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A SEX DIFFERENCE IN THE EFFECT OF PHENAZOCINE AND DIHYDROCODEINE ON THE BLOOD pH OF THE UNANAESTHETIZED RABBIT

BY

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The effects of inhaled carbon dioxide, morphine and dextromoramide on pH homoeostasis in the blood of the rabbit have been described (Rees, 1967). The investigation has been extended to two other narcotic analgesics to determine whether the bicarbonate accumulation and " pH tolerance " observed after morphine are peculiar to this drug alone.

METHODS

Apparatus

This was identical to that used by Rees (1967).

General methods

Four rabbits were used in each group. These were of Flemish strain (weight 2.5–3.5 kg) and of the same sex in each group. Blood was sampled from the marginal vein of the warmed ear and blood pH, carbon dioxide tension P_{CO_2}) and standard bicarbonate were estimated by the method of Andersen, Astrup, Engel & Jorgensen (1960).

Examination of effects of administration of drugs

Control values for each animal under investigation were determined during the 1 hr before drug administration, and the effects of each drug were expressed in terms of change in pH (ΔpH) ΔP_{CO_2} and Δ standard bicarbonate from the mean of these control values.

Administration of drugs

Phenazocine and dihydrocodeine were each injected intravenously to a group of male and a group of female rabbits. Blood was sampled 7, 15, 30, 45, 60, 90 and 120 min after injection, and then at hourly intervals until control values were regained. The dose of phenazocine hydrobromide used was 0.2 mg/kg, and of dihydrocodeine bitartrate 20 mg/kg.

Pretreatment with drugs

Testosterone propionate: a group of female rabbits was pretreated by injection of 5 mg/kg intramuscularly each day for 14 consecutive days.

Oestradiol monobenzoate: a group of male rabbits was pretreated by injection of 30 μg/kg intramuscularly each day for 14 consecutive days.

Phenazocine hydrobromide: a group of male rabbits was pretreated by injection of 0.2 mg/kg intravenously each day for 6 consecutive days.

The effects of phenazocine hydrobromide (0.2 mg/kg) were investigated in all these rabbits on the first day after the pretreatment course was completed. The times of blood sampling were identical to those used in untreated rabbits.

Drugs

These were: dihydrocodeine bitartrate injection (DF 118, Duncan, Flockhart and Evans Ltd.); oestradiol monobenzoate (Dimenformon, Organon); phenazocine hydrobromide injection (Narphen, Smith and Nephew Pharmaceuticals Ltd.); testosterone propionate (Neo-hombreol, Organon).

All doses are expressed in terms of the salts.

RESULTS

Effects of dihydrocodeine

Changes in blood pH, P_{CO_2} and standard bicarbonate after injection of dihydrocodeine (20 mg/kg) in a group of male and a group of female rabbits are shown in Fig. 1. In both groups there was a rise in P_{CO_2}, which was slightly greater in the male group. There was a rise in standard bicarbonate in the female group similar to that seen after intravenous morphine (Rees, 1967), while in the male group P_{CO_2} elevation was accompanied by a fall in the standard bicarbonate. Because of this, normal blood pH was restored much sooner in the female group than in the male.

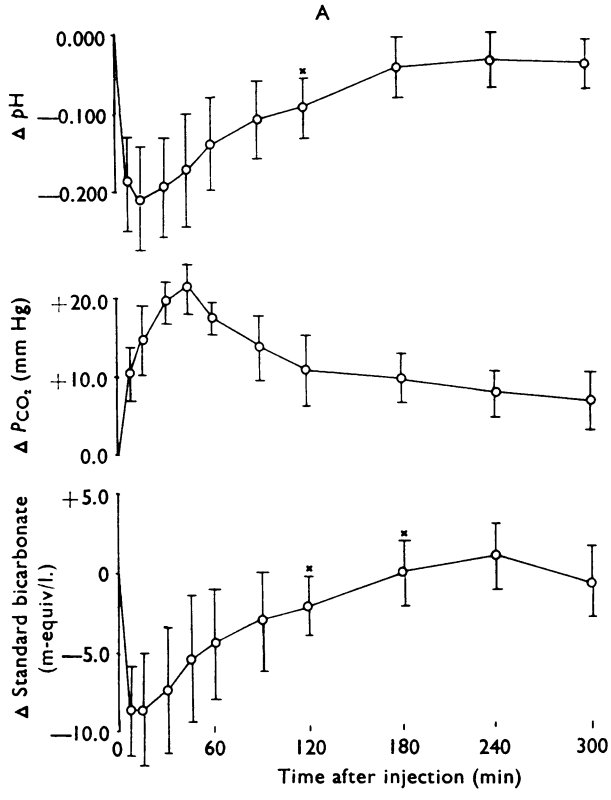

Fig. 1A.

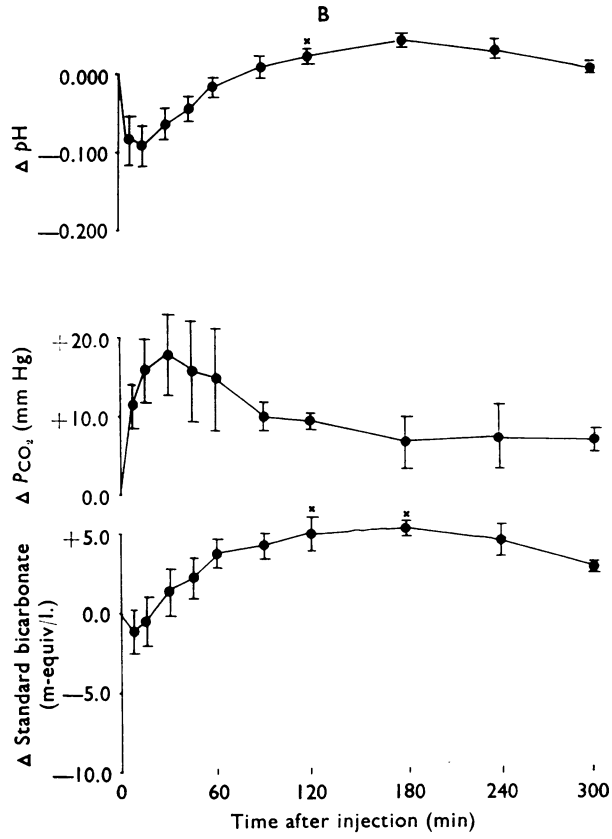

Fig. 1. Effects of dihydrocodeine (20 mg/kg) on blood pH, P_{CO_2} and standard bicarbonate in a group of four male rabbits (A), and in a group of four female rabbits (B). Vertical bars, standard errors of the mean; a cross above a mean indicates that it is significantly different ($P<0.05$) from the corresponding mean in the other sex.

Effects of phenazocine

The effects of phenazocine (0.2 mg/kg) are shown in Fig. 2. The general pattern was similar to that seen after dihydrocodeine. The changes in P_{CO_2} in both sexes were almost identical, but while there were only slight fluctuations in standard bicarbonate in the male group, there was a marked rise in the female. The difference in standard bicarbonate between the two sexes was significant ($P<0.05$) until well after the P_{CO_2} values began to return to normal (60 min). Because of this difference, blood pH was restored sooner in the female than in the male.

Because the difference between the sexes was more significant after phenazocine than after dihydrocodeine, the response to phenazocine was investigated further.

Control pH, P_{CO_2} and standard bicarbonate in the pretreated groups

The control pH, P_{CO_2} and standard bicarbonate values in the three groups after completion of the pretreatment courses, and before challenge with phenazocine are

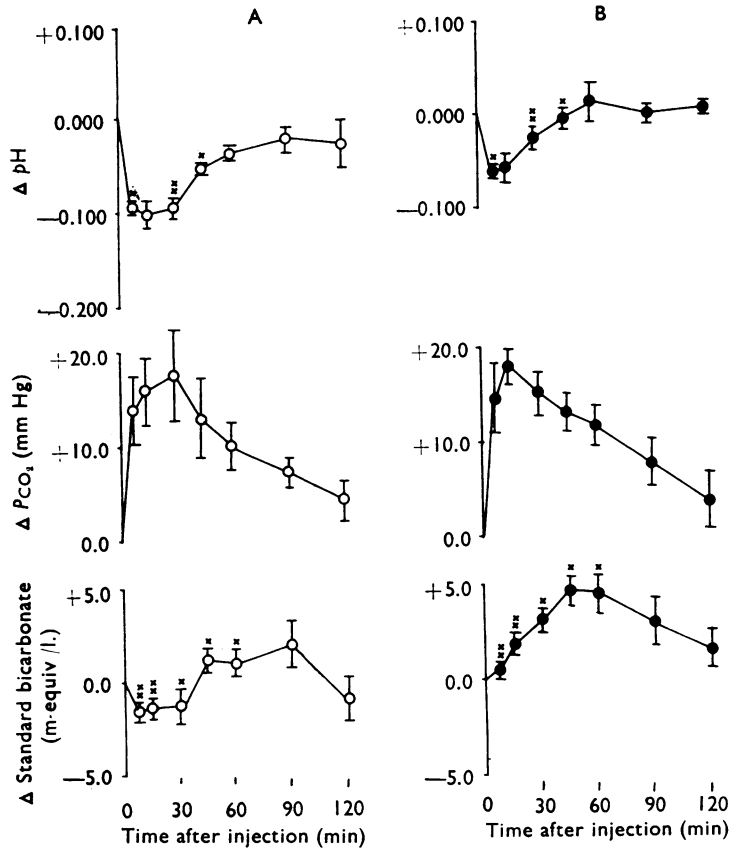

Fig. 2. Effects of phenazocine (0.2 mg/kg) on blood pH, P_{CO_2} and standard bicarbonate in a group of four male rabbits (A), and in a group of four female rabbits (B). Vertical bars, standard errors of the mean; a cross above a mean indicates that it is significantly different ($P<0.05$) from the corresponding mean in the other sex, two crosses denote that $P<0.005$.

TABLE 1

CONTROL VALUES OF pH, P_{CO_2} AND STANDARD BICARBONATE OBTAINED DURING THE 1 hr BEFORE DRUG ADMINISTRATION IN THE NORMAL MALE AND FEMALE RABBIT, THE MALE PRETREATED WITH OESTRADIOL, THE FEMALE PRETREATED WITH TESTOSTERONE AND THE MALE PRETREATED WITH PHENAZOCINE

Mean of four readings ± S.E.

Significance: pH: *B* is significantly lower than *D* and *E* ($P<0.05$); P_{CO_2}: *D* is significantly lower than *A* ($P<0.05$) and *C* ($P<0.005$) and is significantly higher than *E* ($P<0.05$). *E* is also significantly lower than *A* ($P<0.05$) and *C* ($P<0.001$); standard bicarbonate: there is no significant difference ($P>0.05$) between any of the means.

	Normal male *A*	Normal female *B*	Male pretreated with oestradiol *C*	Female pretreated with testosterone *D*	Male pretreated with phenazocine *E*
pH	7·463±0·031	7·415±0·010	7·436±0·033	7·484±0·023	7·473±0·020
P_{CO_2} (mm Hg)	44·1±1·3	43·3±4·3	47·3±1·1	40·4±0·8	37·4±0·8
Standard bicarbonate (m-equiv/l.)	29·8±1·7	26·5±1·8	29·2±1·3	29·1±0·5	27·3±1·5

shown in Table 1. There were no significant differences between the control standard bicarbonate values of the five groups. The mean control carbon dioxide tensions for the testosterone and phenazocine pretreated groups were significantly lower than for the groups of normal males, and males pretreated with oestradiol. During the control period it was observed that while the males pretreated with oestradiol remained generally sedated, both the males pretreated with phenazocine and the females pretreated with testosterone were in an excited state. These differences are reflected in their respective control P_{CO_2} values.

Effects of phenazocine in the pretreated groups

The effects of phenazocine (0.2 mg/kg) in the male group pretreated with oestradiol, and in the female groups pretreated with testosterone are shown in Fig. 3. In general,

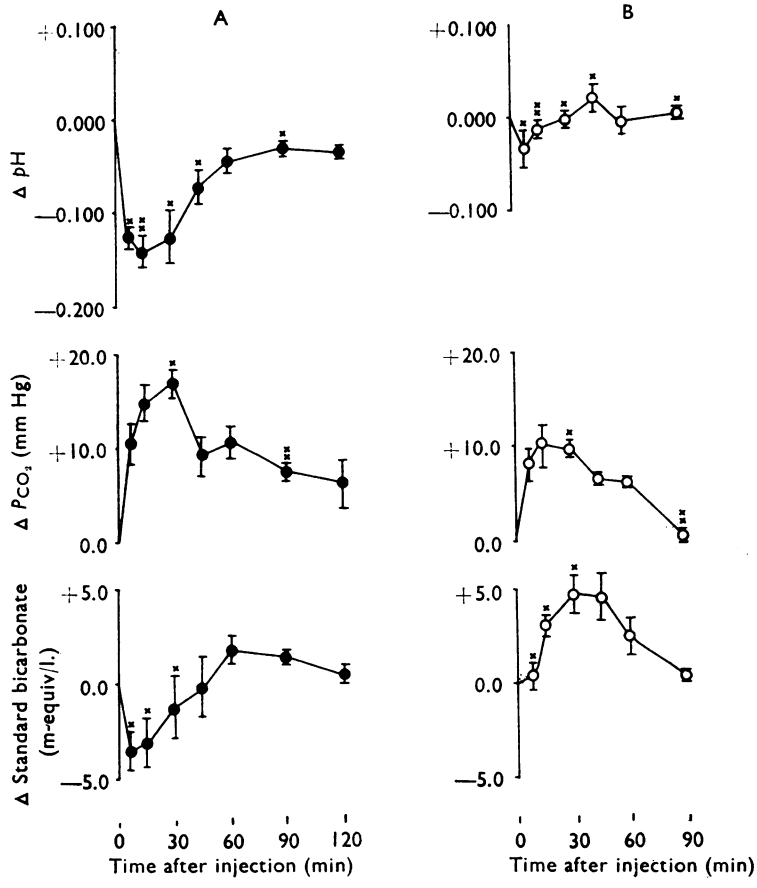

Fig. 3. Effects of phenazocine (0.2 mg/kg) on blood pH, P_{CO_2} and standard bicarbonate in a group of four female rabbits pretreated with testosterone (A), and in a group of four male rabbits pretreated with oestradiol (B). Vertical bars, standard errors of the mean; one cross above a mean indicates that it is significantly different ($P<0.05$) from the corresponding mean in the other sex; two crosses denote that $P<0.005$.

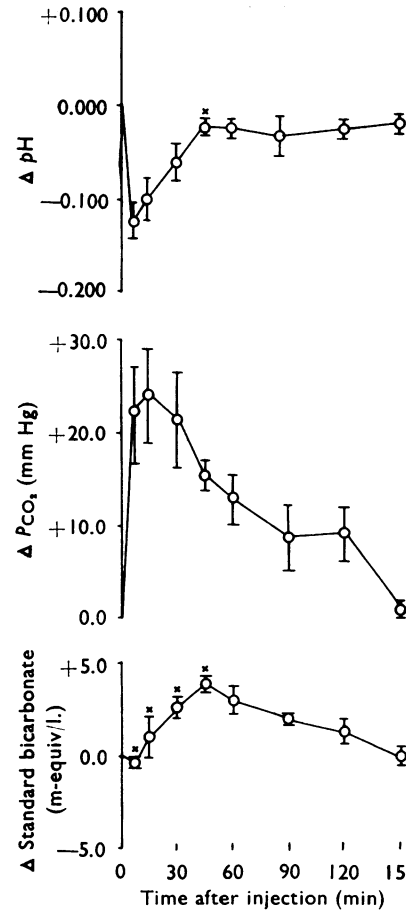

Fig. 4. Effects of phenazocine (0.2 mg/kg) on blood pH, P_{CO_2} and standard bicarbonate in a group of four male rabbits pretreated with phenazocine. Vertical bars, standard errors of the mean; a cross above a mean indicates that it is significantly different ($P<0.05$) from the corresponding mean effects in the normal male shown in Fig. 2.

the responses observed in the pretreated male and female groups were the opposite to those observed in the untreated male and female groups. The *P* values for the differences between the normal and pretreated rabbits are given in Table 2. This shows that the effects of phenazocine are similar in both the male group and the female group pretreated with testosterone. With respect to pH and standard bicarbonate, the effects of phenazocine are similar in the untreated female group and the male group pretreated with oestradiol.

The effects of phenazocine on P_{CO_2} are substantially the same in the four groups, the only exception to this being that the change in P_{CO_2} in the male group pretreated with oestradiol is less than that in the other groups.

TABLE 2

TABLE OF *P* VALUES OBTAINED BY APPLYING STUDENT'S *t* TEST TO COMPARE THE EFFECTS OF PHENAZOCINE (0·2 mg/kg) ON pH, P_{CO_2} AND STANDARD BICARBONATE IN NORMAL MALE AND FEMALE RABBITS WITH THOSE IN PRETREATED MALES AND FEMALES

Note: n.s.=$P>0·05$. Results of applying significance tests to compare normal males with normal females, and males pretreated with oestradiol with females pretreated with testosterone are shown in Figs. 2 and 3.

Comparison of:	Time after injection (min)	7	15	30	45	60	90	120
Normal male and male pretreated with oestradiol	pH	<0·05	<0·005	<0·005	<0·005	n.s.	n.s.	—
	P_{CO_2} (mm Hg)	n.s.	n.s.	n.s.	n.s.	n.s.	<0·01	—
	Standard bicarbonate (m-equiv/l.)	n.s.	<0·001	<0·001	n.s.	n.s.	n.s.	—
Normale male and female pretreated with testosterone	pH	<0·05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	P_{CO_2} (mm Hg)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Standard bicarbonate (m-equiv/l.)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Normal female and male pretreated with oestradiol	pH	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	—
	P_{CO_2} (mm Hg)	n.s.	<0·05	n.s.	<0·025	<0·05	<0·05	—
	Standard bicarbonate (m-equiv/l.)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	—
Normal female and female pretreated with testosterone	pH	<0·005	<0·02	<0·02	<0·02	n.s.	n.s.	<0·02
	P_{CO_2} (mm Hg)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Standard bicarbonate (m-equiv/l.)	<0·02	<0·02	n.s.	n.s.	n.s.	n.s.	n.s.

The effects of phenazocine in the male group pretreated with phenazocine are shown in Fig. 4. The change in standard bicarbonate was significantly different ($P<0.05$) from that seen in the control male group for the first 45 min after injection, but over this same period there was no significant difference between this and the changes either in the normal female group or the male group pretreated with oestradiol.

DISCUSSION

Rees (1967) has shown that after injection of morphine the elevation of blood P_{CO_2} was accompanied by a rise in the standard bicarbonate, a change which did not occur when blood P_{CO_2} was raised to the same extent by inhalation of carbon dioxide. In the present investigation similar bicarbonate changes have been observed after both phenazocine and dihydrocodeine only in the female rabbit. Surprisingly, no such change occurred in the male animal. It was for this reason that experiments were performed to determine whether this unusual sex difference was dependent on hormones.

The results demonstrate that the changes in standard bicarbonate are similar after phenazocine in the untreated female, the male pretreated wih oestradiol and the male pretreated with phenazocine, and differ from those seen in the normal male and the female pretreated with testosterone.

Sex differences in some other aspects of narcotic analgesic action have been reported. *N*-demethylase enzymes have been implemented both in the mechanism of action of narcotic analgesics and in the development of tolerance to them (Adler & Way, 1962, and references cited therein), and the more recent work of Adler (1967). Axelrod (1956) demonstrated that the activity of this enzyme in male rat liver microsomes was far greater than the activity in female rat liver microsomes, although he was unable to demonstrate that this sex difference was characteristic of other species. The central stimulant actions of morphine have often been claimed to be more prominent in women

than in men (Sollmann, 1957) although Lasagna (1964) has described this as an "interesting myth." Contreras, Quijida & Tamayo (1966) have demonstrated an analgesic action of ergometrine in male rats, and in ovariectomized females treated with testosterone, but not in normal females. This analgesic activity was synergistic with morphine in males, but ergometrine was antagonistic to morphine in normal females.

There was no significant difference between the respiratory effects of phenazocine (as shown by ΔP_{CO_2}) between the males and females, but because of the marked elevation of standard bicarbonate in the female group and the male group pretreated with oestradiol, this respiratory depression was accompanied by little significant change in pH. A "pH tolerance" has been reported for morphine in which, after a short course of morphine pretreatment, the rise in P_{CO_2} induced by morphine was accompanied by a marked rise in standard bicarbonate and a negligible fall in pH (Rees, 1967). A similar effect is now reported in the male rabbit with phenazocine. Thus, pretreatment of male rabbits with either oestradiol or phenazocine will lead to a "pH tolerance" on challenge with phenazocine.

TABLE 3

TIME TO MAXIMUM P_{CO_2} ELEVATION AFTER INTRAVENOUS INJECTIONS OF DEXTROMORAMIDE, DIHYDROCODEINE, PHENAZOCINE AND MORPHINE AND THE CONCURRENT CHANGES IN STANDARD BICARBONATE

Results for dextromoramide and morphine after Rees (1967).

Drug	Sex	Dose (mg/kg)	Maximum P_{CO_2} (mm Hg)	Mean time after injection (min)	Number of experiments	Gross changes in standard bicarbonate
Dextromoramide	Male and female	0·05	16·2	7 or <7	5	Both sexes show a fall followed by a slight rise
Dihydrocodeine	Female	20	17·7	30	4	Rise
	Male	20	21·5	45	4	Fall
Phenazocine	Female	0·2	18·3	15	4	Rise
	Male	0·2	17·5	30	4	No significant change
Morphine	Male and female	4·8	17·5	120	8	Both sexes show a rise

The question arises as to why there is a sex difference with dihydrocodeine and phenazocine but not with morphine (after which both sexes show a bicarbonate rise) and dextromoramide (when neither sex shows a rise). If one assumes that these four drugs differ from each other only quantitatively, then the most obvious similarity between phenazocine and dihydrocodeine and difference from morphine and dextromoramide is in the time to maximum P_{CO_2}. Table 3 shows this time for the four drugs at doses which elevate P_{CO_2} to the same extent. Changes in standard bicarbonate after administration of narcotic analgesics will be governed by at least two factors. First there will be bicarbonate redistribution resulting from very rapid rise in P_{CO_2}, giving an apparent fall in the standard bicarbonate (Brackett, Cohen & Schwartz, 1965 ; Brown & Clancey, 1965). This is the probable explanation of the fall reported after dextromoramide (Rees, 1967). Second there is the bicarbonate accumulation characteristic of morphine which has a slow time course of development. In the case of the rapidly acting dextromoramide there is insufficient time for this bicarbonate to accumulate, while with morphine the rise in blood

P_{CO_2} is so gradual that the bicarbonate redistribution would be insignificant. This may then explain the response observed after phenazocine and dihydrocodeine which have a rate of P_{CO_2} elevation intermediate between dextromoramide and morphine, and during which both factors could play a significant part. In such a situation quantitative sex differences could become apparent.

The observation that changes in P_{CO_2} induced by phenazocine in the male pretreated with oestradiol are significantly smaller than in the untreated male or female groups deserves comment. This could be ascribed either to the significantly higher P_{CO_2} in the control state (Table 1), or to the sharing by oestradiol and phenazocine of a common major route of biotransformation (Axelrod, Daly & Inscoe, 1965). Oestradiol could induce enzymes responsible for its own breakdown, and consequently phenazocine would be eliminated faster after pretreatment with oestradiol.

SUMMARY

1. The Astrup technique has been used to investigate the effects of dihydrocodeine and phenazocine on pH, P_{CO_2} and standard bicarbonate in the blood of the unanaesthetized rabbit.

2. After injection of dihydrocodeine, the rise in blood P_{CO_2} is accompanied by a fall in standard bicarbonate in the male rabbit, but by a rise in the female.

3. P_{CO_2} elevation produced by phenazocine is accompanied by little change in standard bicarbonate in the male, but by a marked rise in the female.

4. Male rabbits pretreated with oestradiol when challenged with phenazocine show acid-base changes characteristic of the female, while females pretreated with testosterone and similarly challenged show changes characteristic of the male.

5. Male rabbits pretreated with phenazocine when challenged with phenazocine show acid-base changes characteristic of the female.

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