

10333 P

Colchicine Reactions in Ventral Prostate of Castrated Male Rats Following Androgenic Treatment.*

ELIZABETH Z. BURKHART. (Introduced by C. R. Moore.)

From the Department of Zoology, University of Chicago.

Since Lits¹ and Dustin² showed that colchicine arrests cell division, several workers have utilized the drug to study phases of reproductive processes and hormone activity in the female mouse,³⁻⁶ rabbit,⁷ pigeon,⁸ and the male rat,^{9, 10} and guinea pig.^{11, 12}

I have employed colchicine to study effects of male hormone on the ventral prostate of castrated male rats. The dosage was fixed at 0.1 mg per 100 g weight of rat (0.1 cc of a 1 mg/cc aqueous solution). Testosterone propionate was injected into 80-day-old rats castrated on day 40.

Experiment I. The castrated members of 2 litters were given 3 daily injections of 0.1 mg of testosterone propionate; on the 4th day each animal was injected with colchicine. Castrated controls treated with colchicine were available. At 2-hour intervals following subcutaneous injection of colchicine one animal from each litter was sacrificed. Coagulating glands, seminal vesicles and ventral prostates were removed. Over 1000 nuclei in the glandular epithelium of each ventral prostate were counted (Table I).

During the first 4 hours after colchicine injection all mitotically active cells showed the effect of colchicine by scattering or clumping the chromosomes without spindle formation as described in nor-

* This investigation has been aided by a grant from the Rockefeller Foundation to the University of Chicago. Testosterone propionate was provided by Dr. Gregory Stagnell (Schering Corporation).

¹ Lits, F., *Compt. rend. Soc. de biol.*, 1934, **115**, 1421.

² Dustin, A. P., *Bull. Acad. roy. de méd. de Belgique*, 1934, **14**, 487.

³ Allen, E., and Creadick, R. N., *Anat. Rec.*, 1937, **69**, 191.

⁴ Allen, E., Smith, G. M., and Gardner, W. U., *Am. J. of Anat.*, 1937, **61**, 321.

⁵ Rogers, P. V., and Allen, E., *Endocrinology*, 1937, **21**, 629.

⁶ Lits, F., *Compt. rend. Soc. de biol.*, 1935, **118**, 393.

⁷ Allen, E., Smith, G. M., and Reynolds, S. R. M., *Proc. Soc. Exp. Biol. and Med.*, 1937, **37**, 257.

⁸ LeBlond, C. P., and Allen, E., *Endocrinology*, 1937, **21**, 455.

⁹ Bastenie, P., and Zylberszac, S., *Compt. rend. Soc. de biol.*, 1937, **126**, 891.

¹⁰ Bastenie, P., and Zylberszac, S., *Compt. rend. Soc. de biol.*, 1937, **126**, 1283.

¹¹ Bastenie, P., and Zylberszac, S., *Compt. rend. Soc. de biol.*, 1937, **126**, 1282.

¹² Jailer, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 281.

TABLE I.

Litter No.	Hr after injection	Resting nuclei	Clumped colchicine figures	Scattered colchicine figures	Prophase nuclei	Clusters of small nuclei	Total	Percentage of colchicine figures
1	2	1250	12	51	20	0	1333	4.72
	4	1641	28	82	7	0	1758	6.25
	6	1072	88	43	17	2	1222	10.71
	8	1094	105	11	11	10	1231	9.41
	10	1352	145	3	29	11	1540	9.60
	12	1587	78	0	31	16	1712	4.55
2	2	1006	12	37	7	0	1062	4.61
	4	1185	56	134	4	0	1379	13.05
	6	1165	137	49	4	0	1355	13.50
	8	889	122	19	10	24	1064	13.25
	10	956	85	2	6	7	1056	8.23
	12	1034	39	1	19	9	1102	3.62

mal and tumorous mouse tissue¹³ and in plants.¹⁴ Prophase stages appeared unaffected 12 hours after injection. By the 6th hour most colchicine figures were of the clumped type and the total number of mitotically active cells reached its height. This mitotic index peak appears $3\frac{1}{2}$ hours earlier than in transplanted mouse tumors.^{2, 15} At 8 hours and thereafter abnormal clusters of small nuclei were present and the total number of colchicine figures gradually decreased.

Six hours was selected as the optimal period of colchicine treatment for study of the ventral prostate in the following experiment:

Experiment II. Of 29 males castrated on day 40, 11 received 0.1 mg testosterone propionate on day 80 and were killed at 4-hour intervals in pairs; one of the pair received colchicine 6 hours prior to autopsy, whereas the second animal of the pair received only androgen (Series I, Table II). Sixteen of the castrate males received a second injection of testosterone propionate on day 81 (Series II) and otherwise were handled as those of Series I. Two animals served as castrate controls. Mitotic figure counts were made under the 4 mm lens and an ocular micrometer disc with a field divided into 49 squares. The results are recorded as averages of the mitotically active cells per field.

Mitotic figures are not significantly increased until 36 hours after androgen injection. Only one mitotic cell was found in 50 fields in

¹³ Ludford, R. J., *Arch. f. exp. Zellforsch.*, 1936, **18**, 411.

¹⁴ Eigsti, O. J., *Proc. Nat. Acad. Sc.*, 1938, **24**, 56.

¹⁵ Dustin, A. P., Leeuwenhoek-Vereeniging, IVieme Conference tenue à Amsterdam, Juni, 1935.

TABLE II.

Time of autopsy after 1st dose of test. prop.,hr	Average No. of mitotic figures per field (50 fields)			
	Series I 0.1 mg test. prop.		Series II 0.2 mg test. prop.	
	Control	Colchicine treated	Control	Colchicine treated
15	—	.02		
19	.00	.02		
22	.02	.04		
27	.02	.10		
32	.04	.70	.06	.406
36	1.04	.40	1.50	.504
40			3.08	26.64
44			2.82	32.72
48			2.00	30.62
52			.66	20.88
55			2.54	18.74
60			3.44	40.18

colchicine-treated animals killed at 15 and 19 hours. One can find, very rarely, a mitotic cell in an untreated castrate control rat of the same age. Mitotic activity is highest between 40 and 44 hours, falls, and increases again at 60 hours. Work now in progress will show whether the second increase is due to the second dose of androgen.

Thirty-six hours after the first dose of testosterone propionate there is a sudden increase in the number of mitotic cells in the non-colchicine-treated animals while a corresponding increase after colchicine treatment occurs at 40 hours. This characteristic lag in activity in the colchicine-treated gland suggests that colchicine, having arrested mitosis during the early part of treatment, interferes with the normal mitotic rate just before the animal is sacrificed. Further work is necessary to determine the significance of this interference.

Conclusion. Mitotic activity in rat ventral prostate gland cells is studied to advantage 6 hours after colchicine administration. Testosterone propionate (0.1 mg daily) initiates cell division in castrate prostates 36 hours after injection; activity is maximal at 44 hours, subsides, and rises again. The action of colchicine is apparent only in dividing cells.