X-RAY EFFECTS ON MITOTIC ACTIVITY OF THE ACCESSORY SEX ORGANS OF CASTRATE RATS STIMULATED BY TESTOSTERONE PROPIONATE[1]

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The effects of irradiation of the mouse testis with 320 r of x-rays have been discussed in a previous paper (Bryan and Gowen, 1956). The testis is characterized by a relatively high level of mitosis and in this respect is quite different from most other organs. Our findings indicate that irradiation markedly inhibited mitotic activity in spermatogonia. In addition data were obtained which suggested that irradiation-induced inhibition of desoxyribose nucleic acid (DNA) synthesis contributed to this suppression of mitotic activity. Observations on other tissue cells would broaden the significance of these findings both to the normal mitotic behavior of these cells and to the effects of radiation on them.

The mitotic behavior of the accessory organs of the castrate rat, the seminal vesicle and the dorsal prostate, is of significance to this problem. These organs may have high mitotic rates. They have the further advantage that the rates may be controlled through castration which reduces mitotic activity through removal of hormonal stimulation and/or by testosterone injections which enhance the mitotic activity (Moore *et al.,* 1930; Burrows, 1940; Cavazos and Melampy, 1954; Melampy *et al.,* 1956 and others).

The response of these tissues to irradiation and/or hormone treatments as described herein was measured by mitotic counts coupled with cytophotometric measurements of the DNA-Feulgen content of interphase nuclei. This approach has the advantage that the methods complement one another. Together they provide information with respect to the relations between the visual manifestations of mitotic activity and certain underlying biochemical activities.

Materials and Methods

Male rats of Fischer line 344 were used in the present experiments. The animals were castrated when nine weeks old and the accessory organs allowed to regress for twenty days. All the experimental animals were then given daily subcutaneous injections of testosterone propionate (500 μg) in oil.[2] Half the animals were anesthetized and exposed to 320 r of x-rays (130 pkv; 10 ma; filtration 0.25 mm. Al; anode-target distance 20 cm. in air; dose rate 320 r/min.). The irradiation was delivered to the pelvic region only, the rest of the body being shielded with lead. The irradiation was given coincident with the initial hormone injection.

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[2] Peranderan, Ciba Pharmaceutical Products, Inc., Summit, N. J.

Pairs of animals were killed at 24, 48, 60 and 72 hours following irradiation and/or initial hormone injection. The seminal vesicles and dorsal prostate were rapidly removed, cut into small pieces, blotted to remove any secretion and dropped into the fixative. Tissues were fixed overnight in 10% neutral formalin, washed for 24 hours in running water and then divided into two portions. One was de-

TABLE I

The effects of irradiation and of androgen injection on the mitotic activity of the secretory epithelium of the accessory organs of castrate male rats

Treatment	Seminal vesicle			Dorsal prostate		
	No. cells counted	% Mitosis	Mitosis as % of control	No. cells counted	% Mitosis	Mitosis as % of control
Intact controls	7,697	0.25	—	7,733	0.52	—
20 day castrates	8,753	0.10	40	8,861	0.06	11.5
20 day castrates:						
(a) 24 hrs. after 1st hormone injection	9,280	0.38	152	7,326	0.38	73.1
24 hrs. after 1st hormone injection and x-rays	7,637	0.41	164	6,796	0.43	82.7
(b) 48 hrs. after 1st hormone injection	7,575	1.49	596	8.171	0.71	136.5
48 hrs. after 1st hormone injection and x-rays	13,375	1.30	520	8,752	0.63	121.1
(c) 60 hrs. after 1st hormone injection	7,162	2.76	1,104	11,901	2.05	394.2
60 hrs. after 1st hormone injection and x-rays	7,832	3.12	1,248	7,570	0.99	190.4
(d) 72 hrs. after 1st hormone injection	9,901	3.53	1,412	11,742	1.97	378.8
72 hrs. after 1st hormone injection and x-rays	7,254	2.25	900	7,603	1.82	350.0

hydrated, cleared in benzene and embedded in 56–58° C. Tissuemat; the other was taken up to 70% alcohol and stored in the refrigerator. Kidney tissue from control rats also was fixed and processed in the above manner.

The embedded material was sectioned at 6 μ and the slides therefrom were used for mitotic index determinations. The material stored in alcohol was used to prepare isolated nuclei for photometric purposes since examination of sectioned material indicated that most nuclei were badly overlapped.

Small pieces of tissue were run down to water and hydrolyzed in 1 N HCl for 12 minutes at 60° C., washed, and stained *in toto* by means of the Feulgen reaction for two hours at room temperature (Stowell, 1945). After passing through the bleach baths the tissue pieces were washed in distilled water, passed into 45% acetic acid and left for 10 minutes. Small fragments were then removed into

TABLE II

An analysis of the variation in the mitotic index data

Treatment	Animals compared	Between organs or rats			Among counts			Binomial error	
		M.S.	df	F[1]	M.S.	df	F		df
Control	Rat A—S.V. & D.P.	.000	1		.004	2		.003	6903
	Rat B—S.V. & D.P.	.042	1	8.9**	.002	4		.005	8458
	Rats A & B—S.V.	.000	1		.003	4		.002	7673
	Rats A & B—D.P.	.029	1		.003	3		.005	7688
20-day castrate	Rat A—S.V. & D.P.	.001	1		.000	1		.001	7503
	Rat B—S.V. & D.P.	.000	1		.000	2		.001	10,090
	Rats A & B—S.V.	.000	1		.000	2		.001	8740
	Rats A & B—D.P.	.000	1		.000	1		.001	8853
24-hr. hormone	Rat A—S.V. & D.P.	.001	1		.001	5		.004	8851
	Rat B—S.V. & D.P.	.001	1		.004	4		.003	7707
	Rats A & B—S.V.	.000	1		.003	5		.004	9238
	Rats A & B—D.P.	.003	1		.002	4		.004	7320
24-hr. hormone + x-rays	Rat A—S.V. & D.P.	.000	1		.002	3		.004	7121
	Rat B—S.V. & D.P.	.001	1		.001	3		.004	7242
	Rats A & B—S.V.	.001	1		.001	4		.004	7600
	Rats A & B—D.P.	.000	1		.003	2		.004	6963
48-hr. hormone	Rat A—S.V. & D.P.	.040	1	4.4*	.009	3		.009	7054
	Rat B—S.V. & D. P.	.281	1	23.4**	.003	4		.012	8510
	Rats A & B—S.V.	.118	1	7.9**	.006	4		.015	7450
	Rats A & B—D.P.	.002	1		.006	3		.007	8108
48-hr. hormone + x-rays	Rat A—S.V. & D.P.	.005	1		.008	7		.007	11,591
	Rat B—S.V. & D.P.	.348	1	24.8**	.016	6		.014	10,290
	Rats A & B—S.V.	.404	1	30.3**	.014	8		.013	13,191
	Rats A & B—D.P.	.000	1		.008	5		.006	8690
60-hr. hormone	Rat A—S.V. & D.P.	.010	1		.097	4	4.0**	.024	9304
	Rat B—S.V. & D.P.	.358	1	15.5**	.054	5	2.4*	.023	9304
	Rats A & B—S.V.	.044	1		.093	3	3.4*	.026	6959
	Rats A & B—D.P.	.084	1	4.2*	.063	6	3.1**	.020	11,649
60-hr. hormone + x-rays	Rat A—S.V. & D.P.	1.191	1	56.7**	.025	4		.021	7617
	Rat B—S.V. & D.P.	.689	1	34.4**	.009	4		.020	7454
	Rats A & B—S.V.	.027	1		.029	4		.031	7582
	Rats A & B—D.P.	.007	1		.005	4		.010	7489
72-hr. hormone	Rat A—S.V. & D.P.	1.190	1	37.0**	.064	6	2.0	.032	11,593
	Rat B—S.V. & D.P.	.185	1	9.4**	.009	5		.020	9454
	Rats A & B—S.V.	.744	1	21.2**	.074	5	2.1	.035	9544
	Rats A & B—D.P.	.136	1	6.8*	.010	6		.020	11,503
72-hr. hormone + x-rays	Rat A—S.V. & D.P.	.072	1	3.5	.061	4	3.0*	.021	7246
	Rat B—S.V. & D.P.	.011	1		.081	4	4.0**	.020	7298
	Rats A & B—S.V.	0.012	1		.061	4	2.7	.022	7085
	Rats A & B—D.P.	.003	1		.081	4	4.5**	.018	7459

[1] F values marked * shows significance at the 0.05 level and ** at the 0.01 level.

drops of 45% acetic acid on microscope slides, covered with a coverslip and macerated with the aid of a Burgess Vibro-graver equipped with a plastic tip. By this means epithelial nuclei were isolated in a manner suitable for photometric purposes.

TABLE III

DNA-Feulgen content of seminal vesicle and dorsal prostate nuclei

	Seminal vesicle			Dorsal prostate		
	Class	N	DNA-Feulgen content	Class	N	DNA-Feulgen content
Intact controls	II	50	3.58 ± 0.07	II IIa	49 1	3.26 ± 0.06 4.41
20-day castrates	II	50	3.13 ± 0.06	II III	49 1	3.11 ± 0.07 6.36
20-day castrates: (a) 24 hrs. after 1st hormone injection	II	50	2.92 ± 0.06	II IIa III	48 1 1	2.35 ± 0.08 3.51 4.27
24 hrs. after 1st hromone injection and x-rays	II IIa	49 1	2.58 ± 0.07 4.60	II	50	2.49 ± 0.06
(b) 48 hrs. after 1st hormone injection	II IIa III	25 7 18	3.05 ± 0.10 4.35 5.73 ± 0.12	II IIa III	40 2 8	2.74 ± 0.07 4.13 5.71
48 hrs. after 1st hormone injection and x-rays	II IIa III	38 5 7	3.14 ± 0.08 4.80 6.05	II IIa III	43 3 4	2.74 ± 0.08 4.29 5.35
(c) 60 hrs. after 1st hormone injection	II IIa III	25 9 15	2.61 ± 0.11 3.83 4.96 ± 0.27	II IIa III	37 5 8	1.63 ± 0.06 2.65 3.19
60 hrs. after 1st hormone injection and x-rays	II IIa III	27 6 17	2.61 ± 0.11 4.18 4.74 ± 0.11	II III	48 2	2.10 ± 0.08 3.87
(d) 72 hrs. after 1st hormone injection	II IIa III	39 2 9	3.11 ± 0.08 4.42 5.88	II	50	2.39 ± 0.06
72 hrs. after 1st hormone injection and x-rays	II IIa III	26 14 10	2.74 ± 0.11 4.19 ± 0.10 5.73 ± 0.46	II IIa	49 1	2.24 ± 0.04 3.67

The slides were frozen on a block of dry ice and the coverslips removed. Slides were passed into 95% alcohol, further dehydrated and mounted in oil of matching refractive index.

Measurements of the DNA-Feulgen complex were made with the apparatus as described previously (Bryan and Gowen, 1956). Transmittance data were ob-

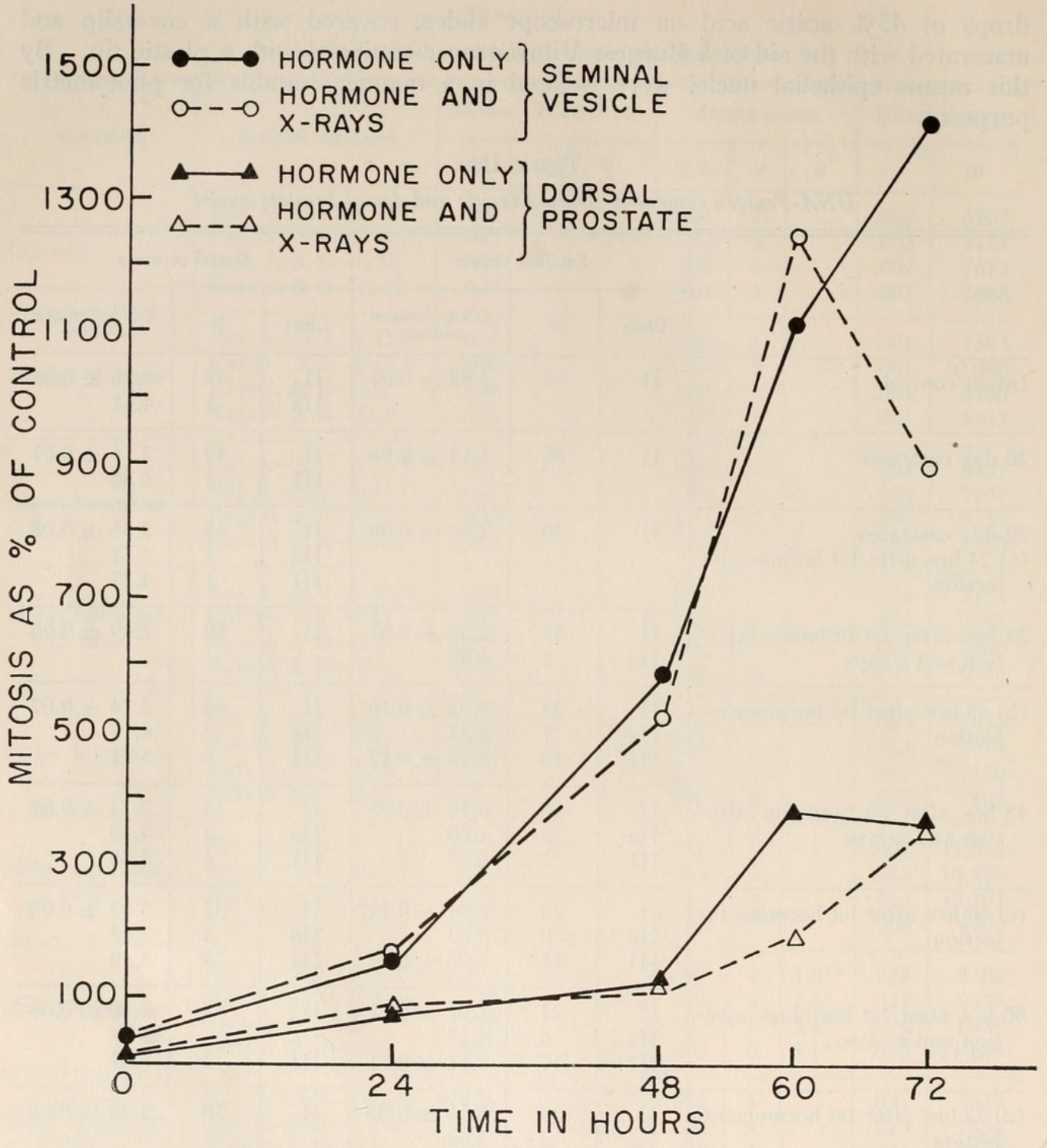

FIGURE 1. The mitotic response of seminal vesicle and dorsal prostate epithelium at different times following irradiation and/or hormonal stimulation.

tained by the "plug" method of Swift (1950); however, since the majority of nuclei were ellipsoidal rather than spherical, the formula derived by Kasten (1956) was substituted for the one devised by Swift.

An important factor in the utilization of the Feulgen procedure for cytophotometric purposes is the precise control of the hydrolysis step. When small pieces of tissue—rather than thin sections—are used, the hydrolysis procedure becomes subject to more variation. The end result is that DNA-Feulgen values for the same tissue processed in two separate procedures may be more variable

than the results obtained with sectioned material processed in a similar manner. However, for any single piece of tissue the staining appears to be quite uniform. Thus in the case of the kidney, DNA-Feulgen measurements made on fragments drawn from different regions of the same piece did not differ significantly from each other. This experiment was repeated at a later date with a fresh batch of Feulgen reagent and similar results were obtained. However, the mean values for the DNA-Feulgen content were about 28% higher. In view of these findings and of the fact that the slides were made at different times involving different batches of the Feulgen reagent, care must be taken not to misinterpret the variation between the mean DNA-Feulgen values reported in Table III.

Feulgen-stained sections were used for mitotic index determinations. A total of 3000–6000 cells was counted and classified per tissue per animal. Fields were chosen at random, every cell in the field being classified and recorded.

Results

A. *Mitotic index determinations*

The data obtained are summarized in Table I. The values in columns 2, 3, 6 and 7 of the table represent the combined counts from each pair of animals. In columns 5 and 9 the mitotic activity is expressed as per cent of control values for seminal vesicle and dorsal prostate, respectively. These values are presented in graphical form in Figure 1.

Seminal vesicle

The data indicate that by 20 days following castration the level of mitotic activity has declined to about 40% of the control value. The hormone-treated animals show a steady increase in activity reaching a peak at 72 hours after the initial injection was given. The level of activity reached at this time represents a fourteen-fold increase over the value obtained for the controls. In the case of the animals receiving irradiation as well as hormone, the rise in mitotic activity is similar over the period 0 through 48 hours. Here, however, the peak of mitotic activity is reached at 60 hours and the level then undergoes a marked decline. This 60-hour value is not very different from the corresponding value of the animals which received hormone alone.

Dorsal prostate

In this organ the response was found to be quite different from that of the seminal vesicle (Fig. 1). At no time during the experiment did the levels of mitotic activity of the dorsal prostate reach levels comparable with those determined for the epithelium of the seminal vesicle. The level of activity increased more slowly over the period 0 through 48 hours. In the case of the animals which received hormone alone the mitotic activity reached a peak at 60 hours and essentially the same level was found at 72 hours. Unlike the conditions prevailing in the seminal vesicle, the mitotic activity in irradiated and hormone-treated animals did not reach a peak until the end of the experimental period, the value being almost identical with the terminal value for the hormone-only group.

In certain pairs of animals some variation in the mitotic index is apparent. As examples of the magnitude of these variations the most extreme cases are presented herewith. In the seminal vesicle, the 48-hour hormone + x-ray data range from 0.75% (rat A) to 1.83% (rat B). Similarly in the case of the dorsal prostate, mitotic counts for the 72-hour hormone animals range from 1.63% to 2.29%. A statistical analysis of the variation in the mitotic index data is set forth in Table II. The variation between seminal vesicles at 48 and 72 hours following the initial hormone injection and at 48 hours after hormone and irradiation is significant at the 1% level. The dorsal prostates show some variation between rats at 60 and 72 hours after the start of the hormone treatment. These differences in response are significant at the 5% level.

On account of this observed variation between animals in the present work, differences in the magnitude of the mitotic response of irradiated versus unirradiated animals should be viewed with circumspection. However, the data do establish the trend in response to the treatments. Within this framework, the mitotic counts when taken together with the DNA-Feulgen data do allow meaningful conclusions to be drawn.

B. *DNA-Feulgen content of nuclei*

The data obtained are presented in Table III. Tissue from each member of pairs of animals was subjected to the photometric procedure. From each animal and tissue, samples of twenty-five nuclei were measured. The means are therefore based on samples of fifty measurements each. The mean amount of DNA-Feulgen complex, in arbitrary units, together with their respective standard errors are listed in columns 4 and 7 of the table. Nuclei possessing the diploid amount of DNA-Feulgen complex are designated as Class II nuclei, those with twice this amount Class III, and Class IIa represents nuclei possessing an amount of DNA-Feulgen complex intermediate between these levels.

The range of values for the DNA classes was determined from measurements of nuclei isolated from kidney tissue of control animals. The spread of values was slightly variable; thus two samples gave highest values of 1.4–1.5 units higher than the lowest (for example, ranging from 2.69 units to 4.17 units) while in two samples measured at a later date a range of 1.2 units was obtained (2.15–3.37 units).

Since the kidney nuclei have a more uniform appearance than the seminal vesicle or dorsal prostate nuclei, the larger range of values was chosen as a better approximation to the range expected for the diploid class. An approximation to the range expected for Class III nuclei was made by doubling the values obtained for the Class II nuclei in each set of measurements. Any values falling between the upper limits of Class II and the lower limits of Class III were assigned to Class IIa.

Seminal vesicle

It is evident from Table III that until 48 hours following irradiation and/or the initial hormone injection, the DNA-Feulgen values fall almost entirely into Class II. At 48 hours significant numbers of Class III nuclei appear but they are

much less frequent in the irradiated material. By 72 hours following the initial hormone injection the frequency of Class III nuclei has declined to about half the 48-hour value. In the case of the irradiated animals the highest number of Class III nuclei appears in the 60-hour material.

This variation in number or lack of Class III and Class IIa nuclei is not due primarily to errors of sampling (though the latter may contribute to the variation) since duplicate samples have yielded essentially the same results.

Dorsal prostate

In the section on mitotic activity it was pointed out that the epithelium of the dorsal prostate was much less mitotically active than that of the seminal vesicle. This behavior is paralleled by the synthetic activity—as judged by the lower frequency of Class IIa and Class III nuclei. Not only are these classes almost absent until the 48-hour period, but also at 72 hours following the start of the hormone treatment. This latter decline is in contrast with the findings with respect to the seminal vesicle.

Discussion

As pointed out earlier, the testis is characterized by a high rate of spermatogonial mitosis. It is this mitotic activity which is markedly inhibited following irradiation. Other data indicate that inhibition of DNA synthesis in interphasic spermatogonial nuclei contributes to this suppression of mitotic activity. It would therefore seem likely that other mitotically active tissues would respond in a similar manner following exposure to similar x-ray doses.

A brief resumé of the spermatogonial response is given here to facilitate comparison with the results of the present work. In the normal mouse testis 4 per cent of the spermatogonia appeared to be undergoing mitosis at a given time. Spermatogonial divisions rapidly declined to less than 5 per cent of the control level by 3 days after exposure to 320 r of x-rays. A rise in mitotic activity was apparent 5–10 days following irradiation. The highest level of activity was not observed until the termination of the experiment, *i.e.*, at 28 days after exposure. At this time the level attained was slightly more than twice that of the controls.

As may be seen from Table I and Figure 1, the mitotic response of the accessory organs follows a pattern quite different from that just described. The onset of activity occurs in a matter of hours rather than days. This suggests that the period of mitotic inhibition is markedly contracted.

In the case of the seminal vesicle peak mitotic activity is reached at 60 hours after irradiation. Moreover, this peak value is approximately 12 times higher than the control level. The mitotic response of this organ is then much stronger and more rapid than in the case of the testis.

With respect to the dorsal prostate the highest level of activity following irradiation was not reached until 72 hours after exposure. This level was 3.5 times higher than the control. The response of the dorsal prostate is clearly on a much lower level than that of the seminal vesicle.

It would appear from the above discussion that the radiation response of somatic and germinal tissue is quite different. This may be a reflection of under-

lying metabolic differences between tissues. This idea receives some support from the recent work of Pelc and Howard (1956). These workers, using C^{14}-labelled adenine, found a difference in incorporation between spermatogonia and somatic tissues such as seminal vesicle, skin and intestine. Their data suggest that a DNA precursor becomes maximally labelled soon after injection and is drawn upon by spermatogonia but not by somatic tissues. There is then the possibility that the DNA of reproductive cells may be synthesized in a somewhat different manner from that of somatic cells. These biochemical processes may differ in their sensitivity to x-rays.

Certain other factors may also contribute to the observed differences in response. As is evident from our present data and from the prior work of Cavazos and Melampy (1954), hormonal stimulation of mitotic activity does not, in these organs, become markedly effective until 48 hours after the initial injection. It has been pointed out by Lea (1955) that the effect of the x-ray dose decays with time (see page 289). So it is possible that the effect of the dose has undergone some decay before the inhibitory action can become effective. In other words, the "effective" dose of x-rays may not be identical for the two experiments.

The response of the seminal vesicle to androgen and irradiation treatments has been studied by several other workers (see for example Cavazos and Melampy, 1954; Fleischmann and Nimaroff, 1954; Melampy *et al.,* 1956). The results reported here differ in some aspects from those of the studies; however, taken as a whole they do show similar trends. These previous workers used colchicine to facilitate mitotic index determinations whereas colchicine was not used in the experiments reported here. Therefore the mitotic indices listed in Table I are much lower than those published by the workers cited above.

The data of Fleischmann and Nimaroff (1954) and of Melampy *et al.* (1956) were interpreted as suggesting that following irradiation there is a delay in the onset of mitotic activity. After doses of 640 r or less (Melampy *et al.,* 1956) the delay was such that the peak level of mitotic activity was observed at 60 hours. Furthermore, Fleischmann and Nimaroff (1954) found that if an interval of 5 days was allowed between irradiation and hormone injection the delaying effect was lost. This latter observation is of interest inasmuch as it clearly demonstrates that recovery occurs after a partial-body exposure to 3000 r.

In the present work, the 60-hour mitotic index following exposure to 320 r was only about 12% higher than that of the controls (hormone alone) whereas Melampy *et al.* found corresponding values to differ by a factor of two. Strain differences may be involved since the Fischer strain (line 344) appears to be more radioresistant than certain other strains (Dunning, personal communication). On this basis a shortening of the delay period would be expected with the consequence that the mitotic index would be depressed towards control levels. Also from a statistical viewpoint, the numbers of animals per time period are rather small so that errors of sampling are of increased significance (see elsewhere in this paper for further discussion of sources of variation which bear on this point).

As shown in Figure 1, the mitotic response of the dorsal prostate following irradiation and/or hormonal stimulation is on a much lower level than that of the seminal vesicle. This is most probably a reflection of innate differences in activity of these organs as well as possible differences in magnitude of the response to the

experimental treatments. The shape of the curves for the dorsal prostate would suggest that recovery from any induced mitotic delay does not occur until close to the end of the experimental period. This is in contrast with the findings for the seminal vesicle.

It has been shown by various workers that the synthesis of chromosomal material in preparation for mitotic division occurs during the interphase. Experiments with radio-active precursors by Pelc and Howard (1952), Taylor and McMaster (1954) and recently, by Taylor *et al.* (1957), point clearly in this direction. Cytophotometric studies (see for example Swift, 1950; Bryan, 1951; Taylor and McMaster, 1954) are in agreement with the results obtained by autoradiographic procedures.

From the above work it should follow that any treatment which suppresses or inhibits mitotic activity should result in the absence or reduction in number of Class III nuclei. In a sample of nuclei selected at random from a mitotically active tissue the relative proportions of the DNA classes will depend upon the speed at which DNA synthesis is accomplished and the rate at which Class III nuclei enter into prophase. Thus if conditions are such that Class III nuclei enter division without any intervening delay, the chances of encountering such nuclei in a sample are rather small. If, on the other hand, chromosomal reduplication is proceeding at a rate appreciably faster than that at which such nuclei enter division, then Class III nuclei should accumulate and should constitute a significant proportion of the measured sample. The mitotic activity—measured in terms of reduplication of the DNA content of interphase nuclei—will then be a measure of the balance between these mechanisms. It should also follow that, within certain limits, a comparison of the DNA-Feulgen data and the mitotic counts should afford some insight with respect to the nature of the response evoked by agents which inhibit or enhance mitotic activity.

In the case of the intact controls, where mitotic activity was found to be rather low and most probably represented replacement of dead cells rather than tissue growth, 98–100% of the nuclei measured fell into the diploid DNA class (Class II). Similar results were obtained with respect to the 20-day castrates where the mitotic index was still lower. Not until the 48–hour period were significant numbers of Class III (and IIa) nuclei encountered. With respect to the seminal vesicle material, the proportion of Class III nuclei at this time was 63% in the case of the unirradiated animals, whereas following irradiation 14% of the sample was composed of such nuclei. At 60 hours after the initial hormone injection the proportion of Class III nuclei remained about the same. In the case of the 60-hour irradiation and hormone-treated material, the proportion of Class III nuclei had increased to 34% (from 14% at 48 hours). At 72 hours after the start of the treatment, the proportion of Class III nuclei had fallen to 18% of the sample from animals receiving hormone alone and to 20% in the case of the irradiated animals.

These cytophotometric data indicate that the tissue response evoked by the irradiation treatment is rather similar to the response to hormone injection but that a difference in timing is apparent. In the case of the animals receiving only hormone, the highest frequency of Class III nuclei is reached at 48 hours following the initial injection. After irradiation, on the other hand, the peak frequency is not attained until 60 hours after exposure. Moreover, following irradiation, the

60- and 72-hour frequencies are rather similar to the corresponding values for animals receiving hormone alone.

It should be recalled that the frequency of Class III nuclei most probably represents a balance between the rate of chromosomal reduplication and the rate at which nuclei enter into prophase of mitosis. From this it would follow that the rise in frequency of Class III nuclei very probably is an indication of increased synthesis following hormonal stimulation. By the same token, the slower rise in frequency noted in the irradiated material suggests that in such material the synthetic rate responds less rapidly to the hormonal stimulus. The present data then may be interpreted to mean that irradiation does interfere with the synthetic mechanism of these hormonally stimulated tissues.

The data pertaining to the dorsal prostate showed little change from the control level until the 48-hour period. At this time the frequencies of Class III nuclei following irradiation and/or hormone were 8% and 16%, respectively. At 60 hours, the frequency of such nuclei in the sample from irradiated material had fallen to 4%, while the corresponding value for unirradiated material remained unchanged from the 48-hour level. At the termination of the experimental period (72 hours) the samples measured contained no Class III nuclei regardless of treatment.

These cytophotometric data show that the response of the dorsal prostate is on a much lower level than that determined for the seminal vesicle. Just as in the case of the mitotic counts, the DNA-Feulgen data point up a response to the experimental treatments which is different from that shown by the seminal vesicle.

As is the case with the mitotic counts, the DNA-Feulgen data show that the response of these somatic tissues to irradiation and/or hormonal stimulation is much more rapid than in the case of the spermatogonia. In the latter the frequency of Class III nuclei reaches its highest level (51%) at about 10 days following irradiation and thereafter declines rapidly to about 5% of the sample at 28 days. These data when considered along with the corresponding mitotic counts indicate a rapid utilization of Class III nuclei during the regenerative period. From the present DNA-Feulgen data it may be seen again that a situation analogous to the spermatogonial one presents itself but over a much shorter period of time.

The mitotic index determinations and the DNA-Feulgen data taken together allow the interpretation that hormonal stimulation of mitotic activity does not, in these organs, become markedly effective until 48 hours after the initial injection. Furthermore, the data prior to the 48-hour period lend themselves to the conclusion that mitotic activity is very closely correlated with the rate of chromosomal reduplication (formation of Class III nuclei). It would also appear that by 48 hours the rate of chromosomal reduplication is beginning to run ahead of mitotic activity and, with respect to the seminal vesicle, that this "imbalance" is maintained to a lesser degree for the duration of the experimental period. With respect to the dorsal prostate similar conclusions may be drawn with the notable distinction that over the period of 48 through 72 hours the "balance" has again changed in a manner such that, at the termination of the experiment, the close correlation evident earlier has been restored.

The results reported here lead to the conclusion that exposure to a similar dose (320 r) of x-rays is much less effective in inhibition of mitosis in these tissues

(under conditions of hormonal stimulation) than in the case of the spermatogonia. The DNA-Feulgen data may be interpreted to mean that, following irradiation, DNA synthesis suffers some impairment but recovers in a relatively short period of time. The demonstrated mitogenic action of testosterone propionate may have in some manner interfered with, or masked, the inhibiting action of the irradiation. Such an idea receives some support from the work of Rugh and Clugston (1954). These authors found that the stage of the oestrus cycle (at the time of irradiation) affected the radiation sensitivity of female mice. They found that females in dioestrus were about twice as sensitive (in terms of the male LD 50/30 days—625 r) to the irradiation as were females in oestrus. Thus an increased level of oestrogen appears to be correlated with increased resistance to x-irradiation. The same may be true also for androgens, especially so since both classes of steroid hormones elicit similar responses in experimental animals (see Bullough, 1952).

The present results, together with our earlier data (mouse testis), not only imply that the male germ line may differ from somatic tissues in its response to irradiation, but also that somatic tissues may differ from one another in this respect. At the present time it would appear that differences between the tissues studied are mainly ones of degree and rate of response. However, the findings of Pelc and Howard (1956) point out the possibility that somatic and germinal tissues may also differ in their metabolic pathways. Until more biochemical and cytological information is at hand the precise nature of these differences must remain an open question.

Summary

1. The accessory organs of castrated male rats have been used in a study of the effects of x-rays on mitosis in somatic tissues.

2. Animals nine weeks old were castrated and the organs allowed to regress for 20 days prior to use. Half the animals received daily injections of 500 μg of testosterone propionate (in oil) until death; the others, in addition to the hormone injections, were exposed to a single dose of 320 r of x-rays, the irradiation being given at the time of the first hormone injection. Pairs of animals were killed at 24, 48, 60 and 72 hours following the irradiation and/or initial hormone treatment.

3. The response to the treatments was followed by means of mitotic index determinations and cytophotometric measurements of the DNA-Feulgen content of interphase nuclei.

4. The cytological data indicate the existence of a difference in response between the epithelium of the seminal vesicle and of the dorsal prostate. At no time during the experiment did the mitotic activity of the latter rise to levels characteristic of the former. In addition, the time-response curves for the two organs indicate that the dorsal prostate responds more slowly than the seminal vesicle.

5. The DNA-Feulgen measurements together with the mitotic index data indicate that in the controls and in the experimental animals killed prior to 48 hours there is a close correspondence between the level of mitotic activity and the rate of chromosomal reduplication. Over the period of 48–72 hours in the case of the dorsal prostate the data show that, during the time of maximal hormonal stimulation, DNA synthesis is proceeding at a rate appreciably faster than the rate at which nuclei enter into prophase.

6. The results obtained have been compared with those obtained from a similar experiment involving the mouse testis. The accessory organs appear to be less sensitive to the irradiation than the testis. Factors bearing on this point are discussed.

LITERATURE CITED

BRYAN, J. H. D., 1951. DNA-protein relations during microsporogenesis of *Tradescantia*. *Chromosoma,* **4**: 369–392.

BRYAN, J. H. D., AND J. W. GOWEN, 1956. A histological and cytophotometric study of the effects of x-rays on the mouse testis. *Biol. Bull.,* **110**: 229–242.

BULLOUGH, W. S., 1952. The energy relations of mitotic activity. *Biol. Rev.,* **27**: 133–168.

BURROWS, H., 1949. Biological Actions of Sex Hormones. Second ed., The University Press, Cambridge, England.

CAVAZOS, L. F., AND R. M. MELAMPY, 1954. Cytological effects of testosterone propionate on epithelium of rat seminal vesicles. *Endocrinology,* **54**: 640–648.

FLEISCHMANN, W., AND M. NIMAROFF, 1954. Combined effects of x-irradiation and testosterone propionate on accessory sex organs. *Proc. Soc. Exp. Biol. Med.,* **85**: 655–658.

KASTEN, F. H., 1956. Cytophotometric studies of deoxyribose nucleic acid in several strains of mice. *Physiol. Zoöl.,* **29**: 1–20.

LEA, D. E., 1955. Actions of Radiations on Living Cells. Second Ed., edited by L. H. Gray. The University Press, Cambridge, England.

MELAMPY, R. M., J. W. GOWEN AND J. W. WACASEY, 1956. Effects of x-irradiation on androgenic response of seminal vesicles of the castrate rat. *Proc. Soc. Exp. Biol. Med.,* **92**: 313–315.

MOORE, C. R., W. HUGHES AND T. F. GALLAGHER, 1930. Rat seminal vesicle cytology as a testis-hormone indicator and the prevention of castration changes by testis-extract injection. *Amer. J. Anat.,* **45**: 109–135.

PELC, S. R., AND A. HOWARD, 1952. Chromosome metabolism as shown by autoradiographs. *Exp. Cell Res., Suppl.,* **2**: 269–278.

PELC, S. R., AND A. HOWARD, 1956. A difference between spermatogonia and somatic tissues of mice in the incorporation of (8-^{14}C)-adenine into deoxyribonucleic acid. *Exp. Cell Res.,* **11**: 128–134.

RUGH, R., AND H. CLUGSTON, 1954. Radiosensitivity with respect to the oestrus cycle in the mouse. *Biol. Bull.,* **107**: 289–290.

STOWELL, R. E., 1945. Feulgen reaction for thymonucleic acid. *Stain Technol.,* **20**: 45–56.

SWIFT, H. H., 1950. The desoxypentose nucleic acid content of animal nuclei. *Physiol. Zoöl.,* **23**: 169–198.

TAYLOR, J. H., AND R. D. MCMASTER, 1954. Autoradiographic and microphotometric studies of desoxyribose nucleic acid during microgametogenesis in *Lilium longifolium*. *Chromosoma,* **6**: 489–521.

TAYLOR, J. H., P. S. WOODS AND W. L. HUGHES, 1957. The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium labeled thymidine. *Proc. Nat. Acad. Sci.,* **43**: 122–127.