TABLE III.
Weight Gains in Experiment II.

	Group	
	I Basal ration A	II Basal ration A + reticulogen
No. animals	9	12
Av initial wt	1.56 kg	1.60 kg
Av final wt at 8 wks	16.0 "	21.6 "
Av gain in wt for 8 wks	14.4 "	20.0 "

Summary. The addition of Reticulogen (a 20-unit anti-pernicious anemia liver extract) to a synthetic milk diet resulted in an increased growth rate in baby pigs over an 8-week period. The final weights of pigs receiving Reticulogen averaged 19.96 and 21.6 kg as compared with 13.53 and 16.0 kg for those not receiving Reticulogen in two comparisons.

16774

Effect of a Progesterone Compound on Growth of a Transplanted Granulosa Cell Tumor.*

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Progesterone has been considered to be inhibitory to, or without effect on, tumor growth. Heimann[1] reported a marked decrease in incidence of mammary carcinoma in the RIII strain of mice treated with progesterone alone and a still lower incidence when this was combined with testosterone. He stated, however, that it did not prevent growth of transplanted tumors in mice. He also found[1b] that it inhibited growth of adenomatous portions of breast fibroadenomata and decreased the number of takes of auto- and homo-transplants in rats. There was no effect on fibroma, myxoma, or sarcoma or on adenomatous growth in pregnant or castrated rats. In the guinea pig Lipschütz and Vargas[2] observed decrease in size, and actual disappearance, of the fibroids caused by oestrogen. Burrows and Hoch-Ligeti,[3] however, found no change in incidence of mammary carcinoma in mice injected weekly with 1 mg of progesterone, and Loesser[4] found slight, if any, improvement in animals or patients with breast carcinoma treated with progesterone.

The effects of larger amounts of progesterone on tumor growth have been studied, using a material containing a high concentration of progesterone.§ Preliminary experiments indicated a growth-stimulating effect on mammary carcinoma in mice.[5] A study of the effect of this material on a transplanted ovarian tumor was then undertaken. Subcutaneous transplantation of a granulosa cell tumor induced by intrasplenic transplants[6] in mice of the C57 strain had been attempted.

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1 a, Heimann, Jacob, *Cancer Research*, 1945, **5**, 426; b, Heimann, Jacob, *Cancer Research*, 1943, **3**, 65.

2 Lipschütz, A., and Vargas, L., *Lancet*, 1939, **2**, 420.

3 Burrows, Harold, and Hoch-Ligeti, Cornelia, *Cancer Research*, 1946, **6**, 608.

4 Loesser, A. A., *Lancet*, 1941, **2**, 698.

§ A crude preparation believed to contain 200-250 I.U. Progesterone/cc made available through the courtesy of E. A. Engstrom, The Glidden Co., Chicago, Ill.

5 Cliffton, E. E., unpublished data.

6 Li, M. H., and Gardner, W. U., *Cancer Research*, 1947, **7**, 549.

TABLE I.

Treatment	Total No. animals	No. animals without growth	No. of animals with growth						
			1 wk	2 wk	3 wk	4 wk	5-7 wk	8-12 wk	12-16 wk
Glidden Progesterone	26	4	1(1)	11(10)	20(9)	22(2)	22(0)	22(0)	22(0)
Oestradiol	18	9	0	0	0	2(2)	4(2)	9(5)	—
Progesterone*	8	7	0	0	0	0	0	0	1(1)
Control	18	14	0	0	0	0	0	1(1)	4(3)

Incidence and time of apparent subcutaneous growth of tumor 18C57) with different hormone treatment.

The numbers in brackets are the numbers of animals showing first growth in that period while the unbracketed numbers are total tumor takes through that period.

*"Proluton" obtained through the courtesy of the Schering Corp., Bloomfield, N. J., containing 5 mg progesterone/cc.

TABLE II.

Treatment	Total No. animals	No. animals showing growth in 6 wk
Glidden Progesterone 0.05 cc two times per week	31	28
Oestradiol 0.05 cc two times per week	9	5
Progesterone* ("Proluton") 0.1 cc two times per week	4	0

Number of animals treated and showing growth of tumor within 6 weeks with 3 types of hormone treatment.

*"Proluton" obtained through the courtesy of the Schering Corp., Bloomfield, N. J., containing 5 mg progesterone/cc.

Growth of one of the transplanted tumors occurred but rarely and only after a long time in intact or castrated animals with and without estrogen treatment. In castrated animals treated with the crude progesterone, growth of the tumor occurred in some cases within 10 days. Subsequent study has confirmed this finding plus additional information.

Methods. Mice of the C57 strain approximately 2 to 3 months of age were castrated and a small piece of tumor was transplanted subcutaneously in each animal. The tumor was obtained from a first generation subcutaneous transplant. Injections were started on the day of transplantation. To facilitate injection, the crude material was diluted by adding 1 part Ethanol to 4 parts of the compound. The test animals in the first group received 0.05 ml of the diluted "progesterone compound" 3 times per week. Subsequent series received the diluted material 2 times per week. Control animals were set up in 4 groups: Group one received estradiolbenzoate (25 μg in 0.05 cc sesame oil 2 times a week); Group 2 received Ethanol (1 part to sesame oil 4 parts, 0.05 cc two times per week); Group 3 received progesterone‖ 0.05 cc daily, and subsequently 0.1 cc daily; and Group 4 received no injections. Groups 2 and 4 have been combined in the study of results (Tables I and II). All animals were kept on a standard diet and water *ad libitum,* with the same number of animals per cage. Alternate animals were used for each treatment.

Three series of animals so treated, confirmed the preliminary opinion that the crude, progesterone-containing compound, when injected into animals receiving a transplant of the granulosa cell tumor, stimulated growth of the tumor (Tables I and II). Seventy-seven per cent of the "progesterone compound" treated animals had growing tumor within 3 weeks, and 85% within 4 weeks, while in the other groups no palpable tumors were ob-

‖ "Proluton" obtained through the courtesy of the Schering Corp., Bloomfield, N. J., containing 5 mg progesterone/cc.

served in the 3 week period and only 2 in the 4 week period. Allowing a period of at least 4 months for the control animals to develop tumors, it was found that 50% of the estrogen treated animals, 22% of the animals receiving Ethanol in sesame oil or no injections, and one, or 25% of the "proluton" treated animals showed tumor growth, to compare with the 85% tumor growth within 4 weeks with the test material.

One small series of intact mice with transplanted tumor, divided into "progesterone compound" treated, estrogen treated, "proluton" treated, and control groups of 3 animals each, was studied. These animals were also allowed to breed. Of this group only one, a progesterone compound treated mouse, developed a tumor.

Subsequent groups of animals with tumor transplants of the second and third generations, observed for only 6 weeks, are shown in Table II. These confirm the previous findings with 90% of the "compound" treated, 55% of the estrogen treated, and none of the "proluton" treated animals showing tumor growth.

Additional facts in evaluating the action of the "progesterone compound" are: (1) 7 of the "progesterone compound" treated animals of the original 26 died of their tumors within the period 1 to 3½ months after transplantation, while none of the other animals of the original 3 series died of their tumors up to 4 months after transplantation. Many animals were sacrificed in all groups but in every instance the animals in the control groups were permitted to live longer because their tumors were smaller; (2) 2 of the "progesterone compound" treated animals developed gross and several had microscopic metastases, while no animals of the other groups showed metastatic spread.

More detailed studies are being carried out. One obvious line of investigation will be the effect of other hormones especially androgens on the growth of this tumor. It is always possible that this growth stimulation is due to some other material present in the "compound", which can be proven only by more detailed chemical work.

16775 P

Effects of Folic Acid on the Anemia Induced by X Irradiation.

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Previous work in this laboratory has shown that the anemia which followed radiations from internal and external sources was frequently macrocytic.[1,2] The macrocytosis may have been the result of damage directly to the bone marrow or indirectly through damage to the viscera, resulting in failure to form an antianemia factor. Folic acid, classified as a vitamin of the B complex, is reported to be similar in its action to liver extract. It has been shown to be effective therapeutically in certain of the macrocytic anemias in man.[3,4] It was without effect, however, upon the macrocytic anemia that resulted from administration of Sr^{89}.[5,6] Total-body X irradiation, unlike radioactive strontium, causes damage to the viscera as well as bone marrow. It is

[1] Stearner, S. P., Simmons, E. L., and Jacobson, L. O., to be published, 1948.

[2] Simmons, E. L., and Jacobson, L. O., Radiotoxicity of injected Sr^{89} for rats, mice and rabbits. Part IV. The hematological effects of enterally and parenterally administered Sr^{89} in mammals. National Nuclear Energy Series, Vol. 22b.

[3] Spies, T. D., Vilter, R. W., Koch, M. B., and Caldwell, M. H., *Southern Med. J.*, 1945, **38**, 707.

[4] Moore, C. V., Vilter, R. W., Manneck, V., and Spies, T. D., *J. Lab. and Clin. Med.*, 1944, **29**, 1226.

[5] Jacobson, L. O., Stearner, S. P., and Simmons, E. L., to be published.