

SOME EFFECTS OF A SINGLE INJECTION OF CHLORMADINONE ACETATE OR METHALLIBURE ON SEXUAL FUNCTION IN THE PUBESCENT MALE ANIMAL

II. THE RABBIT

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Summary. The effect of CAP and of methallibure on sexual function was studied in the male rabbit. CAP was given as a single subcutaneous injection (2 mg or 10 mg/kg body weight) and methallibure as a single intravenous injection (1 mg or 10 mg/kg body weight) at birth, 42 days of age (before the onset of spermatogenesis) and at 63 days of age (after the onset of spermatogenesis). Surgically castrated males and controls were included for comparison. All bucks were subjected to autopsy at 133 days of age, 1 week after the appearance of spermatozoa in the ejaculate of the controls.

Both drugs suppressed male behaviour and testicular growth. Growth rate was adversely affected in bucks treated at birth or 42 days of age. The effect of the drugs was not so marked when given after the onset of spermatogenesis.

INTRODUCTION

Prolonged treatment with progesterone or chlormadinone acetate (CAP, 6-chloro-6-17-acetoxypregesterone) suppresses both libido and spermatogenesis in mature rabbits (Ericsson, Dutt & Archdeacon, 1964). Jöchle & Schilling (1965) found that CAP exerts a long-acting depot effect in the boar and that puberty can be delayed by a single injection of the drug. Continuous treatment with methallibure (ICI 33828, 1-(α -methyl, allyl)-6-methyl-dithio-biurea) causes atrophy of the testis and accessory glands in the rat (Paget, Walpole & Richardson, 1961; Walpole, 1965). In cocks, daily ingestion of this compound leads to sterility, and it has been suggested that this treatment could be used for controlling aggressive behaviour (El Jack & Sykes, 1965). The object of the present experiment was to investigate the possibility of inhibiting sexual development and aggressive behaviour in the pubescent rabbit using a single injection of either CAP or methallibure.

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MATERIALS AND METHODS

CAP and methallibure were injected in crystalline suspension at three stages, namely at birth, at 42 days of age (before the onset of spermatogenesis) and at 63 days of age (after the onset of spermatogenesis). Two dosage levels were used. CAP was injected subcutaneously at 2 mg/kg body weight ('low' level) and 10 mg/kg body weight ('high' level), while methallibure was injected intravenously at 1 mg/kg ('low') and 10 mg/kg ('high'), respectively. Since intravenous injection proved extremely difficult in some new born rabbits, the 'low' dose of methallibure was omitted in this group. Further groups of animals were surgically castrated at birth, 42, or 63 days of age in order to compare their growth with that of animals receiving the two drugs.

The design of the experiment was a randomized block with three blocks of sixteen treatments. Forty-eight rabbits were allocated randomly to the different groups. After weaning and transfer to colony pens, the behaviour of the rabbits was observed and the first attempts at mounting (copulatory behaviour) were noted.

From about 90 days of age, many of the rabbits had to be separated into smaller groups and some had to be placed in single pens as they became aggressive and attacked their companions. This occurred first in the entire, untreated animals but later also in some of the treated bucks. Weaker rabbits were penned together and large docile bucks were kept in another group.

When mounting was observed, ejaculates were collected from the control bucks and these animals were killed 1 week after the appearance of spermatozoa, which occurred at 126 days of age. The reproductive tracts were dissected out and examined. Spermatozoa from each cauda epididymidis were counted, their motility was estimated and the concentration of fructose and citric acid in the gl. vesicularis and gl. seminalis was determined (Mann, 1964). Slices of testis were fixed in Bouin's fluid and after routine paraffin embedding, sectioned and stained with Delafield's haematoxylin and chromotrope 2R for later histological examination and measurement of seminiferous tubule diameter. All of the rabbits were weighed regularly throughout the experimental period.

RESULTS

The mean values for the various parameters under study are presented in Tables 1, 2 and 3. In Table 1, all measurements have been combined for statistical convenience. As the stage of injection had a significant influence on the results obtained, the overall mean for drug effects could not be compared with the controls. The following points are of particular interest:

- (a) Male behaviour was significantly delayed both by CAP and methallibure.
- (b) The stage of injection was important. The injections of CAP at birth resulted in smaller tubules, lower caudal sperm count and lower motility than when given later. Vesicular fructose levels were, however, higher in the CAP-treated animals than in the controls, possibly as a result of the 'rebound' phenomenon. Injections at 42 days depressed the caudal sperm count as well as sperm motility. The injection of methallibure at birth had the same effect as that of CAP. Injections of methallibure at 42 days of age depressed tubule

diameter, number and motility of caudal spermatozoa, and the fructose content of the accessory glands.

(c) The growth rate of CAP-treated rabbits injected at birth and at 42 days of age was lower than that of either the entire or the castrated bucks. This treatment may have had an adverse effect on the growth rate of this group

TABLE 1

THE EFFECT OF CHLORMADINONE ACETATE AND METHALLIBURE ON SEXUAL FUNCTION IN THE PUBESCENT MALE RABBIT

	Control (C)	Chlormadinone (A)	Methallibure (B)	S.E.
Age at first mounting (days)	73.8	97.1**	97.1**	4.40
Weight of testes (g)	3.82	3.11	3.40	0.25
Diameter of seminiferous tubules (μ)	166†	154	164	6.4
Weight of epididymides (g)	1.49	1.43	1.48	0.11
No. of cauda spermatozoa ($\times 10^6$)	2.62	2.53	3.18	0.79
Motility of cauda spermatozoa (%)	43.6	25.3	31.9	5.1
Weight of gl. seminalis and vesicularis (g)	1.72	1.72	1.69	0.19
Fructose concentration (mg/100 g)	70.8	84.1	72.0	7.43
Fructose content (mg)	1.31	1.67	1.33	0.23
Citric acid concentration (mg/100 g)	124.0	106.2	84.8	17.5
Citric acid content (mg)	2.59	2.60	1.50	0.55
Weight of ampullae (g)	0.38	0.37	0.38	0.04
Weight of prostate (g)	0.43	0.41	0.45	0.04

** $0.001 < P < 0.01$.

† Values in bold type refer to those parameters which were markedly affected by stage of treatment (see Table 2).

throughout the remainder of the experiment. The rabbits injected at birth had an average daily weight gain of 14.7 g as compared with 25.7 g for entire control bucks up to weaning, a difference which was highly significant ($P < 0.001$). Although the difference between the castrated and entire, untreated bucks was not significant, the surgically-castrated bucks had a much

TABLE 2

INFLUENCE OF A SINGLE INJECTION OF CHLORMADINONE ACETATE OR METHALLIBURE ON GROWTH RATE, EXPRESSED AS MEAN DAILY GAIN IN G, IN MALE RABBITS

Treatment group (Time of treatment)	Entire	Castrated			CAP (L and H)			Methallibure (L and H)		
		Birth	42 days	63 days	Birth	42 days	63 days	Birth	42 days	63 days
0 to 42 days	25.7	23.7	23.2	26.4	14.7	18.8	27.6	26.7	19.0	28.3
42 to 63 days	18.7	14.6	20.8	15.9	18.1	22.4	18.0	15.7	15.7	18.1
63 to 126 days	19.9	20.1	20.0	19.4	21.4	18.0	16.7	18.3	18.6	17.6

larger amount of omasal fat at autopsy which would have produced a bigger difference in carcase weight in favour of the entire bucks. The growth rate of the bucks injected at 42 days with methallibure was also reduced. This group appeared to be markedly affected by injections at this stage.

TABLE 3
THE EFFECT OF TIME OF TREATMENT WITH CHLORMADINONE OR METHALLIBURE ON CERTAIN SEXUAL CHARACTERISTICS IN THE MALE RABBIT

	Chlormadinone			Methallibure			Control	S.E.
	Stage of treatment			Stage of treatment				
	Birth	42 days	63 days	Birth	42 days	63 days		
Diameter of seminiferous tubules (μ)	147**	156	159	166	141	186	166	6.4
No. of cauda spermatozoa ($\times 10^6$)	2.93***	0.74***	4.12	1.19***	0.84***	6.51	2.62	0.93
Motility of cauda spermatozoa (%)	16.3**	16.5**	38.5	18.3**	15.5**	55.0	43.6	6.8
Fructose concentration (mg/100 g)	144.3**	59.2	79.0	47.7	47.6**	95.0	70.8	8.8
Fructose content (mg)	3.14*	1.10	1.41	1.49	0.62*	1.95	1.31	0.27

* 0.01 < P < 0.05; ** 0.001 < P < 0.01; *** P < 0.001

DISCUSSION

Injections of CAP after the onset of spermatogenesis had no apparent effect on sexual function in the rabbit, apart from delaying the onset of male behaviour which, in fact, occurred in all treated bucks. This suppression of libido and aggressive behaviour is in agreement with reports of the effect of CAP on mature rabbits (Ericsson *et al.*, 1964) and of methallibure on the cock (El Jack & Sykes, 1965).

Neonatal injections of CAP had a marked, and apparently reversible, effect on sexual function. However, not only was sexual function affected at this stage but also growth rate, possibly indicating a wider inhibition of pituitary function. Campbell (1965) injected three male rabbits neonatally with 200 mg progesterone and killed them at 93 days of age. These rabbits showed no significant difference from control bucks with regard to age at first copulation, final body weight, or testis weight.

The growth rate of bucks injected at 42 days of age was also significantly affected by both CAP and methallibure. The latter group was also severely retarded with regard to sexual function, indicating that the pituitary gland may be more susceptible to drugs at some stages than at others. Poppe's (1932) observation that the amount of omasal fat is greater in surgically castrated rabbits was confirmed.

The present results, as in the ram (Skinner & Rowson, 1969), support the hypothesis that progestagens inhibit gonadotrophin secretion and that, after the form of treatment described, reproductive processes return to normal in due course. CAP might be used to control aggressive behaviour in colony-reared rabbits, though the suppression of growth rate in animals treated in the neonatal period could be disadvantageous.

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