THE EFFECTS OF 22,25-DIAZACHOLESTEROL DIHYDROCHLORIDE ON THE PIGEON TESTIS AND ON REPRODUCTIVE BEHAVIOUR

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In recent years, attention has been focused on the potential of reproduction inhibitors for the possible control of harmful birds (Davis, 1961, 1962; Elder, 1964; Becker, 1966). Elder, in his search for an effective avian oral contraceptive, found that the best results were obtained with a hypocholesterolemic agent, 22,25-diazacholesterol dihydrochloride (compound SC-12937, Searle & Co., Chicago). When this constituted 0.1% of the diet of pigeons for 10 days, ovulation was inhibited for 3 months and impaired for a further 3 to 9 months. The present study was designed to investigate the effects of compound SC-12937 on the pre-incubatory behaviour of *Columba livia* and to assess the action of the drug on normal testis cytology.

Males and females in two groups, each of three pairs, and a third group of six pairs, were laparotomized, their gonads measured and seen to be in breeding condition. The individuals were kept in separate cages for a 3-day post-operative recovery period. The members of each pair were then allowed access to each other for 15 min to ensure that the males would all give the initial bowing display. Group 1 was then dosed with 30 mg SC-12937, administered in gelatin capsules, for 5 consecutive days during the 1st week and for 5 days more during the 2nd week. Group 2 birds similarly received five doses of 60 mg/bird in the 1st week, but at this point two birds (one male, one female) died from the effects of the drug and the remaining two pairs were given only two further doses during the 2nd week. The total dose/bird for each group was, therefore, 300 and 420 mg, respectively. Group 3 (six pairs) served as controls and were given only empty gelatin capsules. Observations on the behaviour of all birds began at the start of the 2nd week and continued for a week after dosing had ceased. The method used was that adopted by Fabricius & Jansson (1963), based on the techniques of Carpenter (1933) and Beach (1951), in which the birds were housed in individual cages and were only paired for $\frac{1}{2}$ hr daily. During this time a record was kept of certain activities and displays occurring in each 2-min period throughout the $\frac{1}{2}$ hr and also the frequency with which particular displays followed each other. However, on Days 6 and 7 the birds

were continuously paired and no observations took place. It has been established that, although paired only briefly each day, normal birds will go through all the stages of a complete pre-incubatory behaviour cycle, as if they had been continuously paired (Fabricius & Jansson, 1963).

All subjects were killed on the 14th day, after a total of ten $\frac{1}{2}$ hr observation periods for each pair. Gonads were measured and portions of testis were then fixed in Bouin's fluid, wax-embedded, sectioned at $4\ \mu$ and stained with iron haematoxylin and orange G. Other portions were fixed in formol-saline, embedded in gelatin, and frozen sections were then stained with Sudan black B and carmalum to reveal the lipoidal condition.

At the 60-mg dosage level, virtually all courtship behaviour was completely suppressed. In addition to the two birds that died, the others developed toxic symptoms including standing in abnormal postures, continuous shivering, vomiting and paralysis of the legs; these symptoms agree with those reported by Becker (1966).

At the 30-mg dosage level the number of 2-min periods (maximum possible was 150/bird) during which the bowing display was recorded for males was 25, 26 and 23, compared with 22, 29, 22, 23, 18 and 20 for the six control males. Similarly individual scores for courtship feeding were 9, 3 and 24 against 5, 0, 3, 11, 21 and 7 for controls. There was no significant difference between groups for these displays but there was for displacement preening which averaged 32.0 in treated birds and 12.5 in controls ($t=2.57$, $P>0.05$). It will be evident that displacement preening was less frequently followed by courtship feeding in Group 1 birds. The above three displays all occur during the early stages of the courtship cycle (Fabricius & Jansson, 1963) and other work (Murton, Thearle & Lofts, personal communication) shows that they can be significantly enhanced by exogenous androgen. It would be reasonable to attribute, at least partly, their natural hormonal causation to androgen from the Leydig cells.

Exogenous oestrogen significantly and consistently elevates the incidence of the nest demonstration display of male pigeons and we shall present evidence elsewhere that endogenous oestrogen is implicated in its causation in the normal cycle (Murton *et al.*). Group 1 subjects appeared to display a lower incidence of nest demonstration (44, 75 and 43, mean = 54) than controls (41, 81, 86, 51, 73 and 70, mean = 67) but differences were not significant. Nest-building by males follows nest demonstration in the course of the cycle, but it was inhibited by exogenous oestrogen and could only be maintained, relative to controls, by FSH injection (without oestrogen) and not by other hormone treatment; it is possible that an FSH-oestrogen synergism is involved in nest-building (Murton *et al.*, personal communication). The incidence of nest-building behaviour in the Group 1 birds (0, 2 and 0) was significantly lower ($t = 2.57$, $P>0.05$) than amongst the controls (14, 13, 10, 14, 1 and 4). These results, therefore, suggest that at low dosage the drug interfered with those behaviours associated with FSH and tubular oestrogen but not with the pituitary-Leydig cell axis.

The testes of the birds administered the drug showed no change in size over controls, the mean for all birds being 23×10 mm. There was a slight increase

PLATE 1

Transverse section of testis of feral pigeon stained with iron haematoxylin and orange G. $\times 350$.

FIG. 1. Normal subject in full breeding condition showing orderly sequence of germ-cell stages from spermatogonia to mature spermatozoa.

FIG. 2. Subject treated with 420 mg 22,25-diazacholesterol dihydrochloride, showing effect of interference with germ cell co-ordination in the germinal epithelium, whereby all stages become intermixed in the tubule lumina. Pycnotic nuclei can be seen in the primary spermatocytes.

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in the diameter of the largest follicles of the treated females, the mean increasing from 3 to 5 mm, but three of the control females laid eggs, while the remainder had very enlarged follicles at the end of the experiment.

In transverse section, the gonads of untreated male pigeons, which are continuous breeders, are seen to present an asynchronous condition, with often adjacent seminiferous tubules displaying different stages in the spermatogenetic cycle of the germinal epithelium (Lofts, Murton & Westwood, 1966). A similar asynchrony occurs in the sectioned tubules of the continuously breeding white rat, where there is a regular and successive replacement of germ cells about a radial axis (Leblond & Clermont, 1952). An orderly sequence of germ cell replacement from the periphery to the lumen is also apparent in the pigeon (Pl. 1, Fig. 1). From the histological examination of the testes of the birds dosed at the 30 mg level, it appeared that the drug had little effect on spermatid and spermatozoal stages, but markedly interfered with the continued proliferation of spermatogonia and spermatocytes. Its main effect was an inhibition of meiosis. At the low dosage level, the germinal epithelium of all tubules appeared to contain more germ cells in meiotic prophase and these prophase nuclei were much more condensed than in normal controls. A few metaphase figures were seen, but these were comparatively few. Spermatids and spermatozoa appeared normal, but there had been a reduction in the population of these stages, since mature spermatozoa had evacuated the lumen and had not been succeeded by subsequent generations, as the rate of replacement from the primary spermatocyte stage was retarded. There were fewer interphase primary spermatocytes (Clermont, 1958), but leptotene and zygotene stages were comparatively common. Spermatogonial division had been suppressed and mitotic figures were absent.

At the higher dosage level, in addition to the above effects, many primary spermatocytes were pycnotic and there had been an interference of the germ cell co-ordination in the germinal epithelium, so that the orderly replacement pattern of germ cells was no longer apparent (Pl. 1, Fig. 2). This suggests an interference with the functional activity of the Sertoli cells, reminiscent of cadmium damage (Lofts & Murton, 1967). Since the mammalian (Lacy, Lofts, Kinson, Hopkins & Dott, 1965) and fowl (Woods & Domm, 1966) Sertoli cell appears to be capable of steroid synthesis, it might be expected that such an anti-cholesterol agent would interfere with its functional activity and this is supported by the present results. Endogenous cholesterol production by the avian seminiferous tubules is very extensive at certain times of year (Marshall, 1955).

At the higher dosage level atrophic changes were also produced in the interstitial Leydig cells. In controls, these cells have large rounded nuclei and numerous small sudanophilic droplets in the cell cytoplasm. In the experimental birds, the Leydig cell nuclei became irregular in outline and the cell cytoplasm filled up with a dense amorphous mass of lipid. At the low dosage level, however, the interstitial cells appeared to be relatively unaffected.

The results of the histological examination support those of the behaviour observations. At the 60-mg dosage level, where damage is recorded to both the tubules and the interstitium, all sexual activities were suppressed; at the

30-mg dosage, where only the tubules were affected, the displays under oestrogenic or FSH influence were reduced (oestrogen is thought to originate from the Sertoli cells of the tubules). It is possible to have androgen based displays with only slight spermatogenesis, as happens with the autumnal recrudescence of sexuality in some temperate species.

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