EFFECT OF OESTROGEN ON THE INCIDENCE OF DRUMSTICKS IN THE RABBIT NEUTROPHILS

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Summary: Adult female rabbits were given daily intramuscular injections of 0.5 *mg* of oestradiol diproprionate for four weeks. Weekly drum-stick counts were made from their blood smears and percent incidences of drum-sticks in the neutrophils was recorded. A highly significant increase in the drum-stick counts compared to the control animals was noted after one week's treatment with the oestrogen. Subsequent values after 2nd, 3rd and 4th week's treatment were not significantly different from values obtained from the corresponding untreated animals. It was concluded that oestrogen produces only an initial increase in the drumstick count and subsequent continued treatment neither increases nor decreases the drum-stick counts significantly.

Key words: oestrogen drum-sticks neutrophils

INTRODUCTION

The sex chromatin, generally believed to arise from the genetic inactivation of one of the two x-chromosomes is a distinguishing characteristic of the inter-phase nuclei of females in man and certain other animals. Its presence has been used clinically as a guide to the chromosomal sex in ambisexual patients. Although normally an inactivated x-chromosome will be expected to be present in each nucleus, sex chromatin is not demonstrable in all cells and its reported incdience varies from one study to another. Besides technical factors these variations have been attributed to alterations in the metabolic states within the cells (7). Accordingly steroidal hormones (Estrogens in particular) have been held responsible for the variations in the sex chromatic count during the menstrual cycle (2,3,9,10), pregnancy (14) and at various ages from the neonatal period to over 90 years (1,8,11,13,15). The results are, however, not only variable but at times contradictory. Low sex chromatin counts have been attributed to both higher estrogen levels as in the newborn female chlidren (12,13) and during pregnancy (14), as well as lower estrogen levels as in the post menopausal women (6). High sex chromatin counts on day 12 of the menstrual cycle (2) have been associated with the estrogen peaks. Experimentally an increase in the sex chromatin count after administration of natural as well as synthetic estrogen has been reported (5).

As such a study of the effect of oestorgen on the sex chromatin counts was considered worthwhile. Since it is more convenient to control the experimental conditions in the laboratory animals the study was conducted on the rabbits in which sexual dimorphism of the nuclei is known to exist, though, only in the neutrophils. Most other experimental animals like the guinea-pig, mouse, rat and hamster show no sexual dimorphism in any of their cells (9).

MATERIALS AND METHODS

Twenty adult female rabbits of approximately the same weight (1.5 to 1.7 *kg*) were taken for the study. Ten of these rabbits were given daily intramuscular injections of 0.5 *mg* oestradiol dipropionate (ovocycline, Ciba) in olive oil for four weeks. The treatment was started after ascertaining by vaginal smears that the animal was in the oestrus phase. The remaining ten rabbits were given daily intramuscular injections of olive oil only and served as the control animals. Weekly counts of the neutorphil drum-sticks of all the animals, experimental and control, were done by preparing peripheral blood smears and staining them with Harris Haematoxylin using the method of Carpentier *et al.* (4). The slides were examined under the oil immersion objective and 100 neutrophils were examined for the presence or otherwise of drumsticks, sessile bodies, rackets etc. were excluded. The percent incidence of the drum sticks were recorded.

OBSERVATIONS AND RESULTS

The percent neutrophil drumstick counts from the peripheral blood smears of the rabbits both experimental and control are given in a tabular form. The rabbits number one to ten (Table I) were treated with ovocyline while the rabbits number 11 to 20 (Table II) were the control animals. Under the column 'a' are the counts at the commencement of the experiment, before

TABLE I: Percentage of sex chromatin positive neutrophils in the rabbits before and after treatment with 0.5 *mg* daily parentral oestrodiol dipropionate for 4 weeks.

Animal number	*At the commencement*	*After 1st week's treatment*		*After 2nd week's treatment*		*After 3rd week's treatment*		*After 4th week's treatment*	
	% (a)	% (b)	*Diff.* (b-a)	% (c)	*Diff.* (c-a)	% (d)	*Diff.* (d-a)	% (e)	*Diff.* (e-a)
1	16	23	+ 7	21	+5	21	+5	21	+5
2	18	21	+ 3	13	—5	12	—6	11	—7
3	15	19	+ 4	13	—2	12	—3	11	—4
4	10	23	+13	16	+6	15	+5	15	+5
5	12	23	+11	21	+9	14	+2	14	+2
6	16	18	+ 2	14	—2	12	—6	10	—6
7	14	19	+ 5	18	+4	16	+2	10	—4
8	14	18	+ 4	11	—3	11	—3	11	—3
9	11	23	+12	19	+8	13	+2	10	—1
10	17	29	+ 2	14	—3	12	—5	12	—5
Group Mean	14.3	21.6		16.00		13.8		12.5	
S.D.	2.62	3.37		3.55		2.97		3.43	
S.E.	0.82	1.06		1.12		0.93		1.08	

TABLE II : Percentage of drum-stick positive neutrophils in control animals receiving 0.5 *mg* olive oil daily intramuscularly for 4 weeks.

Animal number	*At the commencement*	*After 1st week's treatment*		*After 2nd week's treatment*		*After 3rd week's treatment*		*After 4th week's treatment*	
	% (a)	% (b)	*Diff.* (b-a)	% (c)	*Diff.* (c-a)	% (d)	*Diff.* (d-a)	% (e)	*Diff.* (e-a)
11	12	10	—2	13	+1	12	—	13	+1
12	14	15	+1	13	—1	12	—2	13	—1
13	13	12	—1	16	+3	12	—1	11	—2
14	15	13	—2	14	—1	13	—2	16	+1
15	16	15	—1	20	+4	15	—1	15	—1
16	18	19	+1	19	+1	19	+1	16	—2
17	17	17	—	20	+3	16	—1	14	—3
18	11	13	+2	12	+1	12	+1	12	+1
19	15	14	—1	12	—3	14	—1	14	—1
20	13	13	—	10	—3	14	—1	14	+1
Group mean	14.4	14.1		14.9		13.9		13.8	
S.D.	2.22	2.55		3·63		2.28		1.61	
S.E.	0.70	0.80		1.14		0.72		0.50	

the injections were started. The subsequent weekly counts are recorded under the columns b,c,d and e. The values have also been compared with those at the commencement of the experiment and the differences have been recorded. The group mean figures of both the experimental and control animals have been cmpared in Table III. Fig. 1 is a graphic representation of the average neutrophil drumstick counts in the experimental animals over the four week's period of treatment.

TABLE III: Mean group comparison between test and control animal's drumstick counts.

S. No.	*Group*	*Mean at the commencement*	*Mean after 1st week's treatment*	*Mean after 2nd week's treatment*	*Mean after 3rd week's treatment*	*Mean after 4th week's treatment*
1	Test animals	14.3	21.6	16.0	13.8	12.5
2	Control animals	14.4	14.1	14.9	13.9	13.8
3	Difference (1-2)	—0.1	+7.5	+1.1	—0.1	—1.3
4	Standard error of the difference	1.077	1.327	1.597	1.175	1.189
5	t, value	0.092	5.651	0.688	0.085	1.093
6	Significance	not significant	highly significant	not significant	not significant	not significant

At the commencement of the study the drum-stick counts were found to be in the range of 11 to 18% in both the test and control animals with a group mean count of 14.3% and 14.4% respectively. After one week's treatment with oestrogen a rise in the counts was recorded. These were in the range of 18 to 29% with a group mean count of 21.5%. Individually, the rabbits of the test group showed an increase of the percent drm-stick count varying from 2 to 13.

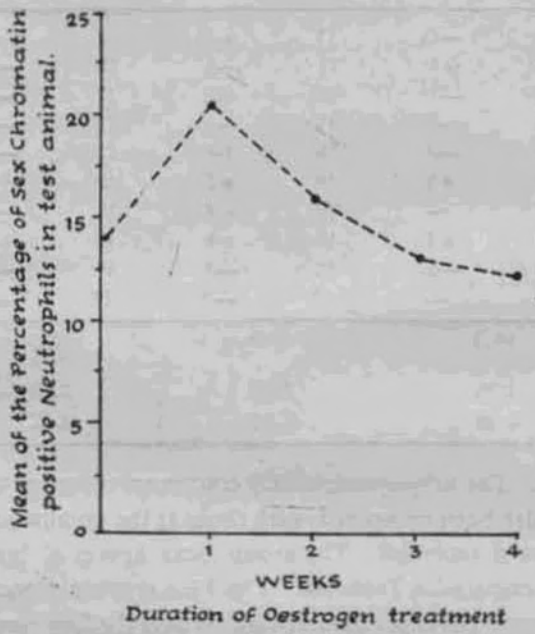

Fig. 1: Showing the pattern of drum-stick count in relation to the duration of oestrogen therapy in the test animals.

The counts in the control animals on the other hand were not much altered after one week's treatment with olive oil, the difference being of the order of $\pm$ 2 only. The alteration in the group mean count was also only fractional. A statistically significant difference in the drum-stick counts was found when the mean counts from the test animals after one week's treatment were compared with the mean count from the corresponding group of control rabbits (Table III).

Lower counts were noted after the second week of treatment with oestrogen. These ranged from 11 to 21% with the group mean of 16% $\pm$3.55. When compared with the corresponding value for the appropriate period from the control set, however, the difference was not found to be statistically significant.

The counts further dropped at the ends of the 3rd and 4th week of treatment with oestrogen with values of 13.8%$\pm$2.97 and 12.5%$\pm$3.43 respectively. But on comparison with the mean values from the corresponding control set the difference was found to be insignificant statistically.

DISCUSSION

The above observation show that oestrogen produces only an initial rise of the drum-stick count. Subsequently the counts come down and inspite of the treatment with oestrogen being continued, they do not differ significantly from those obtained from control animals. As such, although this study does not contradict the Previous reports (5) of high sex chromatin count with estrogen treatment it highlights the fact that the counts are raised with oestrogen only temprorily and revert to the original levels inspite of the treatment being continued. This is possible due to detoxification of the exogenous oestrogen by the metabolic system of the animals body or perhaps the leucocytes acquire some resistence to the influences of the exogenous oestrogen.

At no stage of treatment in the present study were the counts found to be significantly lower than the corresponding values from the control set. Therefore, lower sex-chromatin counts reported in the newborn female children (12,13) and during pregnancy (14) are perhaps found on account of factors other than high estorgen levels during these phases.

Conclusion

The results obtained in the present study indicate that oestrogen treatment brings about an increase in the sex chromatin count. But this increase is seen only during the early period of treatment. With further treatment counts fall down and do not differ significantly from untreated animals.

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