LIGHT AND THE TIMING OF OVULATION IN THE GUINEA-PIG

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Summary. The effect of differing lighting schedules upon the timing of the influx of leucocytes into the vaginal smear of the guinea-pig, and hence of ovulation, was examined. The timing of this event was altered following advancement of the light–dark sequence by 12 hr, and implies that the timing of ovulation in this species is influenced by light.

INTRODUCTION

Light is a potent stimulus influencing gonadal function. Alongside photoperiodic control of the breeding season in many species, the timing of ovulation during the oestrous cycle may be closely integrated with the diurnal sequence of night and day (Fraps, 1962; Critchlow, 1963; Everett, 1964; Donovan, 1970). The guinea-pig has been little studied in this respect although the finding that oestrous behaviour tends to begin at a certain time of night, and varies with changes in the onset of darkness (Dempsey, Myers, Young & Jennison, 1934), suggests that the release of an ovulatory quantum of gonadotrophin in this species may similarly be initiated at a certain time of day. As a preliminary to more detailed studies of the mechanisms governing ovulation, the effects of changing the daily lighting schedule on the vaginal cycle of this species have been explored.

MATERIALS AND METHODS

Adult female guinea-pigs of mixed origin were housed in windowless rooms maintained at 18° C and illuminated for 16 hr daily. The lights were switched on either at 08.00 or 20.00 hours and the animals examined for vaginal opening at 12.00 and 24.00 hours, with a vaginal smear being taken when appropriate. Thus, the animals were always examined 4 hr after the end of the dark period and just before it began. Vaginal smears were taken by lavage, dried on to a slide, and stained with Ehrlich's acid haematoxylin and eosin Y.

The influx of leucocytes into the vaginal smear after the appearance of cornified cells in the sample was taken as the point of reference for this study. The first smear taken after vaginal opening often contains epithelial cells, cornified cells and leucocytes but, as oestrus approaches, there is a progressive reduction in the number of leucocytes, with the appearance of brightly stained rounded cornified cells, and the end of vaginal oestrus is signalled by a marked rise in the leucocyte count.

TABLE 1
TIMING OF THE VAGINAL LEUCOCYTIC INFUX IN A GROUP OF GUINEA-PIGS UNDER A LIGHTING
SCHEDULE OF 8 HR DARKNESS FOLLOWED BY 16 HR LIGHT

Guinea-pig no.	Experiment 1				Experiment 2			
	First	Second	Third	Fourth	First	Second	Third	Fourth
	leucocytic influx	leucocytic influx	leucocytic influx	leucocytic influx	leucocytic influx	leucocytic influx	leucocytic influx	leucocytic influx
3273	Dark	Dark	Dark	Dark	Dark	Light	Dark	Died
3387	Dark	Dark	Light	Light	Light	Dark	Dark	Died
3391	Light	Dark	Light	Dark	Dark	Dark	Dark	Dark
3393	Dark	Light	Light	Light	Dark	Light	Dark	Dark
3402	Dark	Light	Dark	Dark	Dark	Dark	Light	Light
3406	Light	Light	Light	Light	Dark	Dark	Light	Dark
3407	Dark	Dark	Light	Dark	Dark	Light	Dark	Dark
3420	Light	Light	Light	Dark	Dark	Light	Light	Light
3429	Dark	Dark	Dark	Dark	Dark	Dark	Dark	Dark
3451	Light	Dark	Dark	Dark	Light	Dark	Light	Dark
3452	Dark	Dark	Dark	Dark	Light	Light	Light	Light
3460	Dark	Dark	Dark	Dark	Dark	Dark	Dark	Dark
3462	Dark	Dark	Dark	Light	Dark	Dark	Light	Dark
3463	Light	Dark	Dark	Dark	Light	Dark	Dark	Dark
3467	Dark	Light	Light	Dark	Light	Dark	Dark	Light
3469	Dark	Dark	Dark	Dark	Dark	Light	Dark	Died
3475	Dark	Dark	Light	Dark	Dark	Light	Dark	Light
3477	Dark	Light	Dark	Dark	Light	Light	Dark	Dark
Totals	43	21	43	26	43	26	43	26

Dark—the 12 hr that commenced at the beginning of darkness; light—the remaining 12 hr. The dark period commenced at 12.00 hours in Exp. 1 and at 24.00 hours in Exp. 2.

In a preliminary experiment, all the guinea-pigs in the departmental colony of approximately 100 females were examined at 12.00 and 24.00 hours, in addition to the routine daily examination at 09.00 hours, for 7 days. For these animals, the lights were on from 08.00 to 24.00 hours.

Subsequently, in Exp. 1, eighteen females were transferred to a room lit from 20.00 to 12.00 hours and allowed to become accustomed to this regimen for 1 month. Twice daily vaginal examination, at 12.00 and 24.00 hours, was

TEXT-FIG. 1. The effect of a change of lighting on the timing of the leucocytic influx into the vaginae of a group of guinea-pigs. Between Exp. 1 and 2, the light-dark sequence was altered by 12 hr, but the proportion of animals showing a leucocytic influx during the 12 hr including the dark period remained the same.

then begun and maintained for periods of 1 or 2 weeks with intervals of 1 or 2 weeks over a span of 5 months. In the middle of this period, the lighting was readjusted to be on from 08.00 to 24.00 hours (Exp. 2) so that several oestrous periods could be studied for each animal under each lighting schedule.

RESULTS

Thirty-nine oestrous periods were recorded in the preliminary experiment and, in twenty-seven instances, the influx of leucocytes occurred between 24.00 and 12.00 hours, the time interval that contained the dark period. This asymmetric distribution is significant (two-tailed exact binomial test) at the 2.5% level, and exhibits a pattern similar to that observed with behavioural oestrus by Dempsey *et al.* (1934).

The effects of a change in the timing of illumination are summarized in Table 1 and Text-fig. 1. Sixty-four periods of vaginal oestrus were observed

while the animals were illuminated from 20.00 to 12.00 hours and in forty-three of these, the influx of leucocytes into the vaginal smear occurred between 12.00 and 24.00 hours. Sixty-nine oestrous periods were observed in the same group of animals after altering the onset of illumination by 12 hr and, on forty-three occasions, the influx of leucocytes into the vagina occurred between 24.00 and 12.00 hours. Statistical analysis of these findings showed that the tendency for this vaginal change to occur during the 12 hr containing the dark period was significant at the 5% level. Oestrous cycles of normal length continued during the experiments.

DISCUSSION

These results demonstrate that the occurrence of vaginal oestrus in the guinea-pig is correlated with the light-dark sequence of environmental illumination, and that the influx of leucocytes tends to take place during or soon after the dark period.

In evaluating these findings, the significance of the vaginal index employed needs to be established. Stockard & Papanicolaou (1917) first related changes in the vaginal smear to concurrent events taking place in the ovaries and found that ovulation and the entry of leucocytes into the vaginal fluid tended to be coincident. Leucocytic infiltration of the vaginal smear after complete cornification has been taken to indicate ovulation by Young, Myers & Dempsey (1933) and Hermreck & Greenwald (1964). It is likely that the influx of leucocytes arises under the influence of progesterone. Oestrogen alone has failed to cause normal vaginal cornification and typical changes following oestrus were lacking after withdrawal of this steroid but, when oestrogen-primed ovariectomized guinea-pigs were given progesterone, the vaginal smears were similar to those of intact animals at oestrus and leucocytes entered the vaginal lumen 15 hr after steroid injection (Ford & Young, 1951). We have confirmed this finding in experiments involving the hourly collection of vaginal smears after the subcutaneous administration of 5 mg progesterone to oestrogen-primed (25 µg oestradiol benzoate/day) spayed females. The change from an oestrous to a metoestrous smear was rapid and was completed between consecutive examinations of the vagina. Measurements of the concentration of progesterone in arterial plasma indicate that a surge of progesterone secretion from the ovaries occurs approximately 8 to 12 hr before ovulation (Feder, Resko & Goy, 1968), while the relationship between gonadotrophin secretion and ovulation can be assessed from the finding of Myers, Young & Dempsey (1936) that ovulation took place some 10 to 12 hr after the onset of oestrus, and that the latency between gonadotrophin administration and the manifestation of oestrous behaviour was 6 hr (Dempsey, Hertz & Young, 1933), thus giving an estimate of 16 to 18 hr for the time interval. Following the intravenous injection of 500 µg LH (Armour Lot No. E 13210), ovulation has, in fact, been reported to occur within 16 to 18 hr (J. E. Booth, unpublished work). Thus, the influx of leucocytes into the vaginal smear occurs around the time of ovulation.

It is clear that, in the guinea-pig, the timing of gonadotrophin release, as measured by the onset of oestrus (Young, Dempsey & Myers, 1935) or the

timing of the leucocytic influx into the vagina, shows considerable individual variation, unlike the situation in some strains of rat where this event occurs at a constant time of day among females kept under similar lighting conditions (Everett, 1964). Whether this is due to a lesser influence of environmental factors in the triggering of gonadotrophin secretion in the guinea-pig, or whether it arises as an after-effect of the action of progesterone during the long luteal phase of the oestrous cycle, remains to be determined. Hoffmann & Schwartz (1965) indicate that the mechanism controlling ovulation in the rat operates in a different manner after an influence of progesterone has waned than in the normally cyclic animal.

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