mals. 3) Forty-eight hours' starvation reduces the citrate accumulation in the liver of fluorocitrate-poisoned rats to one-sixth the value obtained in fed animals.

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## Effects of X-Irradiation on Androgenic Response of Seminal Vesicles of the Castrate Rat.* (22462)

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In investigating cytological effects of testosterone propionate on secretory epithelium of rat seminal vesicles, Cavazos and Melampy (1) observed no mitoses in epithelium of castrates or 24- and 36-hour hormone-treated animals. However, mitotic activity of 7.5% was noted 60 hours after initial hormone treatment. Following this maximum there was a sharp decline in mitoses and in animals treated with male hormone for 20 days a value of 0.1% was observed. Colchicine was used in these experiments.

Regressed seminal vesicles of the castrate rat provide useful test tissue for such investigation because cells of secretory epithelium are in the resting stage and can be reactivated with androgen. Observations are reported on effects of various levels of X-irradiation on 60-hour response of seminal vesicles to testosterone propionate (T.P.).† Data were obtained on mitotic index and cell height of secretory epithelium as well as total nitrogen content of the organ.

*Materials and methods.* 159 rats of Holtzman strain were used and fed Purina Laboratory Chow. At time of castration average animal weights ranged from 266 to 312 g and seminal vesicles were allowed to regress 20 days before X-irradiation and/or hormone treatment. The initial dose of androgen was administered at time of irradiation. Hormone-treated castrates received daily 500 μg of T.P. in oil injected subcutaneously, total amount 1500 μg. Control groups received 0.05 ml of oil daily and no irradiation. Experimental animals were sacrificed 60 hours following treatment. Animals were exposed to X-rays from General Electric Maxitron operated at 130 kvp and 10 ma with 0.25 mm Al filtration and at 20 cm target distance. Nembutal anesthesia was employed during irradiation (2.5 mg/100 g body wt). Animals were shielded with lead except in pelvic area. Individuals used to furnish data on mitotic index and cell height of secretory epithelium were injected subcutaneously with 0.1 mg of colchicine per 100 g body weight 6 hours prior to killing according to Burkhart(2). Percentages of mitotically dividing nuclei, mitotic index, and cell heights were determined by procedure described earlier(1). Total nitrogen data were obtained by a micro-Kjeldahl method.

*Results.* The results of this investigation are summarized in Tables I and II. Data are presented as means with their standard errors.

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† Peranderen, Ciba Pharmaceutical Products, Summit, N. J.

TABLE I. Effects of X-Irradiation on Body Weight and Androgenic Response of Secretory Epithelium of Seminal Vesicles of Castrate Rat.

| Animals per group | Treatment Irradiation, r | Treatment Hormone daily, $\mu$g | Body wt at castration, g | Body wt at irradiation, g | Body wt 60 hr after treatment, g | Seminal vesicles wt, mg | Mitotic index, % | Cell height, $\mu$ |
|---|---|---|---|---|---|---|---|---|
| 4 | 0 | 0 | 275 ± 8* | | 325 ± 9* | 96 ± 8* | 0 | 8 ± .4* |
| 7 | 0 | 500 | 294 ± 9 | | 344 ±10 | 219 ± 9 | 8 ± 1* | 17 ± .3 |
| 6 | 80 | " | 288 ±10 | 319 ±11* | 320 ±10 | 207 ±13 | 14 ± 1 | 19 ± .3 |
| 5 | 160 | " | 296 ± 8 | 325 ±10 | 325 ± 9 | 218 ± 7 | 20 ± 1 | 19 ± .6 |
| 5 | 160 | 0 | 304 ± 7 | 346 ± 8 | 330 ± 8 | 95 ± 5 | 0 | 7 ± .3 |
| 7 | 320 | 500 | 274 ± 6 | 305 ± 7 | 306 ± 8 | 214 ±11 | 17 ± 1 | 18 ± .4 |
| 7 | 640 | " | 276 ± 5 | 315 ± 5 | 303 ± 5 | 205 ±19 | 15 ± 1 | 18 ± .0 |
| 5 | 1000 | " | 281 ± 6 | 318 ± 3 | 285 ± 4 | 185 ± 5 | 5 ± 1 | 17 ± .3 |
| 7 | 1500 | " | 291 ± 9 | 327 ± 7 | 309 ± 7 | 181 ± 7 | 4 ± 1 | 18 ± .4 |
| 7 | 2000 | " | 261 ± 5 | 314 ± 5 | 293 ± 6 | 184 ± 7 | 3 ± 1 | 18 ± .3 |
| 8 | 3000 | " | 308 ± 4 | 337 ± 3 | 311 ± 4 | 198 ± 5 | 1 ± 0 | 17 ± .7 |

* Mean ± S.E.

*Discussion.* Mitoses were not observed in regressed seminal vesicles of control animals but a mitotic index of 8% was obtained in animals 60 hours after initial dose of hormone and no X-irradiation. In androgen-treated groups receiving 80 and 160 r this value increased to 14 and 20%. Mitotic activity was absent in secretory epithelium of animals which received 160 r without androgen. A mitotic index of 10 ± 0.5% was obtained in a group of 5 rats irradiated with 160 r one hour before colchicine treatment (53 hours after initial hormone injection). Bloom(3) indicated there is no experimental evidence that X-irradiation induces cell division. In hormone-treated castrates it is possible that irradiation at levels between 80 and 640 r caused a delay in mitotic response of secretory epithelium and as a result a greater mitotic index was observed at 60 hours. Animals receiving 3000 r showed 1% of epithelial nuclei dividing indicating destructive action of higher dosage. Fleischmann and Nimaroff (4) have demonstrated X-irradiation of accessory sex organs of castrate male rats and immediate subsequent androgen treatment resulted in cellular hypertrophy with delayed mitoses. However, if an interval of 5 days was allowed between irradiation and administration of male hormone this delaying effect was lost. Data suggesting recovery from irradiation effects were also obtained. An average mitotic index of 2% was found in a group of 8 animals irradiated with 3000 r 48 hours prior to initial hormone injection, whereas no mitoses were observed in a similar group of 8 rats given same dosage 48 hours following androgen.

Data presented in Table I indicate that under conditions of these experiments X-irradiation from 80 to 3000 r had no significant effect on cell height in animals receiving male-sex hormone. The weight of seminal vesicles in hormone-treated animals without irradiation (Table II) was more than double the weight of controls. A decrease was noted in weights of seminal vesicles of animals receiving 500 $\mu$g of hormone daily and increasing amounts of X-irradiation (Table II). There was a 3-fold increase in nitrogen content of seminal vesicles following androgen administration without irradiation when compared to controls. However, at 3000 r the nitrogen content was more than twice that of controls which received neither irradiation nor hormone. The decreasing amounts of nitrogen per gland as shown in Table II are the result of a fall in organ weight rather than a significant change in percentage of nitrogen.

Table II indicates that irradiation at highest levels (1500, 2000, and 3000 r) reduced seminal weight by approximately 30% when compared with hormone-treated animals. The same levels of irradiation resulted in lowest observed mitotic indices (Table I). This finding is in agreement with observations of Fleischmann and Brackin(5) that growth induced by testosterone propionate in accessory sex organs of castrate male rats is only partially inhibited by irradiation with X-rays.

*Summary.* Mature male rats were cas-

TABLE II. Effects of X-Irradiation and Testosterone Propionate on Body and Seminal Vesicle Weights and Nitrogen Content of Seminal Vesicles of Castrate Rat.

| Animals per group | Treatment Irradiation, r | Hormone daily, μg | Body wt at castration, g | Body wt at irradiation, g | Body wt 60 hr after treatment, g | Seminal vesicles wt, mg | N/gland, mg |
|---|---|---|---|---|---|---|---|
| 13 | 0 | 0 | 305 ± 5* | | 347 ± 6* | 107 ± 4* | 1.8 ± .1* |
| 13 | 0 | 500 | 304 ± 6 | | 344 ± 7 | 233 ± 8 | 5.5 ± .1 |
| 6 | 80 | " | 293 ± 9 | 337 ± 7* | 337 ± 6 | 207 ± 7 | 4.9 ± .1 |
| 6 | 160 | " | 294 ± 3 | 351 ± 6 | 347 ± 7 | 222 ±10 | 5.1 ± .2 |
| 4 | 320 | " | 270 ±10 | 326 ±11 | 324 ±12 | 209 ± 3 | 5.2 ± .1 |
| 8 | 640 | " | 303 ± 3 | 334 ± 7 | 318 ± 5 | 193 ± 9 | 4.7 ± .2 |
| 5 | 1000 | " | 312 ± 4 | 342 ± 5 | 320 ± 4 | 197 ± 4 | 4.6 ± .1 |
| 5 | 1500 | " | 296 ± 1 | 348 ± 3 | 312 ± 3 | 180 ± 5 | 3.6 ± .3 |
| 5 | 2000 | " | 285 ± 7 | 334 ± 7 | 298 ± 7 | 159 ± 8 | 3.9 ± .4 |
| 5 | 3000 | " | 284 ± 3 | 337 ± 5 | 301 ± 3 | 166 ± 5 | 4.0 ± .2 |

* Mean ± S.E.

trated and accessory organs regressed 20 days before X-irradiation and/or hormone treatment. Androgen-treated castrates received daily 500 μg of testosterone propionate in oil, total amount 1500 μg. Animals were sacrificed 60 hours following initial treatment. The mitotic index with hormone was 8%, whereas following androgen and irradiation at 80, 160, 320 and 640 r it was 14, 20, 17 and 15% respectively. At levels of 1000, 1500, 2000, and 3000 r these values were 5, 4, 3 and 1%. These data indicate inhibition of mitotic activity at levels of 1000 r and above. It is suggested that irradiation between 80 and 640 r caused a delay in mitotic response of secretory epithelium and as a result the mitotic index was greater than in those which received androgen alone. X-irradiation had no significant effect on cell height. There was a decrease in seminal vesicle weights of animals treated with hormone and increasing amounts of irradiation.

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## Effect of Malonate on Selected Body Defenses.* (22463)

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Previous work from this laboratory(2-7) has shown that repeated injections of sublethal doses of malonate reduce survival time of mice infected with *Salmonella typhimurium* from 64-84 hours to 8-24 hours. Survival time is also reduced by injections of fluoroacetate, arsenite, citrate, and succinate. Bacteria multiply more rapidly in malonate treated mice than in saline treated mice as revealed by total viable pathogen counts in homogenates prepared from mouse carcasses (7). Survival time seems dependent, therefore, upon time required for microorganisms to reach a fairly constant lethal number. The greater rate at which this lethal number of pathogens is reached in mice, given malonate, may be attributed to one or both of the following conditions: (a) decreased rate of destruction due to impairment of animal's body defenses or (b) more rapid rate of reproduction due to more favorable nutritive environ-

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