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Received March 29, 1965. P.S.E.B.M., 1965, v119.

Protective Effect of Estrogen Against Vascular Damage to the Testis Caused by Cadmium.* (30331)

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The complete necrosis of the testis induced by a single subcutaneous injection of soluble cadmium salt to rats and mice has been amply documented(1-4). It has also been shown that the primary effect of cadmium is on the vascular system of the testis and that damage to the interstitial tissue and seminiferous tubules is secondary(5-7). In unpublished observations we have confirmed the report of Parizek(8) that cadmium does not produce testicular injury in Wistar rats before the age of 9-16 days. This suggests there may be a particular hormonal balance existing in the immature animal which prevents the testicular vasculature from being damaged by cadmium. We raised the question of whether the testis of the mature animal could be protected from cadmium injury by administration of hormones. This paper presents results of experiments undertaken to determine whether androgen or estrogen would alter the vascular response of the mature mouse testis to cadmium.

Materials and methods. Mature male C. D. mice (Charles River Breeding Laboratories, Inc.), 12-16 weeks of age, weighing approximately 40 g, were used. Preliminary experimentation showed that the degree of vascular injury in the testis caused by cadmium varied directly with the dosage. Cadmium was given as $CdCl_2$ in a single subcutaneous injection in the interscapular area. Administration of 0.03 mmole/kg of $CdCl_2$ (0.2 ml of 0.006 M solution) to mature C. D. mice consistently produced marked edema, capillary hemorrhage and thrombosis with resulting interstitial tissue and tubular necrosis. This degree of response was graded as a maximal reaction and is illustrated in Fig. 2; compare with the uninjected control, Fig. 1. With a lower dose of $CdCl_2$, 0.01 mmole/kg (0.2 ml of 0.002 M solution), there was edema, but no capillary hemorrhage and no interstitial or tubular necrosis; this was graded as a minimal response (Fig. 3). In the hormone treatment experiments to be presented, the degree of vascular injury in the testis from 0.03 mmole/kg of $CdCl_2$ was graded as a maximal, minimal or negative response, based on the classifications described above. A total of 87 male C. D. mice was used. Mice were subjected to saline, sesame oil, testosterone propionate, estradiol benzoate or stilbestrol treatment of various duration as described below. At the end of the treatment period, one testis was removed surgically under ether anesthesia, fixed in Bouin's fluid and processed for microscopic study by routine histological techniques. Following surgery, mice were given a single subcutaneous injection of 0.03 mmole/kg of

* Supported by U.S.P.H.S. research grants CA-06045 and CA-07004 from Nat. Cancer Inst.

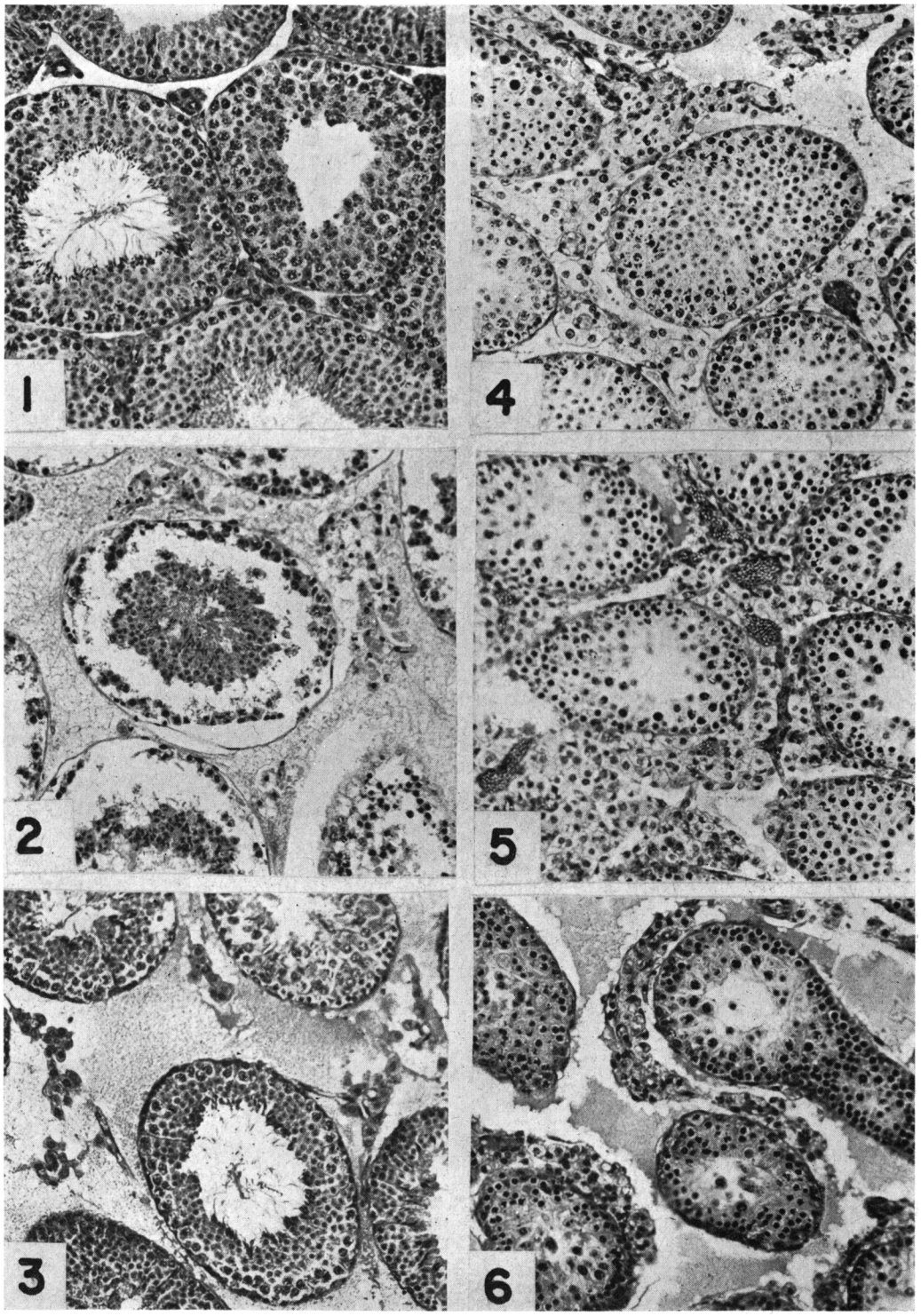

FIG. 1. Testis of control mouse. H & E × 135.

FIG. 2. Testis of mouse 48 hr after subcutaneous injection of 0.03 mmole/kg of $CdCl_2$, showing maximal degree of vascular injury from cadmium. H & E × 135.

FIG. 3. Testis of mouse 48 hr after subcutaneous injection of 0.01 mmole/kg of $CdCl_2$, showing minimal degree of vascular injury from cadmium. H & E × 135.

FIG. 4. Testis of mouse after 7 weeks of estradiol benzoate treatment (100 μg 3 times weekly), showing that spermatogenesis progressed only through the spermatid stage. H & E × 135.

FIG. 5. Testis of estradiol benzoate treated mouse (as in Fig. 4) 48 hr following subcutaneous injection of 0.03 mmole/kg of $CdCl_2$, showing negative vascular response to cadmium. H & E × 135.

FIG. 6. Testis of estradiol benzoate treated mouse (as in Fig. 4) 48 hr following subcutaneous injection of 0.03 mmole/kg of $CdCl_2$, showing minimal vascular response to cadmium. H & E × 135.

$CdCl_2$ in the interscapular area (0.02 ml of 0.006 M solution). Mice were killed 48 hours later, and the remaining testis was removed, fixed and processed for microscopic evaluation of vascular damage. With this experimental protocol, each mouse served as its own control prior to cadmium injection.

Results. Effect of testosterone propionate and estradiol benzoate treatments on vascular damage to the testis caused by $CdCl_2$. Four groups of 7 mice each were used. Each group received 0.1 ml subcutaneous injections of either saline, sesame oil, testosterone propionate† (100 μg) or estradiol benzoate† (100 μg) 3 times weekly for 7 weeks prior to testing the vascular response of the testis to cadmium.

The testes of all mice of the saline, sesame oil and testosterone propionate treated groups showed maximal vascular damage from $CdCl_2$ (as in Fig. 3). Treatment with estradiol benzoate produced some tubular regression; spermatogenesis progressed only through the spermatid stage (Fig. 4). After administration of $CdCl_2$, the testes of 4/7 estradiol benzoate treated mice showed no vascular injury (Fig. 5), 2/7 showed only minimal damage to testicular vasculature (Fig. 6) and 1/7 showed maximal damage.

Effect of estradiol benzoate treatments of various duration on vascular damage to the testis caused by $CdCl_2$. Four groups of mice (5-10 per group) were used. The control group received 0.1 ml subcutaneous injections of sesame oil 3 times weekly for 7 weeks prior to testing the vascular response of the testis to cadmium. Three estradiol benzoate treated groups received 100 μg of hormone in 0.1 ml subcutaneous injections 3 times weekly. One group was treated for 2 weeks, another group for 7 weeks; these 2 groups were tested for testicular response to cadmium immediately following the last estradiol benzoate injection. The 3rd group of estradiol benzoate treated mice received hormone injections for 7 weeks, but were allowed to rest without additional hormone for 18 weeks before they were checked for vascular response of the testis to cadmium.

Treatment with estradiol benzoate for 2 weeks, which was not sufficient to produce tubular regression, did not alter the testicular vascular response to $CdCl_2$; the testes of 9/10 mice showed maximal damage, identical to that seen in Fig. 3. Seven weeks' treatment with estradiol benzoate which resulted in tubular regression (as in Fig. 4), protected the testes of 6/7 mice against vascular injury from cadmium (as in Fig. 5). When mice were allowed to rest for 18 weeks following the 7-week hormone treatment, there was no longer tubular regression nor protection against the cadmium induced vascular injury. The response was maximal damage in 7/7 mice, identical to that seen in Fig. 3.

Effect of stilbestrol treatments on vascular damage to the testis caused by $CdCl_2$. Three groups of mice (4-7 per group) were used in this study. The first 2 groups received 0.1 ml subcutaneous injections of sesame oil or stilbestrol (100 μg) 3 times weekly for 7 weeks; after the last injection, they were tested for vascular response of the testis to cadmium. A 4-6 mg stilbestrol pellet, 25% stilbestrol in cholesterol prepared according to Shimkin, Grady and Andervont(9), was inserted subcutaneously in the 3rd group of mice; 12 weeks later these mice were checked for vascular response of the testis to cadmium. Although neither form of stilbestrol treatment resulted in testicular regression at the

† Solutions of testosterone propionate and estradiol benzoate in sesame oil were generously furnished by Schering Corp.

conclusion of this experiment, both treatments gave protection against the vascular response of the testis to $CdCl_2$. This was most striking in the stilbestrol pellet series in which the testes of 5/5 mice showed a completely negative response to cadmium; the microscopic picture was identical to that of controls which had not received $CdCl_2$ (as in Fig. 1).

Discussion. These experiments have demonstrated that following treatments with estrogenic compounds, cadmium failed to produce the vascular damage characteristically seen in the testes of mature C. D. mice. Treatment with estradiol benzoate, which resulted in tubular regression, protected the testes from cadmium induced vascular injury. This protection was reversible since following cessation of estradiol benzoate treatment and disappearance of tubular regression, the testes were again susceptible to vascular damage from cadmium. The induction of tubular regression by estrogen was not essential for this vascular protection since stilbestrol, which did not produce tubular regression, also protected the testis against vascular injury caused by cadmium. This indicated that the presence or absence of certain seminiferous tubular elements was not necessary for protection to take place and suggested that there was a direct effect of compounds with estrogenic activity on the testicular vasculature. The vascular reaction of the testis to cadmium was not altered by treatment with testosterone propionate. Parizek(10) suggested that cadmium damage to the testis might occur specifically at sites of estrogen biosynthesis in the male gonad. This hypothesis was not borne out by the results of this study which demonstrated a protective effect of estrogenic compounds against cadmium injury to the testis.

It was previously demonstrated that following administration of cadmium to the male, vascular damage occurred specifically in one set of blood vessels, the internal spermatic artery and its branches and the pampiniform plexus, the complex supplying the testis and proximal end of the caput epididymis(5). This specificity of vascular response suggested that the vascular system may be more than just a simple conduit taking blood to and removing it from various areas. Since each organ has its own specialized functions, each organ may have its own specialized vascular endothelium so that permeability may be controlled, not only by intra- and extraluminal pressure differences, but by different enzyme systems that allow specific permeation for the handling of a multitude of differences in function. Others have emphasized that dynamic biochemical reactions are associated with inflammation and that enzymatic mechanisms are important features in the regulation of vascular permeability(11). Altschul(12) has presented ideas on "morphologic organ specificity" and discussed specific permeation and reactions to injury as related to differences in endothelial structure. An organ specificity for the vasculature of the testis, the internal spermatic artery and pampiniform plexus complex, has already been established by virtue of its specialized functions of counter-current heat exchange(13-15).

The vascular system of the uterus is known to be under hormonal control. Following administration of estrogen to many animal species, the uterus specifically undergoes changes resembling those of acute inflammation; estrogens fail to induce these vascular changes in any tissue other than uterus(16). Our results have shown that a critical factor of hormone balance may be implicated in vascular response in another organ of reproduction, the testis.

Summary. A single subcutaneous injection of $CdCl_2$ (0.03 mmole/kg) to mature male C. D. mice consistently produced vascular damage to the testis characterized by marked edema, capillary hemorrhage and thrombosis with resulting interstitial tissue and tubular necrosis. Previous treatment with testosterone propionate did not alter this response. A treatment period with estradiol benzoate, which resulted in tubular regression, protected the testis against vascular injury from cadmium. This protection was reversible since following cessation of estradiol benzoate treatment and disappearance of tubular atrophy, the testes were again susceptible to cadmium induced vascular damage. The induction of tubular regression was not essential

for this vascular protection by estrogen since stilbestrol, which did not produce tubular regression in the doses used, also protected the testis against vascular injury caused by cadmium.

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Received April 7, 1965. P.S.E.B.M., 1965, v119.

Thyroidal Coupling of Tyrosine *in vitro*.* (30332)

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The mechanism by which tyrosines are coupled to form thyronines by living tissues is unknown. Coupling occurs spontaneously *in vitro* at alkaline pH(1) in the presence of oxygen, and it is possible that non-enzymatic coupling may occur *in vivo*. It is not known whether the spontaneous coupling that occurs at pH 7.0 is sufficient to account for thyronine synthesis in the intact thyroid gland. *In vivo*, synthesis of thyronines proceeds at a rapid rate, which may be faster than can be accounted for by spontaneous coupling.

The experiments described here were designed to investigate the ability of the thyroid gland to couple 2 molecules of tyrosine for formation of one molecule of thyronine. Attempts were made to demonstrate incorporation of tyrosine-UL-C^{14} into thyronine *in vitro* with calf and pig tissue slices and rabbit thyroid homogenates.

* This investigation was supported by Grant AM-05446 from Division of Grants, Nat. Inst. Health, U.S.P.H.S.

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Materials and methods. A. Preparation and incubation of tissue slices. (1) Fresh pig thyroids were obtained from a local slaughter house, chilled until processed and sliced on a Stadie-Riggs microtome. The slices were then weighed and placed immediately into 25 ml flasks containing 5.0 ml Krebs-Ringer phosphate buffer, pH 7.4. The flasks were incubated in a Dubnoff metabolic incubator at 38°C for 2 hours. The incubated material was then extracted exhaustively with n-butanol. The residue was digested with pancreatin, reextracted with n-butanol and chromatographed.

(2) Tissue slices from beef thyroid glands were prepared in the same way. In flask No. 1, 500 mg of thyroid tissue was incubated in 10 ml of Krebs-Ringer bicarbonate buffer. In flask No. 2, 50 μc (90 mc/mM) tyrosine-UL-C^{14} was added to 500 mg thyroid tissue and 10 ml Ringer bicarbonate buffer. The flasks were incubated in the Dubnoff incubator at 37°C for 3 hours. Additional buffer was then made up and pH adjusted to 9.0 with NaOH. To 12 ml of buffer 250 mg of pancreatin was added. Six ml of this mixture were added to each flask. Both were layered