THE INFLUENCE OF HORMONES ON CELL DIVISION

II. TIME RESPONSE OF EAR, SEMINAL VESICLE, COAGULATING GLAND AND VENTRAL PROSTATE OF CASTRATE MALE MICE TO A SINGLE INJECTION OF TESTOSTERONE PROPIONATE[1]

J. M. ALLEN

Department of Zoology, University of Michigan, Ann Arbor, Michigan, U.S.A.

Received June 30, 1957

A PREVIOUS report has described the time-course mitotic response of seminal vesicle, coagulating gland, ventral prostate and ear epidermis to a single injection of estradiol benzoate [1]. This paper deals with the time-course mitotic response of these same tissues to a single injection of testosterone propionate.

MATERIALS AND METHODS

The methods employed in this investigation are identical to those used in prior work [1]. Testosterone propionate (Schering) has been administered as a single subcutaneous injection of 16 micrograms in 0.25 cc sesame oil (N.F.). Statistical comparison of data has been made by application of standard methods.

RESULTS

Comparison of mitotic indices obtained in tissues from intact control animals with those obtained in tissues from castrate control animals (Table I) indicates that orchidectomy results in a depression of mitotic activity in all cases. The *T*-test shows this drop to be significant in ear, coagulating gland and ventral prostate ($P < 0.01$) but not significant in the case of seminal vesicle ($P = 0.4$).

The response of ear epidermis to testosterone propionate is presented graphically in Fig. 1. Significant increases in mitotic activity over castrate levels are noted only during the periods 18–24 hours and 36–42 hours ($P < 0.02$) following hormone administration. Inspection of this curve reveals a series of fluctuations which have an apparent period of 18–30 hours.

[1] This research has been supported by grants from the Horace H. Rackham School of Graduate Studies, the University of Michigan and the National Cancer Institute of the U.S. Public Health Service (C-2751).

TABLE I. *Mitotic activity at six-hour intervals following the injection of 16 μg testosterone propionate into castrate male mice.*

For each tissue mean mitotic indices as log (per mille mitotic index × 100) followed by standard error values are given. The number of donor mice for tissues examined follows in brackets.

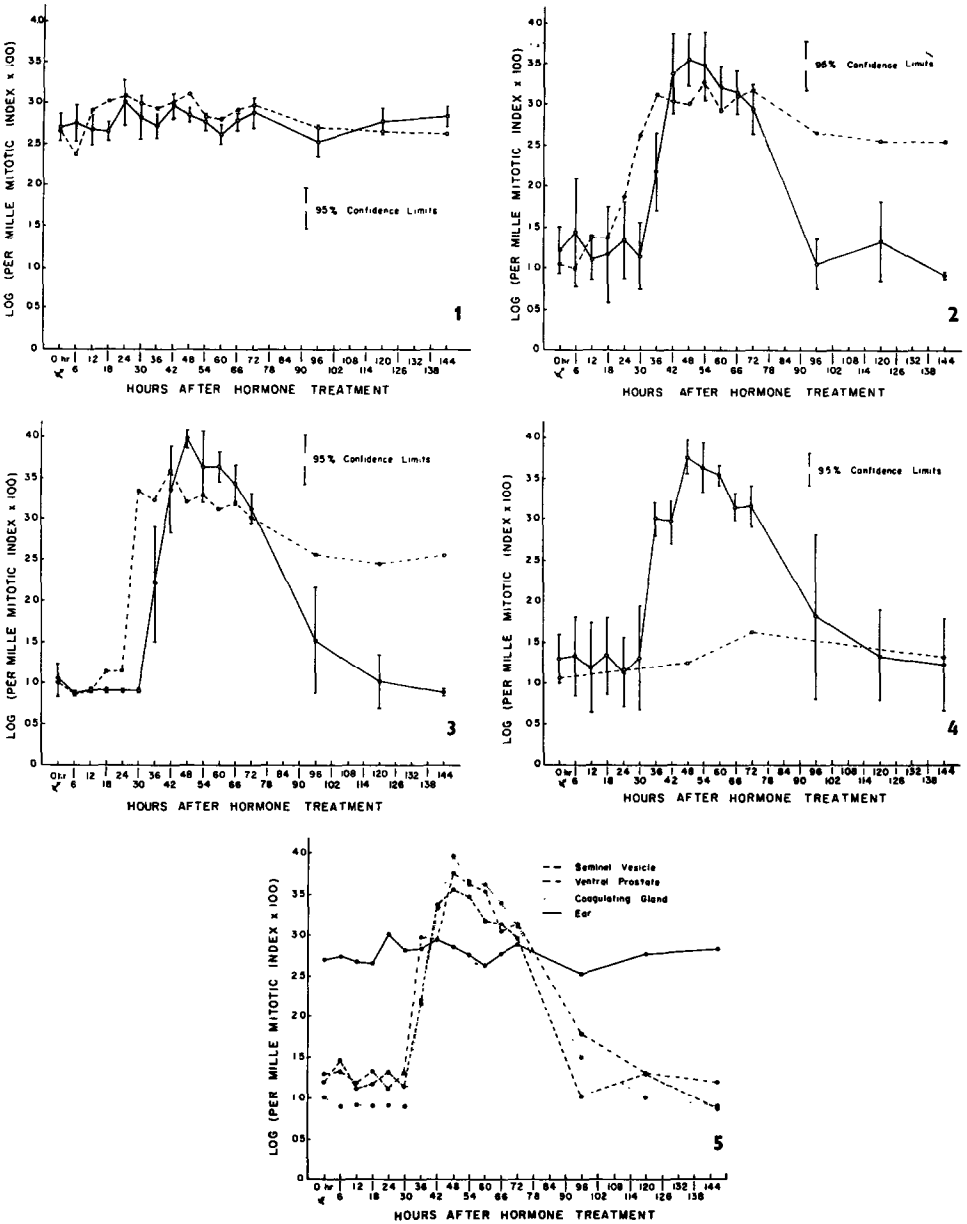
Sample category	Ear	Seminal vesicle	Coagulating gland	Ventral prostate
Normal	2.910 0.033 (10)	1.328 0.119 (10)	1.860 0.052 (10)	2.060 0.064 (10)
Castrate	2.702 0.062 (9)	1.208 0.123 (9)	1.034 0.088 (9)	1.301 0.127 (9)
0–6 hrs.	2.744 0.073 (5)	1.486 0.235 (5)	0.897 0.006 (5)	1.335 0.180 (5)
6–12	2.673 0.070 (6)	1.110 0.106 (6)	0.905 0.005 (6)	1.190 0.199 (5)
12–18	2.656 0.037 (5)	1.187 0.210 (5)	0.093 0.007 (5)	1.325 0.178 (5)
18–24	3.040 0.105 (5)	1.338 0.179 (5)	0.909 0.002 (5)	1.144 0.146 (5)
24–30	2.822 0.096 (5)	1.154 0.145 (5)	0.897 0.006 (5)	1.305 0.231 (5)
30–36	2.716 0.068 (7)	2.179 0.181 (6)	2.201 0.252 (5)	2.999 0.081 (7)
36–42	2.957 0.051 (5)	3.376 0.185 (5)	3.367 0.185 (5)	2.957 0.094 (5)
42–48	2.835 0.026 (5)	3.554 0.117 (5)	3.971 0.038 (5)	3.774 0.071 (5)
48–54	2.757 0.035 (5)	3.480 0.132 (5)	3.616 0.135 (4)	3.625 0.099 (4)
54–60	2.608 0.044 (6)	3.203 0.093 (5)	3.620 0.071 (6)	3.555 0.039 (5)
60–66	2.776 0.040 (9)	3.146 0.106 (7)	3.404 0.099 (7)	3.135 0.068 (7)
66–72	2.877 0.071 (5)	2.952 0.105 (5)	3.112 0.067 (5)	3.158 0.098 (5)
90–96	2.522 0.070 (5)	1.036 0.119 (5)	1.507 0.210 (4)	1.806 0.316 (4)
114–120	2.765 0.057 (5)	1.338 0.180 (5)	1.026 0.121 (5)	1.313 0.193 (5)
138–144	2.840 0.028 (5)	0.911 0.002 (5)	0.909 0.005 (5)	1.215 0.192 (5)

Analysis of variance between 0–72 hours indicates that these points form a heterogeneous series ($P < 0.01$) thus suggesting the fluctuations to be real.

The response of seminal vesicle epithelium is presented graphically in Fig. 2. A significant increase in mitotic activity above castrate levels is noted during the period 30–36 hours following hormone administration ($P < 0.01$). Peak activity is reached 42–48 hours after commencement of treatment. Mitotic activity has returned to castrate levels 96 hours after beginning of hormone treatment ($P > 0.10$).

Development of mitotic activity in the epithelium of coagulating gland is presented graphically in Fig. 3. Mitotic activity rises significantly above castrate levels during the period 30–36 hours after hormone injection ($P < 0.01$). Peak activity is developed 42–48 hours following treatment. Mitotic activity has returned to castrate levels at 120 hours ($P > 0.10$).

The response of ventral prostate epithelium is represented graphically in Fig. 4. Significant increase in mitotic activity in this tissue is noted during the period 30–36 hours after hormone administration ($P < 0.01$). Peak ac-



Figs. 1–5.—The response of epithelial cells to a single injection of 16 μg of testosterone propionate (solid line) and to a single injection of 16 μg of estradiol benzoate (1) (dashed line). Ninety-five per cent confidence limits are indicated by vertical lines. Each point on the abscissa (X) indicates the six-hour period during which mitoses were arrested by colchicine, and the actual time period involved is (X–6) to X hours. *Fig. 1.* Time response of ear Malpighian layer epithelial cells. *Fig. 2.* Time response of seminal vesicle epithelial cells. *Fig. 3.* Time response of coagulating gland epithelial cells. *Fig. 4.* Time response of ventral prostate epithelial cells. *Fig. 5.* Time response of the epithelial cells of Malpighian layer of ear, acinar epithelial cells of seminal vesicle, coagulating gland and ventral prostate.

tivity is reached 42–48 hours after institution of treatment. Mitotic activity has returned to castrate levels at 96 hours ($P > 0.10$).

The responses of all tissues examined are plotted on the same set of coordinates in Fig. 5. Examination of the data presented in this fashion emphasizes certain points. (*a*) All tissues exhibit a latent period before increased mitotic activity is evident. This latent period is constant in the case of seminal vesicle, coagulating gland and ventral prostate and is 30 hours in length. The latent period of ear epidermis is 18 hours. (*b*) Seminal vesicle, coagulating gland and ventral prostate reach peak mitotic activity 48 hours after hormone administration. The level of activity reached at this time by coagulating gland is significantly higher than that exhibited by seminal vesicle ($P < 0.02$). Ventral prostate is intermediate in response ($P > 0.05$ *vs.* seminal vesicle and coagulating gland). Seminal vesicle, coagulating gland and ventral prostate reach significantly higher levels of activity (48 hours) than ear epidermis (24 or 42 hours) ($P < 0.01$).

DISCUSSION

Comparison of the above results with those obtained following administration of estradiol benzoate [1] is of interest.

The response of ventral prostate epithelium to estradiol benzoate is in marked contrast to the response obtained with testosterone propionate (Fig. 4). This tissue shows no significant increase in mitotic activity until 72 hours after administration of estradiol benzoate. Testosterone propionate, however, elicits a significantly greater mitotic response (by inspection) and does so within 30–36 hours after hormone treatment. The response following treatment with this androgen is about 100 times greater than that obtained with estradiol benzoate.

The response of coagulating gland epithelium to estradiol benzoate and testosterone propionate is compared graphically in Fig. 3. Inspection of this figure indicates that the latent period is different under the two treatment conditions. With testosterone propionate it is 30 hours in contrast to 24 hours with estradiol benzoate. Testosterone propionate educes greater mitotic activity than does estradiol benzoate ($P < 0.01$; estradiol benzoate 42 hours *vs.* testosterone propionate 48 hours). Mitotic activity under estrogen treatment is more protracted than under androgen treatment. Activity following administration of estradiol benzoate remains significantly above castrate levels 144 hours after hormone treatment but with testosterone propionate has returned to castrate levels at 120 hours.

The response of seminal vesicle epithelium to estrogen and androgen is compared graphically in Fig. 2. Examination of this figure indicates that the latent period following administration of estradiol benzoate is 18 hours as opposed to 30 hours with testosterone propionate. The levels of mitotic activity reached under the two treatment conditions are equivalent ($P > 0.10$; testosterone propionate 48 hours *vs.* estradiol benzoate 54 hours; testosterone propionate *vs.* estradiol benzoate between 42 and 66 hours). Mitotic activity remains significantly higher than castrate levels for 144 hours following estradiol benzoate administration in contrast to the return to castrate levels at 96 hours following testosterone propionate treatment.

The mitotic activity evoked by androgen and estrogen in ear epidermis is represented graphically in Fig. 1. It will be noted that the latent period under estrogen treatment is 6 hours while under androgen treatment the latent period appears to be 18 hours. The mitotic response of this tissue to estradiol benzoate is significantly greater than the response to testosterone propionate ($P < 0.001$ between 24–72 hours).

From the above discussion it appears that the responses of the investigated tissues to testosterone propionate and estradiol benzoate differ in several respects. First, the latent period observed under estrogen treatment is variable while under androgen treatment it is constant at least in the case of seminal vesicle, coagulating gland and ventral prostate. Second, under estrogen treatment the time at which seminal vesicle, coagulating gland and ventral prostate reach peak activity is variable while under androgen treatment it is constant. Third, the level of mitotic activity reached following administration of these hormones differs significantly in the case of ear and ventral prostate. Ear epidermis is more responsive to estradiol benzoate while ventral prostate is more responsive to testosterone propionate. Seminal vesicle and coagulating gland respond nearly equally to both hormones. Fourth, return of mitotic activity to castrate levels in seminal vesicle, coagulating gland and ventral prostate is more rapid following testosterone propionate treatment than following estradiol benzoate treatment.

The time-course mitotic response of seminal vesicle and ventral prostate reported above compares favorably with the time-course mitotic response of these same tissues under androgen stimulation in the rat [4]. Thus, in the rat these tissues respond significantly 35 hours following hormone administration and reach their maximal activities at about 48 hours. The return to castrate mitotic levels is comparable in the two species and occurs in the rat about 100 hours after commencement of treatment.

The difference in response of the ventral prostate epithelium to testosterone

propionate and estradiol benzoate appears important in terms of the mechanism through which these hormones exert their effects on mitotic activity. Currently, it is common to assume that hormonal materials exert their characteristic effects by an influence on enzyme systems. Interpreted in this fashion the differential response of ventral prostate epithelium to androgen and estrogen suggests that these hormones exert their mitogenic actions through an influence upon different metabolic systems. The slight mitotic response elicited in the epithelium of ventral prostate by estradiol benzoate suggests that an estrogen sensitive metabolic step which is critical in terms of mitotic activity may be absent in this tissue. This step, of course, is present in those tissues responding mitotically to estrogen administration (e.g. seminal vesicle, coagulating gland or ear). Evidence in the literature suggests that one primary enzymatic reaction affected by estrogenic materials is the hexokinase reaction. Thus, Bullough [2] finds that estrogen treatment significantly enhances the mitotic activity of ear epidermis (measured *in vitro*) in the presence of gluose but has no enhancing effect when fructose or pyruvate are supplied. These results were interpreted as indicating the hexokinase reaction to be rate limiting in terms of estrogen-stimulated cell division in ear epidermis. Biochemical evidence also suggests that the hexokinase reaction is stimulated by estrogenic materials [9]. No information is available concerning the response of this enzyme in rodent ventral prostate to estrogen.

Seminal vesicle and ventral prostate of the rat may show decreased levels of oxidative metabolism following orchidectomy [6, 8]. Administration of androgen reverses this change [6, 8]. The activity of the cytochrome oxidase, succinic dehydrogenase, aconitase, fumarase and malic dehydrogenase systems in these tissues is depressed by castration [5, 10]. Administration of androgen reverses the changes in the activity of these enzymes [5, 10]. These facts indicate that androgens may affect primarily the reactions of the tricarboxylic acid cycle. If this assumption is correct it might be expected that the enzymatic steps which are critical in the development of mitotic activity under androgen treatment lie in this metabolic area. No direct evidence bears on this question. However, Bullough [3] has shown that testosterone treatment does not enhance the ability of glucose to maintain mitotic activity in ear epidermis (measured *in vitro*). This fact suggests that the metabolic points of androgenic stimulation of mitotic activity lie distal to the preliminary phosphorylation of glucose.

One time-course study in the literature presents data on the development of oxidative metabolism in secretion-free rat seminal vesicle following androgen treatment [7]. Q_{O_2} values under these conditions reached maximal levels 48

hours after commencement of hormone treatment and then declined to control levels even under continued hormone administration. The correlation of this increase in Q_{O_2} level with development of mitotic activity is apparent.

SUMMARY

The development of mitotic activity following administration of a single dose of testosterone propionate has been followed in the epithelia of ear, seminal vesicle, coagulating gland and ventral prostate of castrate male mice. All tissues respond to androgen treatment by significantly increased mitotic activity. Ear epidermis shows significantly increased activity 18 hours following hormone treatment while all other tissues show significantly increased activity 30 hours after treatment. Seminal vesicle, coagulating gland and ventral prostate reach peak activity 48 hours following hormone injection. Mitotic activity in these tissues has returned to castrate levels 96–120 hours after commencement of treatment. The response of these tissues to testosterone propionate is compared with the response to estradiol benzoate [1]. The results are discussed in terms of hormonal effects upon metabolic systems which may be critical in the development of mitotic activity.

REFERENCES

1. Allen, J., *Exptl. Cell Research* **10**, 523 (1956).
2. Bullough, W., Ciba Foundation Colloquia on Endocrinology, Vol. VI, Hormonal Factors in Carbohydrate Metabolism, p. 278. Little, Brown and Company, Philadelphia, 1953.
3. —— *Vitamins and Hormones* **13**, 261 (1955).
4. Burkhart, E., *J. Exptl. Zool.* **89**, 135 (1942).
5. Davis, J., Meyer, R. and McShan, W., *Endocrinology* **44**, 1 (1949).
6. Nyden, S. and Williams-Ashman, H., *Am. J. Physiol.* **172**, 588 (1953).
7. Porter, J. and Melampy, R., *Endocrinology* **51**, 412 (1952).
8. Rudolph, G. and Samuels, L., *Endocrinology* **44**, 190 (1949).
9. Walaas, O., *Acta Endocrinol.* **10**, 175 (1952).
10. Williams-Ashman, H., *Endocrinology* **54**, 121 (1954).