turn, pursuing another female. During the next three days she was absent from the covey by herself, a behavior rarely seen in females. On the tenth day, she was paired with the male she had been chasing four days before, and apparently was assuming the major rôle in keeping the pair together. Ten days later her mate disappeared and she was found loosely paired with another male (the initiative in this second pairing was not determined).

This same bird, given testosterone pellets in the second experiment two months later, continued to exhibit a restless behavior of the type observed in experiment 1. Another female treated with testosterone in the second experiment showed a similar delayed-pairing response. When first paired, this bird assumed the male rôle over her stilbestrol-treated mate, but on the twelfth day she submitted to copulation and thereafter became the less attentive of the two.

Authors are generally agreed that male sex hormone in female birds produces certain features of male courtship behavior. The present study shows that certain other traits may not be affected in the quail. More detailed studies with chickens have shown that only certain aspects of the psychological state are altered by gonad transplantations and that many fundamental attributes of behavior are unaffected (Finlay, 1925).

Effect of stilbestrol.—No direct behavior response was detected in any of the birds treated with stilbestrol. The acceptance of males as mates cannot be considered a response since it differed in no visible way from the corresponding behavior in untreated females. No indications of 'squatting' were noted, and only one of the three observed instances of copulation was with a stilbestrol-treated bird. The single stilbestrol-treated male (416) paired at about the same time as the untreated males. He was, it is true, less attentive than his mate at first, but this was probably due to the testosterone induced activity of the latter.

Lack of behavioral response to estrogens has also been reported for canaries (Shoemaker, 1939a) and night herons (Noble and Wurm, 1940). It should be noted, however, that in the chicken, Goodale (1916) has produced female behavior by transplanting ovaries into capons.

Effect of experiments on untreated birds.—Untreated birds were expected to retain normal behavior and thus serve as controls, permitting direct comparisons with treated individuals. Definite pairing behavior, however, appeared in eight of the ten 'control' males

between the tenth and sixteenth days of the experiments. In experiment 1, this occurred during the first part of December, more than two months earlier than pairing has ever been detected in five years of observation at Davis. In experiment 2 the reaction came about two weeks before natural pairing might be expected.

Pairing behavior in these untreated males differed from that in testosterone-treated birds only in lacking the initial phase of aggressiveness toward the female. In this respect it resembled normal pairing. One untreated male (411) showed traces of aggressiveness on acquiring the mate released by another male (412) on the sixteenth day. Due to the unbalanced sex ratio in the covey, no females had been available until this time, and this may have been reflected in his behavior. Unmated males are extremely aggressive toward mated females during the breeding season (Emlen, 1939).

Pairing in the normal, untreated males of the experimental covey was probably due to contagious behavior. Further studies on this reaction and on morphological effects that may accompany it are being planned.

SUMMARY

1. Compressed pellets of crystalline sex hormones (testosterone and stilbestrol) were implanted subcutaneously in wild Valley Quail at Davis, California during the non-breeding season. Follow-up observations were made in the field over a six-weeks' period in each of two experiments.

2. The three males treated with testosterone became pugnacious toward other males, but this had no effect on their position in the peck order of the covey.

3. Each of the three males and two females treated with testosterone pursued and then paired with a bird of the opposite sex. The female reaction was slower and less intense than that of the male. Courtship and copulation did not appear in these birds. Two males, upon losing their mates, assumed the crowing behavior typical of unpaired males during the breeding season.

4. The male and the four females treated with stilbestrol showed no behavioral response.

5. Untreated (control) males showed contagious behavior in eight out of ten cases by pairing off in the manner of treated males.

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