HAEMOLYSIS FOLLOWING COPULATION IN MALE RATS

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(Received 3rd June 1971)

In the course of a study on the effects of mating on plasma LH concentrations in the rat, a serendipitous finding was that plasma samples collected from male rats shortly after copulation often showed obvious evidence of haemolysis. These observations prompted a more detailed investigation.

Adult Wistar rats were maintained under reversed lighting (lights on from 21.00 to 11.00 hours). Mating tests were carried out under observation in subdued lighting between 14.00 and 18.00 hours. Females were made highly receptive by the subcutaneous injection of 100 µg of oestradiol benzoate in oil 48 hr before tests were begun. Under these conditions, a normal ejaculatory series in the male rat consists of some ten to fifteen mounts with brief penile intromissions, separated by short intercopulatory intervals, over a period of 5 to 10 min. The series terminates with an ejaculation which is followed by a postejaculatory interval of several minutes during which no interest in the female is exhibited.

In some tests, males were allowed to mate for 15 or 30 min, during which time one or more ejaculations occurred; in others, the test period was terminated after ten to fifteen intromissions and before ejaculation ('Intromissions only'). In other tests, a strip of smooth zinc oxide plaster was applied to the female so as to occlude the vaginal orifice and the males were allowed to mount over a period of 30 min but without intromission occurring ('Mounts only'). Erections were induced by the method developed by C. H. Rodgers and J. M. Davidson (unpublished). Rats were injected with sodium pentobarbitone (25 mg/kg body weight intraperitoneally), immobilized in a glass cylinder and light pressure applied to the base of the penis. This resulted in continuous moderate venous congestion of the penis with intermittent brief periods of full erection, ten to twenty-nine in 30 min.

Blood samples (2.0 to 2.5 ml) were obtained from the jugular vein with a heparinized plastic syringe and 25-gauge needle under Avertin (tribromoethanol plus amylene hydrate) anaesthesia and centrifuged under standardized conditions to obtain plasma which was frozen until assayed. No rat was bled more than once in 2 weeks. Plasma haemoglobin (Hb) concentrations were determined

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colorimetrically by a benzidine method. Duplicate 50- μ l samples of plasma or rat Hb standard in saline (5 to 160 mg/100 ml) were added to 1 ml of a solution of 0.5 g benzidine in 100 ml of 90% glacial acetic acid A.R. After careful mixing, 1 ml of a 1% (w/v) solution of hydrogen peroxide A.R. was added, the reagents mixed and allowed to stand for 1 hr at room temperature. At the end of this time, 10 ml of a 10% (w/v) solution of acetic acid were added and the optical density at 5150 Å determined spectrophotometrically against a reagent blank after allowing at least 10 min for colour stabilization. The standard curve was linear from 0 to 120 mg/100 ml and all plasma samples fell in this range. A difference spectrum of a plasma sample showing obvious pink coloration had peaks identical to those of authentic rat Hb.

TABLE 1
PLASMA HAEMOGLOBIN CONCENTRATIONS

	Status	Test			
		15-min copulation	30-min copulation	Mounts only (30 min)	Test cage 30 min; no females
Exp. 1	Experienced	39.2 $\pm$ 12.6 (9)	50.1 $\pm$ 2.9 (13)	—	4.8 $\pm$ 0.5 (11)
Exp. 2	Experienced	22.3 $\pm$ 3.1 (5)	32.8 $\pm$ 9.5 (9)	3.9 $\pm$ 0.3 (7)	—
		Intromissions only	Intromissions plus ejaculation	Test cage 15 min; no females	
Exp. 3	Naïve	16.8 $\pm$ 4.3 (8)	14.0 $\pm$ 5.6 (6)	3.0 $\pm$ 1.2 (4)	
Exp. 4	Same rats as Exp. 3 when experienced	25.8 $\pm$ 5.4 (11)	27.8 $\pm$ 7.2 (9)	—	

Values expressed in mg/100 ml plasma, group means $\pm$ S.E. with number of observations in parentheses in male rats under different conditions.

In Exp. 1, male rats, all of which had had three to five previous mating tests during which ejaculation(s) had occurred ('Experienced males') were allowed to copulate for 15 or 30 min under observation or were placed in the observation cage alone for 30 min, after which blood samples were obtained. The results are given in Table 1. A marked increase in plasma Hb concentration was observed in rats that had copulated as compared to the control value of 3.6 $\pm$ 0.7 (twelve observations) in plasma from rats bled immediately after being taken from their home cage. Simply placing the rats in the test cage for 30 min in the absence of a female had no effect (Exp. 1, Table 1). In a second experiment with 'experienced males', high values were again observed in rats that copulated for 15 or 30 min (Exp. 2, Table 1). The control group in this experiment consisted of rats which were allowed to mount receptive females for 30 min, intromission being prevented by taping the female as described above. Plasma Hb concentration in this group remained low despite the occurrence of between sixty-one and eighty-four vigorous attempts by each rat to achieve intromission. In both Exps 1 and 2, rats copulating for 30 min had higher mean plasma Hb concentrations than those copulating for only 15 min, but the differences were not statistically significant. In Exps 3 and 4, the effects of intromission alone

and intromission plus ejaculation on plasma Hb concentration were compared. The animals were bled immediately after their first behavioural test during which they were allowed ten to twelve intromissions over a period of about 15 min or ten to fifteen intromissions plus an ejaculation. Subsequently, they were allowed to mate under observation and were observed to ejaculate at least once in each of four or five test sessions; the original experimental procedure was then repeated. Neither in naïve nor in 'experienced' animals was there a significant difference between the plasma Hb concentrations of the two groups (Exps 3 and 4, Table 1). Plasma Hb concentrations were higher in the 'experienced' animals but the differences were not statistically significant. Erections alone, induced under pentobarbitone sedation, had no effect on plasma Hb concentrations. The mean value in nine rats showing erections was 4.1 ± 1.8 and 4.1 ± 1.3 in four rats manipulated in the same way but failing to show erections and these were not significantly different from the values for any other control group in the present series of experiments.

It is clear from these results that normal copulation promptly increases plasma haemoglobin concentration in male rats. Penile intromissions without ejaculation are sufficient to produce this effect but repeated mounts without intromission even in far greater numbers than those occurring in a normal ejaculatory series do not have this effect nor does the artificial induction of repeated erections under barbiturate sedation. The cause of these changes in the male rat are not known but the failure of repeated mounts to increase plasma haemoglobin concentration suggests that it is not a non-specific effect related to the test situation. It would be of interest to know whether this consequence of copulation on the part of the male is peculiar to the rat or is of more general occurrence.