Effect of Oral Treatment with Chlormadinone Acetate on Time of Ovulation in Rats. (32596)

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It is well established that ovulation in 4-day cyclic rats, maintained in an environment with the lights on from 5 AM to 7 PM, occurs between 1:10 and 2:30 AM in the morning following the day of proestrus(1). Recently, Hoffmann and Schwartz(2) demonstrated that the time of luteinizing hormone (LH) release and ovulation, after withdrawal from 7 to 9 days of progesterone treatment, occurred during a prolonged period. During the course of experiments on the effects of gestagen on the estrous cycle and fertility in rats, satisfactory results with respect to a synchronized resumption of estrus and subsequent fertility were obtained with oral treatment with chlormadinone acetate (CAP) in our laboratory(3). It was thought desirable, therefore, to determine whether the time of the first ovulation in rats thus treated is modified by the treatment, in comparison with that in the normally cyclic rats.

Materials and methods. Nulliparous Wistar rats had been kept for one month in a room where illumination (lights from 6 AM to 6 PM) and temperature (25 ± 1°C) were controlled. The regularity of the estrous cycle (4 days) had been ascertained by vaginal smear for at least 2 cycles before the treatment started.

The body weight of rats was about 200 g at the start of treatment. The treated group was given CAP* in 0.5 ml of propylene glycol daily (9-10 AM) by stomach tube for 5 consecutive days. The daily dose was 10 mg. Treatment was started at random in the estrous cycle, since the day of recurrence of estrus was not influenced by the stage of the cycle when the treatment started(3). The smear was examined once every day (9-10 AM) during and after treatment. The vaginal smear became diestrous in most rats by the third day after treatment started, regarding the day of the start as day 0. Of 77 rats treated, 51 (61%) showed a proestrous smear on the third day after the end of the treatment. Nineteen (25%) showed an estrous smear on the same day, with or without having shown a proestrous smear on the previous day. These percentages of recurrence of proestrus and estrus are consistent with the previous results(3). The treated rats used in this study for the timing of ovulation were those which showed vaginal proestrus on the third day after withdrawal from CAP. The control rats consisted of non-treated rats in proestrus.

Rats of both groups were sacrificed within 10 minutes of 10 PM of the day of proestrus, or 12 M, 1, 2 and 6 AM of estrous morning. Examination for ovulation was performed by the detection of fresh ova in the oviducts. After careful excision of oviducts, the number of ova was counted under dissecting microscope (×20), lightly pressing the oviduct between 2 glass slides(4). The ovaries of all animals which had shown no ovulation at 1, 2 and 6 AM were histologically examined for the presence of large, stimulated follicles which would be expected to ovulate imminently. The weight of ovaries and uterus was recorded. The results obtained were compared with either the analysis of variance or χ^2-test. (Most of the rats which showed vaginal estrus one day earlier, that is, on day 3 after the end of treatment, were also killed during the morning of estrus(10-11 AM) to ascertain whether ovulation had occurred.)

Results. The results on time of ovulation are presented in Table I, together with the weights of ovaries and uteri. One positive rat with only one ovum was found at 12 AM in the treated group. Positive cases in ovulation in both control and treated groups began to appear at 1 AM and had increased rapidly by 2 AM.

In the histological examination of the ovaries it was seen that among the negative

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TABLE I. Ovulation Times After Withdrawal from Oral Treatment with Chlormadinone Acetate, and Weight of Ovaries and Uteri.

	No. ovulated/ No. autopsied	No. of ova	Wet weight (mg) of Ovaries	Uterus
Control				
10 PM	0/10	0	84 ± 13.0†	541 ± 73.4†
12 M	0/12	0	86 ± 13.0	504 ± 51.3
1 AM	5/11*	1.0 ± 1.26†	77 ± 11.8	480 ± 5.3
2 AM	10/10	5.9 ± 3.84[a]	80 ± 8.8	481 ± 76.2
6 AM	9/9	12.3 ± 1.41[b]	79 ± 12.7	469 ± 56.4
Treated				
10 PM	0/9	0	79 ± 11.6	488 ± 55.0
12 M	1/9	0.1	90 ± 14.6	465 ± 39.9
1 AM	2/9 *	1.1 ± 2.63	70 ± 10.9	454 ± 37.5
2 AM	9/10*	9.9 ± 2.69[c]	72 ± 13.0	443 ± 66.9
6 AM	8/9 *	12.8 ± 2.48[d]	76 ± 9.9	401 ± 31.8

* Group contains one rat which would not have ovulated on the day of examination, judging from histological check of follicles. Data on these rats have been excluded from the table.

† Mean ± S.D.

Statistical difference on ova counted: a *vs* b, (a + c) *vs* (b + d): $P < 0.01$.
a *vs* c, c *vs* d: $P < 0.05$.

rats at 1 AM both the control and treated groups contained one rat each whose follicles had not shown any preovulatory change. In the 2 and 6 AM treated groups, there was also one animal each which was obviously not going to ovulate that night (Table I). Data on these rats were eliminated from the Table.

The differences in numbers of ova between control and treated groups were significant ($P<0.05$) only at 2 AM. There was a significant increase in the number of ova from 1 to 2 AM and 6 AM in both groups of rats.

Of the 19 rats which came into a cornified vaginal smear on day 3 after the withdrawal, 11 were examined on the morning of day 3. Ova were found in 9 animals, the average number of ova being 13.5 ± 1.1.

Weight of ovaries and uteri. The differences in ovarian weight at different times in the treated groups were significant ($P<0.05$), but not in the controls. The difference in ovarian weight between the two groups was not significant; average ovarian weights for control and treated groups were 81 and 77 mg, respectively.

The uterine weight tended to decrease with time during the experiment in both groups, being significant within the treated groups ($p<0.05$), but not in the controls. The average uterine weights in control and treated groups were 495 and 450 mg, respectively, and the overall difference between the two groups was significant ($p<0.01$).

Discussion. All rats of both control and treated groups (except one in the latter) had started ovulating by 2 AM, but not by 12 M, starting ovulation at around 1 AM. In our colony LH release occurs between 1 and 3 PM on the day of vaginal proestrus judging from the results of one of us (Y.T.) with an ovulation blocking experiment (unpublished data). The object aim of the present experiment was to determine whether the time of ovulation after withdrawal of CAP would be altered in comparison with normal cyclic rats. This did not appear to be the case. From the results obtained, it is possible to say that some rats of our strain began to ovulate by 1 AM and all had by 2 AM. The number of ova detected at 2 AM has not yet reached the full number (11-13 ova), referring to the average number of corpora lutea found at a late stage of pregnancy(3), or the number of ova found in the rats which had returned to estrus one day earlier in the present study (13.5 ova).

Everett(5) described, in rats running 4-day cycles, the release of LH between 2 and 4 PM of proestrus and showed that this LH release was responsible for ovulation which began between 1:10 and 1:55 AM, and finished between 1:45 and 2:30 AM, regarding ovulation as complete when 8 or more ova were found in the oviducts. The present data (Table I) are similar. Hoffmann and Schwartz (2) showed that in most rats, 7 to 9 days after withdrawal of progesterone treatment, ovula-

tion occurred by the morning of day 5, but not by day 4, after the last injection. They considered from ovulation-blocking experiments that the time of LH release in this case was rather different from that of 4-day cyclic rats, *i.e.*, the LH release began about 2 PM but lasted longer. Subsequently most rats ovulated between about midnight and 5:30 AM. In addition, they claimed that the vaginal smear was not a reliable indicator of the day of ovulation. The apparent discrepancies between the results of the present experiment and theirs might be due to differences in the kind and dosage of steroids employed, and the route of steroid administration. Our results would appear to show by contrast that (i) the vaginal smear is reliable in predicting the day of ovulation, (ii) time of ovulation in rats treated orally with CAP and then withdrawn is similar to normal cyclic rats and (iii) ovulation occurred by day 4 after withdrawal from treatment.

Summary. After oral treatment with 10 mg chlormadinone acetate (CAP) for 5 days, the time of ovulation was examined by the detection of tubal ova in rats which came back into vaginal proestrus on day 3 after withdrawal (66% of treated rats). This was compared with that of normal cyclic rats. In both control and treated rats, some animals had started ovulating at 1 AM, and nearly all of the rats had ovulated by 2 AM. The number of ova increased from 2 to 6 AM. From the evidence obtained it can be said that in CAP-treated rats the time of ovulation did not differ from that of the normal cyclic (4 days) rats, *i.e.*, the timing of ovulation occurred in the same relation to the diurnal rhythm of lighting.

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Immunoglobulin Classes of Serum Neutralizing Antibody Formed in Response to Immunization with Dead Influenza Virus Vaccine. (32597)

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The immunological basis for protection against influenza virus infection is complex. Protection does not entirely correlate with serum antibody level(1) and seems to be higher after infection with live virus than after dead virus immunization(2). The present study was undertaken to determine, in humans, which class of immunoglobulins possessed neutralizing antibody activity in serum after parenteral immunization with dead influenza virus vaccine. This was done to establish whether this difference in protection could be explained on the basis of different classes of immunoglobulins.

Materials and methods. Serum specimens were obtained before and for 4 weeks after the initial dose of vaccine from 6 normal adult male volunteers hospitalized at the National Institutes of Health. Each volunteer received 2 weekly subcutaneous injections of 1 cc of the commercially available killed influenza

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