isolated, the following may be eliminated as analogues if their proposed structures are confirmed: α, since it is apparently a racemate, Cristol(16) having obtained the dextrorotatory enantiomorph; β (e,e,e,e,e,e)(17, 18); γ (p,p,p,e,e,e)(7,8); and zeta (p,e,e, p,e,e)(17). Our data also rule out the first 3 isomers mentioned and the δ isomer on biological evidence. The question of which isomer of BHC is analogous in structure to i-inositol must await further isolation and characterization studies.

The marked differences in physical properties (solubility, melting points, dipole moments) and in chemical reactivities between the hexahydroxy- and hexachlorocyclohexanes are not in accord with the accepted antimetabolite concept of biological competition. Therefore, true specific competitive antagonism will probably not be demonstrated between such physically dissimilar classes of molecules. However, the antithetical physiological properties of the BHC stereoisomers are unique and may therefore become of great interest in elucidating the mechanisms of narcosis and idiopathic convulsive disorders, such as epilepsy.

Summary. The toxicities of the α, β, γ, and δ isomers of hexachlorocyclohexane were not affected by inositol in the rat. The convulsive and narcotic effects of the γ and β isomers are discussed in detail.

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Some Effects of Testosterone Propionate on Mice Irradiated with X-rays.* (17994)

FRIEDRICH ELLINGER

From the Naval Medical Research Institute, Bethesda, Md.

We have recently demonstrated that administration of desoxycorticosterone acetate after exposure of mice to lethal doses of X-rays reduces the mortality produced by irradition(1,2). It has also been demonstrated that the administration of this hormone protects the liver of mice against radiation induced fatty changes(3) and prevents the radiation induced depletion of sudanophile substances(4) of the adrenal cortex. In order to obtain more complete information on the significance of these findings for the mechanism of total body irradiation, our studies on the pharmacological analysis of this process have been extended to include testosterone propionate. The use of this substance suggested itself because testosterone is not only a chemical relative of desoxycorticosterone but also exercises some similar metabolic effects.

Material and methods. Swiss male white mice (Rockland Farms Stock) 22 g $\pm$ 15% of body weight were used in these experiments. Irradiation took place in the previously described manner(5). The X-ray dose was in all instances 500 r/air (HVL 0.75 mm Cu).

* Based on experiments carried out at the Long Island College of Medicine, N. Y., aided by grants from the John and Mary R. Markle Foundation, New York, and the Schering Corporation, Bloomfield, N. J.

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This dose was administered in one exposure and represents the $LD_{50}/30$ days for these mice under our conditions of exposure. For the hormone treatment a solution of 25 mg of testosterone propionate in 10 cc of sesame oil (Oreton†) was used. Hormone treatment of irradiated animals started on the day of exposure and was given in 6 weekly doses over a period of 2 weeks.

All animals were observed for at least 28 days and some up to 90 days after the start of the experiment. The following experimental groups were used:

A. 34 mice received injections of .25 mg of testosterone propionate and irradiation.

B. 30 mice received injections of .5 mg of testosterone propionate and irradiation.

C. 20 mice received .5 mg testosterone only.

D. 18 mice served as unirradiated untreated controls.

For the study of the influence of testosterone propionate on the X-ray induced mortality rate, their death rate was noted day by day and the mortality curve thus obtained compared with that of 102 mice having been exposed to 500 r/air only.

Autopsy material obtained from the X-rayed and testosterone treated animals, which died during the course of the experiment, served for our study of the influence of testosterone administration on the X-ray induced histological changes. This material was supplemented by organs from 17 mice killed at various time intervals. The organs were fixed in Zenker's solution, embedded in paraffin, sectioned at 4-6 μ and stained with hematoxylin-eosin. In addition, liver and adrenal tissues were also fixed in 10% formalin, sections were made with the freezing microtome and stained with sudan III. The grading system previously described(2,4) was used for the quantitative analysis of the histological changes.

Results. General appearance of the animals: The irradiated animals treated with testosterone developed a ruffled fur about the second or third day after the start of the experiment. This change in the coat of the animals was independent of the dose of testosterone used. At the end of the first week the animals treated with testosterone after irradiation looked very sick in comparison with the animals which had received irradiation only. This change in general appearance was supported by the weight curves. Normal unirradiated animals of our stock gained about 7% of their initial weight during the first week while in 56 animals after exposure to 500 r/air a loss of about 7% of the starting weight was found at that time. The irradiated mice treated with testosterone lost about 11% of their initial weight at the end of the first week. Their general appearance became increasingly worse during the second week. One animal (No. 206/3) was almost bald, when it died on the twelfth day after the start of the experiment and its weight had dropped to 16 g. The general appearance of this group contrasted sharply not only with that of the irradiated control group but also with that of unirradiated animals which received testosterone. The latter showed a slight ruffling of the fur about the fifth day and appeared healthy at the end of the second week.

The influence of testosterone treatment on X-ray induced mortality rate is shown in Fig. 1. In agreement with their general appearance, the testosterone-treated animals showed increased susceptibility to the lethal effects of X-ray. But it appears noteworthy that there was a difference in the onset of death between the 0.25 mg and 0.5 mg dose, even though this difference is not statistically significant. No deaths occurred in the group treated with hormone alone. The study of *the influence of testosterone treatment on the X-ray induced organ changes* indicated no changes in all investigated organs with the exception of the liver, where changes in appearance of sudanophile substances were observed. The data are summarized in Fig. 2. The comparison of the fat accumulation in untreated but irradiated animals (section A) with that found in the group of mice treated with 0.25 mg testosterone and irradiated (section B) indicated no protective action of the hormone in this report. But a similar comparison with the results obtained in the

† Oreton was supplied by the Schering Co. through the courtesy of Dr. E. Henderson.

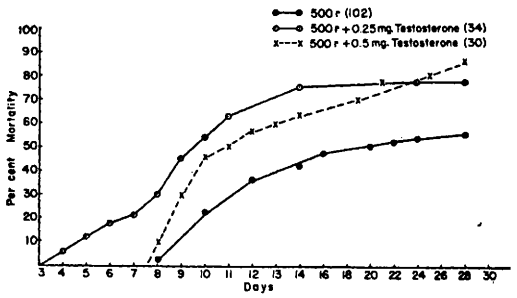

FIG. 1.

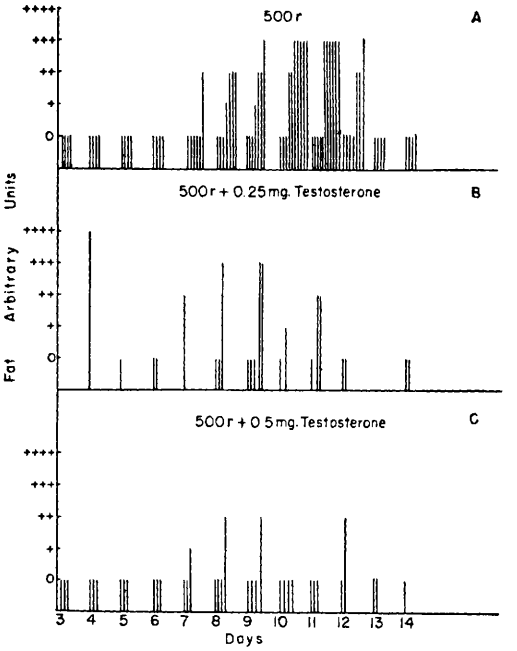

FIG. 2.

group treated with injections of 0.5 mg (section C) shows a definite and statistically significant protective action of the larger hormone dose (probability $p = 0.02$).

Discussion. The foregoing data clearly indicate that administration of testosterone propionate, in contrast to the closely related desoxycorticosterone acetate, enhances the lethal effect of X-rays. Our findings are in agreement with observations made by Edelmann(6) in rats. It appears likely that the antagonistic effect of testosterone and desoxycorticosterone demonstrated by our studies is based on their opposing effects on potassium metabolism. "Potassium diuresis induced by desoxycorticosterone acetate and very important in the repair of adrenal insufficiency is quite opposed to potassium retention induced by testosterone and unlike the negative effects of estradiol usually obtained" (Koch)(7). The similarity in metabolic effects of testosterone and desoxycorticosterone is seen in their effects on fat metabolism. Both steroids are capable of suppressing the radiation-induced fatty changes in the liver. This property was pronounced with the larger testosterone dose used in these experiments, and might possibly explain the somewhat delayed onset of the lethal effect observed in our group B animals. The absence of any protection of the adrenal cortex against radiation-induced depletion of sudanophile substance by testosterone treatment should be emphasized. The impression is given that testosterone administration rather enhances this process. As an indirect support for this contention, some observations made in our group C mice should be mentioned: Our results concerning the testosterone tolerance of unirradiated mice are in agreement with those of Kochakian(8). But histologic examination of the adrenals of 11 mice killed between 4 and 14 days after testosterone administration revealed in 3 animals complete depletion and in 4 a definite reduction of sudanophile substance of the adrenal cortex. In the light of these findings it appears possible to conclude that testosterone, when given in conjunction with irradiation, may augment stimulation of the adrenal produced by irradiation. The worsening in the general appearance of the testosterone-treated irradiated animals, over that of those only irradiated or only hormone-treated could readily be understood on this basis. Assumption of hormone-induced adrenal stimulation permits also under-

6. Edelmann, A. (Brookhaven National Laboratory) private communication. It appears noteworthy that the rats used by Edelmann were about 10 times as heavy as the mice used by us. It is therefore interesting that Edelmann used 5 mg of testosterone in his rats which is exactly 10 times the dose used in our experiments with mice.

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standing of the opposite effects of estradiol administration prior to or after irradiation (9,10). Finally, the observations concerning the action of testosterone administered to animals exposed to lethal doses of X-ray seem to support the opinion of Koch(7) that "neither testosterone nor the estrogens serve to repair adrenal failure."

Our findings concerning increased radiosensitivity in total body irradiation after testosterone administration appear interesting also from the point of view of general radiation biology, inasmuch as they offer an interesting parallelism to observations concerning thyroxin:

In previous studies we have been able to demonstrate that thyroxin increases radiosensitivity of the skin to X-ray(11) as well as to ultraviolet rays(12,13), and Blount and Smith recently demonstrated an increased radiosensitivity in total body X-irradiation (14). We have also been able to demonstrate that skin radiosensitivity increases significantly at puberty in both sexes(12) when sex hormones are produced more abundantly. From the previous and the present studies it appears that hormones in general may alter radiosensitivity locally and also as far as the response of the entire body is concerned, and that this change in general radiosensitivity is not specific for the type of radiation used over a large part of the spectrum(15). This appears to be of interest not only from a theoretical point of view but also from the standpoint of clinical medicine, because of the now generally accepted use of sex hormones in the treatment of certain malignant tumors.

Summary. 1. Administration of testosterone propionate in daily doses of 0.25 and 0.5 mg, respectively, following exposure of mice to the LD_{50} of X-ray, up to 14 days, markedly increased the lethal effect of irradiation. 2. No changes in the X-ray induced organ effects were accomplished in all investigated organs with the exception of the liver. Radiation induced accumulation of sudanophile fat in this organ was definitely suppressed with larger dose of testosterone. 3. Radiobiological and some clinical implications of these findings are discussed.

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