tical with prothrombin.

Summary. A further dissociation between coagulase-globulin and prothrombin has been demonstrated by means of adsorption on CaF_2 and by repeated filtrations through a Seitz filter. This indicates that coagulase-globulin is not identical with prothrombin.

Received August 28, 1950. P.S.E.B.M., 1950, v75.

Ovarian and Lymphoid Tumors in Female Mice Subsequent to Roentgen-Ray Irradiation and Hormone Treatment.* (18222)

W. U. GARDNER.

From the Department of Anatomy, Yale University School of Medicine, New Haven.

Ovarian tumors of the granulosa cell type have developed in mice subjected to total body irradiation(1) with roentgen rays and after intrasplenic transplantation of ovaries into castrated mice(2). The development of ovarian tumors under the latter experimental conditions has led to the hypothesis that prolonged exposure to excessive amounts of gonadotrophic hormone of the pituitary constitutes a carcinogenic stimulus(3). Ovarian tumors did not appear when estradiol benzoate or testosterone propionate were injected into mice with intrasplenic ovarian grafts. Furthermore, preliminary experiments indicated that gonadal hormones prevented the appearance of ovarian tumors in mice subjected to roentgen irradiation(4).

Many investigators, since the original observations of Furth(5), have noted the leukemagenic action of x-rays in mice and more recently the effects of additional treatment with estrogens(6).

Materials and methods. Three groups of 50 female mice of the BC† stock were used in the following experiments. The groups were so constituted that littermates were distributed throughout all groups as uniformly as possible. At ages ranging from 34 to 92 days the mice received a total body irradiation of 280 to 380 r. An equal number of mice in each of the groups received comparable doses. From 42 to 45 animals in each group survived irradiation, the earlier periods of the experiment and are included in the following data. The animals were maintained in an air conditioned room (72-74° F temperature and 50-60% relative humidity). They were fed a commercial food (Purina Fox Chow) and water *ad libitum*. The mice were examined daily throughout the course of the experiments. One group received weekly subcutaneous injections of 0.05 cc of sesame oil, one group 1.25 mg of testosterone propionate‡ dissolved in 0.05 cc of sesame oil; and one group 16.6 μg of estradiol benzoate§ dissolved in 0.05 cc of sesame oil. Injections were omitted on rare occasions in

* This investigation has been supported by grants from The Anna Fuller Fund, the Jane Coffin Childs Memorial Fund for Medical Research and the United States Public Health Service.

1. Bali, T., and Furth, F., *Cancer Res.*, 1949, v9, 449.

2. Li, M. H., and Gardner, W. U., *Cancer Res.*, 1949, v9, 35.

3. Gardner, W. U., *Cancer Res.*, 1948, v8, 397.

4. Li, M. H., Kaplan, H. S., and Gardner, W. U., unpublished.

5. Furth, J., *Am. J. Roentgenol.*, 1934, v32, 377.

6. Kirschbaum, A., Shapiro, J. R., and Mixner, H. W., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 632.

† BC refers to mice obtained as follows (PM♀ x C_3H♂) (F1♀ x PM♂) and subsequent brother to sister inbreeding. "PM" refers to mice sent to the writer by Dr. H. B. Andervont and derived from the colony of Drs. Pybus and Miller in England. The mice used in the present study were from the eleventh to the fifteenth generations of inbreeding—and largely from the twelfth to the fourteenth.

‡ The testosterone propionate was obtained from Organon, Inc., through the courtesy of Dr. Kenneth Thompson.

§ The estradiol benzonate was obtained from Ciba Pharmaceutical Products, through the courtesy of Dr. E. Oppenheimer.

TABLE I. Mice with Ovarian Tumors and Average Survival Time of Mice of the BC Strain Subjected to Total Body Irradiation and Treated with Hormones.

Group	Avg at death			
	Without tumors	With tumors	Small tumors†	Large tumors
Sesame oil	320(21)*	513(24)	496(9)	524(15)
Testosterone propionate	381(16)	512(29)	506(9)	515(20)
Estradiol benzoate	295(42)	—	—	—

* Numbers in () indicate the No. of mice.
† Tumors measuring <5 mm greatest diameter.

TABLE II. Survival and Number of Ovarian and Lymphoid Tumors in Irradiated Mice (280-380 r) Given Weekly Subcutaneous Injection of 16.6 μg of Estradiol Benzoate or 1.25 mg of Testosterone Propionate Dissolved in 0.05 cc of Sesame Oil or Sesame Oil Alone.

Age group	Estradiol benzoate	Testosterone propionate	Sesame oil
100-199	7(–/4)*	1(–/1)	2(–/1)
200-299	18(–/11)	4(–/2)	9(–/8)
300-399	8(–/6)	4(1/3)	7(2/6)
400-499	9(–/2)	15(10/3)	8(6/3)
500-599		20(17/–)	18(15/3)
600-699		1(1/–)	1(1/–)
Total	42(0/23)	45(29/9)	45(24/21)

* Figures in () indicate the number of ovarian tumors/No. of lymphomas.

the latter group when the general condition of an animal seemed to contraindicate treatment for one or 2 weeks. At necropsy the ovaries of all animals were fixed in Bouin's fluid and after sectioning they were stained in hematoxylin and triosin. Other tumors and organs were saved and similarly prepared when the gross observations indicated some abnormality.

Observations. More than one-half of the irradiated mice given weekly injections of testosterone propionate dissolved in sesame oil, or sesame oil alone, had ovarian tumors at death; and none of the estrogen-treated mice had ovarian tumors (Table I). Although the average ages of the mice of the estrogen-treated group was much less than that of the other two groups 17 mice of the former groups survived as long as did 19 of the mice with ovarian tumors in the 2 latter groups (Table II). The early death of mice of the estrogen-treated group was due primarily to the early appearance and high incidence of leukemia. Some of the small ovarian tumors were so small that they were diagnosed only after examination of the serial sections of the ovaries. They were composed of from 1 to 3 nodules of granulosa cells, usually less than 1 mm in diameter. Histologically they were not unlike the larger tumors.

All of the ovarian tumors, except 2 in one animal, were granulosa cell tumors not unlike those described previously. The larger tumors ranged up to 2 cm in diameter. Three of the tumors contained large cysts. Two tumors in one of the mice receiving testosterone propionate were luteomas. Fourteen of the mice given testosterone propionate and 9 of the mice given sesame oil had bilateral tumors. Nine and 10 of the mice in the 2 groups, respectively, had large unilateral tumors. Most of the small tumors appeared near the hilus of the ovary. The impression was obtained that they arose from germinal epithelial ingrowths in this area. Ducts or cysts lined by a pseudostratified or columnar epithelium almost always occupied the hilus or ovarian stalk region but no evidence of their contribution to the tumors could be obtained. Occasionally these ducts were surrounded by the tumors. The ducts were also present in the ovaries of the estradiol-treated mice.

Histologically the non-tumorous ovaries of the testosterone-treated and control groups were very small—less than 1 mm in diameter—and contained ingrowths from the germinal epithelium and large clear or brown interstitial cells. Pituitary deficiency-cells were noted only in ovaries in mice in which the contralateral ovary was tumorous. The ovaries of the estrogen-treated mice were also small—less than 1 mm unless involved in leukemia—and consisted of multiple small anovular follicles obviously arising from the germinal epithelium and a cellular interstitial tissue showing nuclei with the peripheral chromatin aggregates characteristic of the gonads of hypophysectomized animals.

The lymphomas were not unlike those previously described in irradiated mice and ranged considerably in their histological structure and extent of involvement of other organs or tissues.

Three of the mice given testosterone propionate had subcutaneous fibrosarcomas and but one occurred in mice of each of the other 2 groups.

Summary and conclusion. Irradiated mice given estradiol benzoate did not have ovarian tumors whereas mice given testosterone propionate (1.25 mg weekly) or sesame oil did. Irradiated mice given testosterone propionate had fewer lymphomas than did the mice given estradiol benzoate or sesame oil.

Received August 29, 1950. P.S.E.B.M., 1950, v75.

Comparative *in Vitro* Response of Tubercle Bacillus (H37RV) to Dihydrostreptomycin, Para-aminosalicylic Acid, and Dihydrostreptomycin-para-aminosalicylate.* (18223)

K. VENNESLAND, J. ALTONA AND R. G. BLOCH.

From the Frank Billings Medical Clinic, Department of Medicine, University of Chicago.

The authors reported results of *in vitro* experiments(1) indicating that the two drugs streptomycin and PAS, when used together, were more effective in inhibiting the growth of tubercle bacilli than either drug used alone. This was also found to be true in *in vivo* studies(2). Hobby(3), studying a PAS salt of streptomycin consisting of 56 parts of streptomycin and 44 parts of PAS, reported that this compound was twice as effective as an equivalent amount of streptomycin used alone. She further reported early studies indicating that strains of tubercle bacilli resistant to the compound did not readily emerge, none having been isolated after a three-month period of trial. This is in sharp contrast to the well known fact that streptomycin-resistant organisms emerge quite rapidly both *in vitro* and *in vivo* when exposed to this drug, but in keeping with reported *in vitro* studies indicating that the presence of PAS together with streptomycin prevents the emergence of resistance(4). PAS-resistant strains of tubercle bacilli have been reported in patients who have received prolonged therapy(5). *In vitro,* however, such resistant strains emerge only after many months of exposure to PAS(6). Attempts to isolate either streptomycin-resistant or PAS-resistant strains from cultures repeatedly exposed to the two drugs used together have been unsuccessful.

The present report deals with a comparative study of the *in vitro* effects of dihydrostreptomycin-para-aminosalicylate, of dihydrostreptomycin and para-aminosalicylic acid used together in amounts equivalent to those present in dihydrostreptomycin-para-aminosalicylate and of para-aminosalicylic acid and dihydrostreptomycin, each used alone, both with regard to the *in vitro* growth inhibiting effect and to the development of resistance.

Method. The dihydrostreptomycin-para-aminosalicylate (DHSM-PAS) used was a chemical compound in which one mole of dihydrostreptomycin (DHSM) was combined with three moles of para-aminosalicylic acid (PAS). DHSM and PAS were used in a 1:3 molecular ratio in experiments in which the 2 drugs were used together. For convenience in this paper, the use of the 2 free drugs together will be designated by the symbol

* This investigation was supported by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

1. Vennesland, K., Ebert, R. H., and Bloch, R. G., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 250.

2. Bloch, R. G., Vennesland, K., Ebert, R. H., and Gomori, G., *Am. Rev. Tuberc.,* 1949, v59, 554.

3. Hobby, G., Regna, P., and Levert, T., *Am. Rev. Tuberc.,* 1949, v60, 808.

4. Graessle, O. E., and Pietrowski, J. J., *J. Bact.,* 1949, v57, 459.

5. Delaude, A., Karlson, A. G., Carr, D. T., Feldman, W. H., and Pfeutze, K. H., *Proc. Staff Meet. Mayo Clin.,* 1949, v24, 341.

6. Steenken, Jr., W., personal communication.