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Received December 3, 1962. P.S.E.B.M., 1963, v112.

Effect of Triethylenemelamine (TEM) on Endocrine System of the Male Rat.* (28119)

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In the search for anti-fertility drugs that can be used in the male, it becomes important to determine whether there is interference with the male endocrine system. Several investigators have demonstrated that the radiomimetic agent, triethylenemelamine (TEM), interferes with fertility in male rodents(1,2,3,4). Although the data presented by Steinberger *et al.*(4) showed some depression in weights of accessory sex glands following treatment with TEM, these authors felt there was no indication of interference with androgen level since the body weights of the animals were also lowered. The consensus has been that other agents which produce damage to the seminiferous epithelium, such as X-ray, do not damage the more resistant Leydig cells. Studies conducted in this laboratory have shown, however, that within 12 days after whole-body exposure to X-ray there is an androgen depression, even in the face of normal-appearing Leydig cells(5). Since TEM is classified as a radiomimetic agent, it seemed pertinent to investigate more thoroughly its possible effects on the male endocrine system.

Materials and methods. Mature, male Wistar rats, 16 weeks of age (350 to 400 g), were used in these studies. TEM was administered once daily for 5 days in a dose of 0.2 mg/kg. TEM was made up fresh in physiological saline on the day of the first injection and was stored at 4°C during the 5-day injection period. TEM was administered by the intraperitoneal route in volumes of 1 ml/kg body weight.

Since both the weight of the dorsolateral prostate and its capacity to take up administered Zn^{65} have been shown to be under androgen(6), or ICSH control(7,8), these two parameters were used to assess possible endocrine disturbances following TEM treatment. On the 5th, 15th or 19th day following termination of a 5-day treatment with TEM, rats were injected intracardially with Zn^{65}† (0.04 μc/g). A group of non-TEM injected rats of the same age also received Zn^{65}. Twenty-four hours later, the rats were sacrificed; and dorsolateral prostates were dissected as described previously(9). Tissues were placed on tared aluminum foil, dried, weighed and their radioactivity determined in a large, well-type scintillation detector.

The effect of administration of hormones was also studied in TEM-treated rats. During the 5 days following TEM administration, rats were injected daily with 200 μg of testosterone propionate‡ (subcutaneous in 0.2

* Supported in part by a research grant from U. S. Atomic Energy Commission and in part by a U. S. Public Health Service research grant from Nat. Inst. of Arthritis and Metab. Dis.

† Zn^{65} was purchased from Union Carbide Nuclear Co. as $Zn^{65}Cl_2$ in HCl solution with a specific activity of approximately 1000 mc/g. The solution was diluted with physiological saline containing sufficient NaOH to neutralize some of the acid present compatible with solubility.

ml sesame oil), 20 μg of ICSH§ (intramuscular in 0.2 ml physiological saline), or 2 i.u. of chorionic gonadotrophin‖ (subcutaneous in 0.2 ml physiological saline). Controls for the testosterone group received daily injections of 0.2 ml of sesame oil. Controls for the ICSH and chorionic gonadotrophin groups received daily injections of 0.2 ml of physiological saline. On the 5th and final day of hormone administration, *i.e.*, 5 days following termination of TEM treatment, rats were administered Zn^{65}. Twenty-four hours later, studies on dorsolateral prostate were conducted as described above.

Food consumption and body weight were checked in control and TEM-treated rats. These studies were also conducted in rats injected with TEM plus testosterone, TEM plus ICSH and TEM plus chorionic gonadotrophin. TEM was administered as before for 5 days. The hormones were given for these 5 days and, in addition, for 6 days following TEM. The daily dose of hormone was the same as described in the preceding paragraph.

One testis from each experimental and control rat sacrificed was fixed in Bouin's and processed for histological study.

Results. Effect of TEM on weight and Zn^{65} uptake of rat dorsolateral prostate. Fig. 1 shows that 5 to 6 days following termination of a 5-day treatment with 0.2 mg/kg of TEM, there was a statistically significant lowering of weight ($P<0.00001$) and capacity of the dorsolateral prostate to take up Zn^{65} ($P<0.002$). Weight ($P<0.01$) and Zn^{65} uptake ($P<0.005$) values were still low 15 to 16 days following termination of TEM treatment. By 19 to 20 days post-termination of TEM treatment, neither dorsolateral prostate weight nor Zn^{65} uptake differed significantly from control values.

Effect of testosterone and ICSH on weight and Zn^{65} uptake by the dorsolateral prostate of rats treated with TEM. Fig. 2 shows that the fall in both weight ($P<0.00001$) and

‡ Oreton in sesame oil (Schering).

§ NIH-LH-S-1, ovine dissolved in physiological saline.

‖ Antuitrin S (Parke Davis) dissolved in physiological saline.

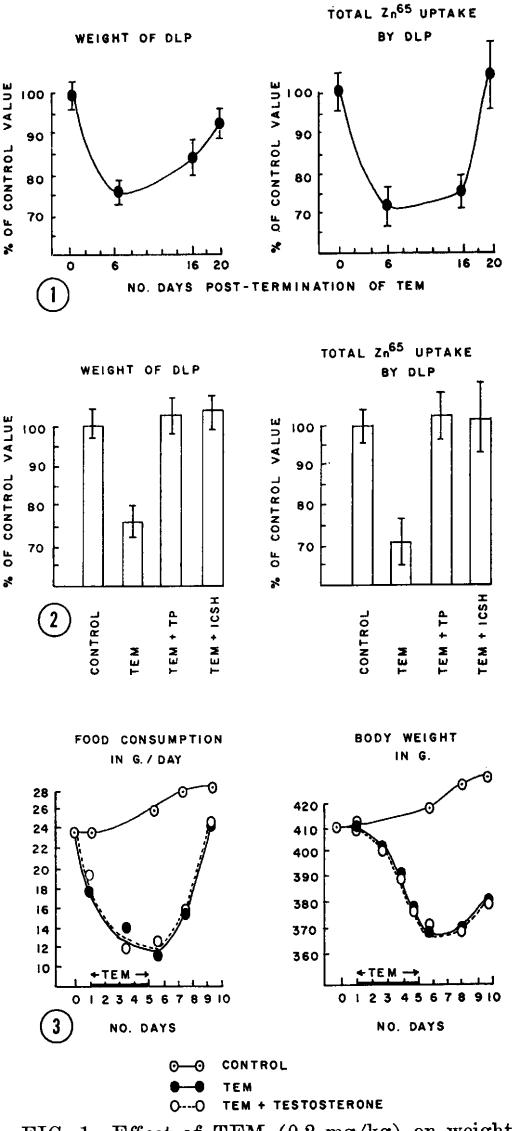

FIG. 1. Effect of TEM (0.2 mg/kg) on weight and Zn^{65} uptake of rat dorsolateral prostate at various times following termination of treatment. Control group and 5- to 6-day study were each comprised of 25 rats. Ten animals each were used in 15- to 16- and 19- to 20-day study. Values represent mean $\pm$ standard error.

FIG. 2. Effect of testosterone and ICSH on weight and Zn^{65} uptake by dorsolateral prostate of rats treated with TEM (0.2 mg/kg). Control group and TEM-treated group were each comprised of 25 rats. Nine animals each were used in hormone-treated groups. Values represent mean $\pm$ standard error.

FIG. 3. Effect of TEM (0.2 mg/kg) and testosterone treatment on food consumption and body weights of rats. Values represent mean from 9 to 10 rats.

Zn^{65} uptake ($P<0.002$) noted 5 to 6 days following termination of TEM treatment could be prevented by administration of either testosterone or ICSH. Although not shown in the Figure, dorsolateral prostate weight and function were also maintained in TEM-treated rats by administration of chorionic gonadotrophin.

Effect of TEM and hormone treatment (testosterone and ICSH) on food consumption and body weights of rats. Fig. 3 shows that during the 5-day treatment with TEM there was a 50% decrease in daily food intake, which returned to near control levels within 4 days following termination of the TEM injections. Loss of body weight became evident about the 3rd day of TEM treatment. Maximum decrease in body weight, about 12% below control values, was noted between the 1st and 3rd day following termination of TEM treatment. Administration of testosterone (or ICSH, or chorionic gonadotrophin, not shown in the Figure) did not alter the depression in food consumption and body weight.

Effect of TEM on testicular histology. The only microscopic change noted 6 days following termination of a 5-day treatment with TEM was loss of spermatogonia. Other germinal elements and interstitial tissue were apparently unharmed. Administration of hormones (testosterone, ICSH and chorionic gonadotrophin) did not alter the microscopic picture seen in testes of TEM-treated rats. The interstitial tissue was also normal in appearance at 16- and 20-day sacrifice. At these times, tubular elements were undergoing maturation depletion as a result of the initial damage to spermatogonia. The histological picture of testis following TEM was identical with that reported by Steinberger *et al.*(4).

Discussion. These experiments have shown that 5 to 6 days following termination of a 5-day treatment with TEM, there was a lowering of dorsolateral prostate weight and Zn^{65} uptake, indicating a diminished androgen level. Although data presented by others showed a similar depression in weights of seminal vesicles and prostate following TEM, the authors attributed these effects to lowered body weights caused by TEM, rather than any depression of androgen level(4). Our results indicated, however, that TEM did cause an androgen depression since the weight and Zn^{65} uptake of the dorsolateral prostate in TEM-treated rats were maintained by administration of testosterone, chorionic gonadotrophin and ICSH. That the lowered accessory gland function was due to an androgen depression, and not merely the result of lowered body weights, was further borne out by the observation that testosterone and ICSH, which maintained the dorsolateral prostate within control limits, did not alter the pattern of lowered food consumption and lowered body weights resulting from the TEM treatment. The cause of the diminished androgen output following TEM appeared to be due to insufficient ICSH elaboration by the pituitary, and not due to any abnormality of the Leydig cells themselves. The morphologically normal-appearing interstitial tissue responded functionally to administered ICSH and chorionic gonadotrophin, since these hormones maintained the dorsolateral prostate weight and Zn^{65} uptake in TEM-treated rats.

A similar lowering of ICSH level has been noted following a single whole-body exposure to X-ray(5) and following exposure of the scrotum to microwaves(10). With all 3 agents, TEM, X-ray and microwaves, there was no microscopic evidence of injury to the interstitial tissue. In the TEM and X-ray studies, the only morphological damage noted in the testis, initially, was a depletion of spermatogonia. In the microwave studies, only mild edema was noted in the testis. In all 3 cases, however, this degree of damage was a sufficient stimulus to evoke a temporary decrease in pituitary output of ICSH, resulting in lowered accessory gland function. It is known that following castration, there are pituitary changes which ultimately result in increased LH (ICSH) output; these same alterations even follow damage to seminiferous epithelium, such as that resulting from X-ray or cryptorchidism(11). Other investigators have confirmed these findings and have also shown that after castration the pituitary changes follow a definite time se-

quence(12,13,14). Initially, within the first 10 days following castration, there is a lowering of gonadotrophic potency which is predominantly FSH with little or no LH (ICSH). Twenty-one days post-castration, the pituitaries become increasingly rich in LH (ICSH). Our studies have shown that TEM, X-ray(5), microwaves(10) and surgical cryptorchidism(15), experimental conditions which do not injure Leydig cells morphologically or functionally, but which do damage seminiferous epithelium, also produce a temporary decrease in ICSH output followed by an increase similar in time sequence to that following castration. These studies furnish more evidence for the concept that the state of seminiferous epithelium is an important regulator of pituitary function.

Summary. Five to six days following the termination of a 5-day treatment of rats with TEM (0.2 mg/kg daily), the weight and capacity of the dorsolateral prostate to take up Zn^{65} was diminished, indicative of a diminished androgen level. Dorsolateral prostate weight and Zn^{65} uptake were maintained in TEM-treated rats by administration of testosterone, pituitary ICSH or chorionic gonadotrophin.

We are indebted to the American Cyanamid Co. for the generous supply of triethyenemelamine (TEM) used in this investigation. We also wish to express our appreciation to the Endocrinology Study Section, Nat. Inst. of Health, for NIH-LH-S-1, ovine, to the Schering Corp. for testosterone propionate and to Parke Davis & Co. for chorionic gonadotrophin.

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Received December 3, 1962. P.S.E.B.M., 1963, v112.

Inhibitory Effect of Antiviral Compounds on Viruses *in vivo* and in Mouse Ascites Cells *in vitro*.* (28120)

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Antiviral effects of several compounds, such as hyroxybenzylbenzimidazole, 5-iodo-2′-deoxyuridine, fluorophenylalanine, N-methylisatin-thiosemicarbazone, ammonium salts, Statolon, and Helenine have been demonstrated by a number of workers(1-19). We have compared the effects of several such compounds against adapted strains of Columbia SK and LCM viruses propagated in mouse ascites tumor cells. Effects of certain of the compounds *in vivo* are also included.

a. In vitro *tests. Materials and methods.* 1. *Virus-host system.* Hemagglutination(HA)-positive and HA-negative strains of Columbia SK (Col SK) virus, and LCM virus, strain NY621, isolated from a leukemic L_2C

* Supported in part by California Division Am. Cancer Soc. and Nat. Cancer Inst.