

Hypervolemia and Associated Changes in Mice Bearing a Transplanted Granulosa Cell Tumor*

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Mice bearing a transplanted granulosa cell tumor have an increased blood volume associated with sinusoidal dilatation in the liver, spleen, and adrenal glands. These changes were first described by Furth, Boon, and Sobel (3, 4, 5, 6). Growth of transplanted granulosa cell tumors has been obtained in a large percentage of castrated mice of the C57 strain¹ that received injections of a crude compound² (2). In connection with other studies, it was noted that a much larger quantity of heart blood was obtained from these animals than had been obtained previously from mice of a similar size of this or other strains either with or without tumors. At post-mortem an increase in the size of the liver and spleen was noted in these animals as compared to normal mice and mice bearing other tumors.

METHODS

A total of 116 mice of the C57 strain were used. The granulosa cell tumor (18 C57) was induced in an animal of the C57 strain by transplantation of an ovary into the spleen. The animals were castrated at 60 to 90 days of age and a small fragment of the tumor transplanted subcutaneously immediately following castration. The tumor transplants became palpable between 10 and 35 days after transplantation and attained a size of 1 × 1 cm. in 40 to 65 days. Early in the experiment the animals were sacrificed only when death seemed imminent; later in the experiment other animals were sacrificed with tumors of different sizes, the smallest being 6 × 6 mm. Sixty-two animals were treated with the crude compound diluted 4 parts to 1 part of ethanol. These animals received 0.05

cc. of the diluted crude compound 2 times weekly. Sixteen animals received 0.05 cc. of estradiol benzoate (25 μg in 0.05 cc. sesame oil) 2 times weekly. Ten animals received progesterone,³ containing 5 mg. progesterone/cc., 0.05 cc. daily, and 5 animals were treated with 0.05 cc. sesame oil diluted with ethanol (4 parts of sesame oil to 1 part of ethanol) 2 times weekly. Twenty-three animals received no injections. All animals were kept on a standard diet and water *ad libitum*.

OBSERVATIONS

The animals with and without tumor growth gained weight progressively. The animals with granulosa cell tumors increased in weight more rapidly than the controls when the tumor attained an approximate diameter of 1 cm. The maximum gain in weight of tumor bearing animals, after subtraction of the tumor weight determined at necropsy, ranged from 3.3 to 13.6 gms.; 27 of 33 animals gained over 5.0 gms. and 7 animals gained over 10.0 gms. Animals without tumors gained from 0.0 to 9.7 gms.; only 7 of the 35 animals gained over 5.0 gms. The average increase in weight, less tumor weight, of the tumor bearing animals was 7.1 gms., while that of the animals without tumors was 3.0 gms.

The differences of weight increment were greater in animals that were mature at the beginning of the experiment. The animals without tumors that gained more than 5.0 gms. were immature at the start of the experiment; they weighed from 13.2 to 17.0 gms. Because the animals without tumors lived an average of 3 months longer than the tumor bearing animals before being sacrificed, they should have increased relatively more in weight.

The tumors were removed from 3 animals and in each instance the animal's weight returned to and remained at the level of the control animals. A local recurrence of the tumor developed 2 months later in one animal that subsequently in-

³ Proluton obtained through the courtesy of Shering Corporation, Bloomfield, N.J.

* This investigation was supported by a research grant from the U.S. Public Health Service and a fellowship grant from the American Cancer Society.

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¹ Original mice obtained from L. C. Strong.

² Crude compound containing approximately 200–250 I.U. progesterone, obtained from the Glidden Company through the courtesy of A. E. Engstrom.

creased in weight in a manner similar to the other tumor bearing animals.

The blood, obtained by cardiac puncture from tumor bearing animals, weighed from 1.0 to 3.3 gms.; from animals without tumors the blood weighed from 0.4 to 1.3 gms. The average weight of blood from tumor bearing animals was 2.1 gms. and from animals without tumors was 0.6 gms. (Chart I). At necropsy an increase in the size of the heart was noted without a concomitant increase in its weight, indicating that the increase in size was due to dilatation.

The livers of the animals with tumors showed marked vascular congestion; their surfaces presented an unusual mottled appearance. The weights of the livers in 51 tumor bearing animals ranged from 1.3 to 6.0 gms., an average weight of 2.5 gms. The livers of 30 animals without tumors weighed from 1.0 to 2.3 gms., an average weight of 1.5 gms. (Chart II).

Microscopic examination of the livers from animals with tumors revealed many dilated blood sinuses. All stages were noted from slight dilatation of the blood sinuses (Fig. 1) to marked dilatation of all blood channels and the formation of new blood-filled sinuses, in many instances isolating single cords of liver cells in pools of blood (Fig. 2). Necrosis of liver cells was not noted except in livers with extreme sinusoidal dilatation. The number of macrophages was increased.

The spleens of the 51 tumor bearing animals were congested and enlarged; they weighed from 0.19 to 1.46 gms. as compared to 0.11 to 0.60 gms. for the 30 animals without tumors. The average weight of the spleens of tumor bearing animals was 0.52 gms. and of the animals without tumors was 0.20 gms. (Chart III).

Microscopic examination of the spleens from animals with tumors showed evidence of increased formation of erythrocytes and an increased number of macrophages. Microscopic examination of the adrenal glands from animals with tumors revealed vascular engorgement of the sinusoids of the cortico-medullary area and destruction of the adjacent cortical cells (Fig. 3).

The uteri of animals with tumors were enlarged and did not show the usual atrophy of castration; their weights ranged from 0.08 to 0.23 gms., averaging 0.14 gms. Microscopic examination of these uteri revealed metaplasia of the epithelium and definite estrogenic effects were observed in smears of vaginal secretions. The uteri of animals without tumors showed the usual atrophy of castration.

The seminal vesicles and prostate of male animals with tumors also showed evidence of stimulation; their weights varied from 0.02 to 0.17 gms.,

averaging 0.10 gms. Microscopic examination showed secretion in these seminal vesicles.

Another unusual change was the presence of hemorrhagic lymph nodules in the submaxillary glands in the male animals bearing large tumors. These nodules showed markedly dilated blood channels and evidence of hemopoiesis.

DISCUSSION

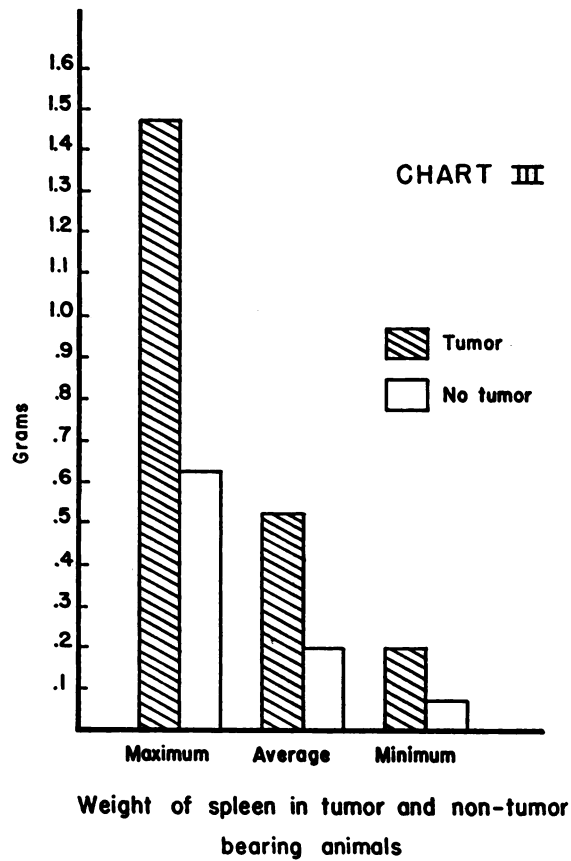
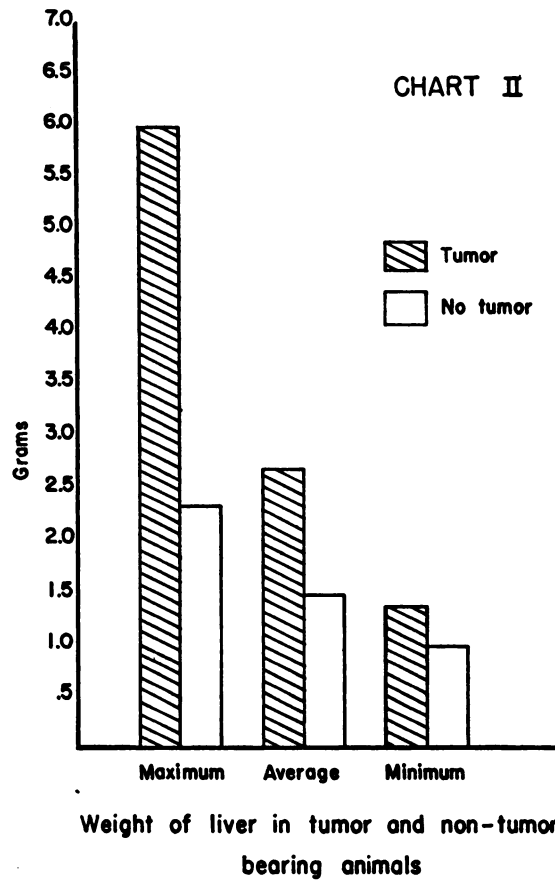
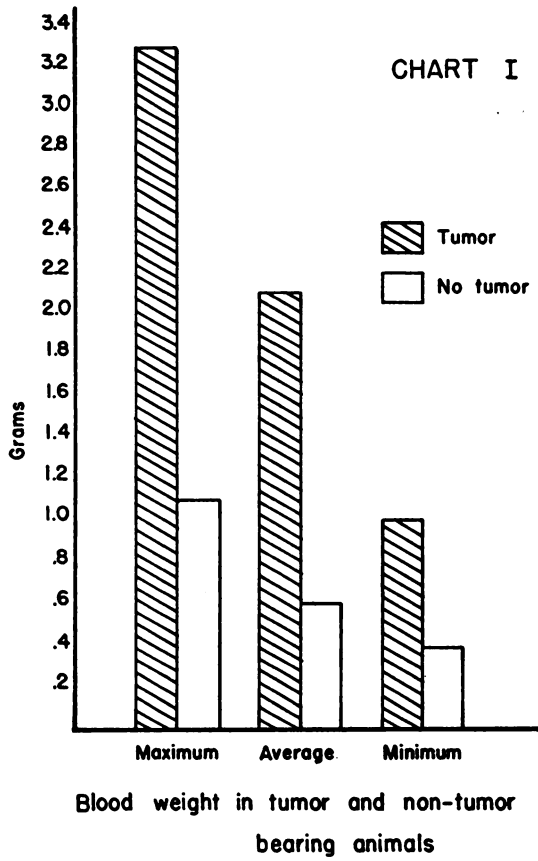
An analysis of the results presented indicates that the most significant difference between the tumor bearing and the non tumor bearing animals is in the blood weight. The difference in the weight of the spleen is next in significance, followed by the weight of the liver. This would indicate that the primary change of the condition described is in the blood volume. Further studies are necessary to discover whether this is due to a fluid volume change resulting from water retention. The observations previously reported indicate that only slight hemodilution occurs (5).

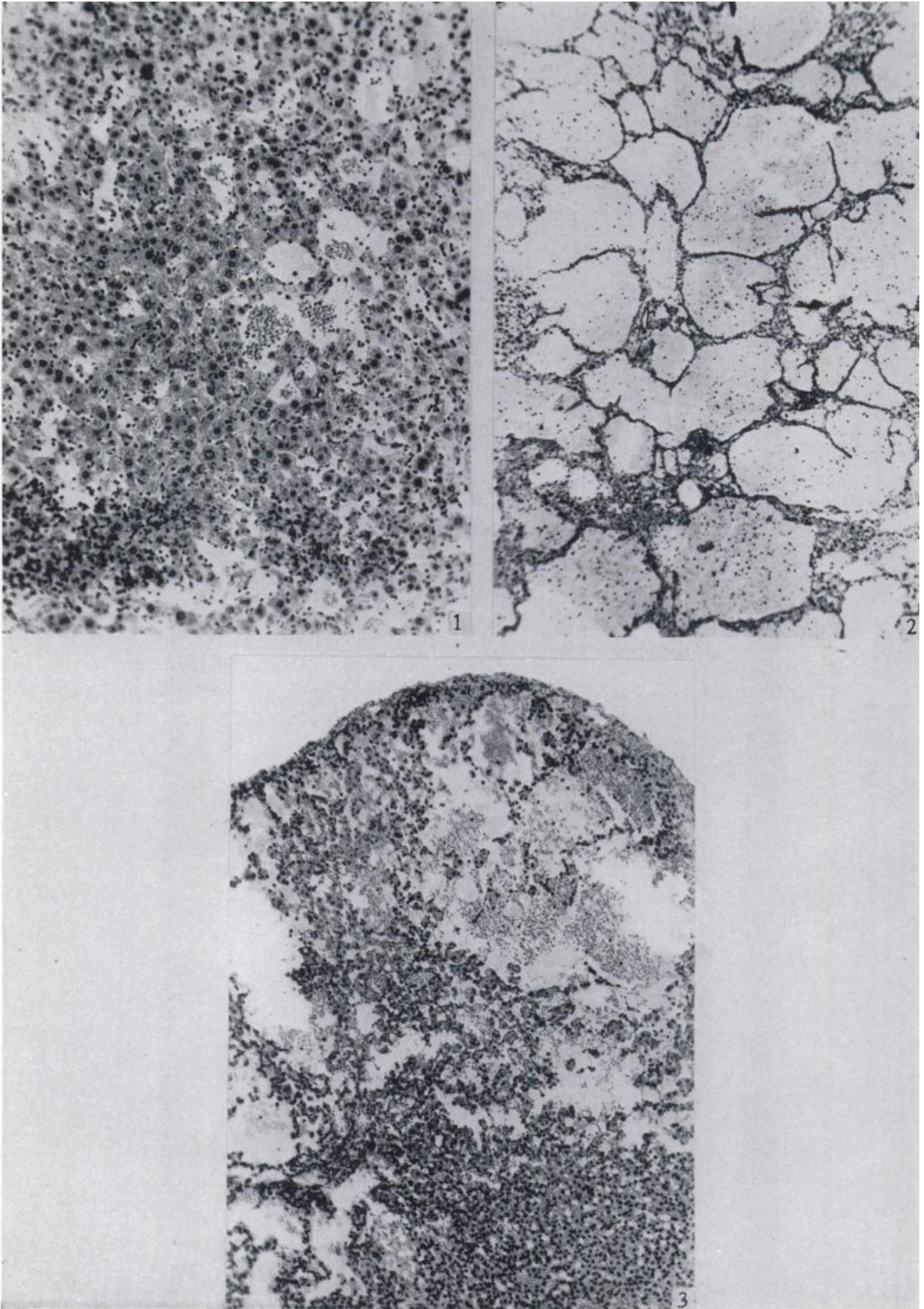
The changes described are not dissimilar to those found in pregnancy. The increase in weight of pregnant mice is greater than can be accounted for by the products of conception (1). Weight loss in excess of the weights of the uterine contents occurs in the first 24 hours following delivery and is probably due primarily to loss of body water. Weights of pregnant mice after killing the fetuses by pressure are maintained so long as both placentae and ovaries are present but the absence of either one causes a definite weight loss (7, 8). These changes are not unlike the loss of weight in animals from which the tumors were removed.

In women there is a progressive increase in the plasma volume from early pregnancy through the ninth lunar month, amounting to an increase of 65 per cent above the average non-pregnant value and 2 to 3 weeks before delivery it decreases to approximately 50 per cent above the non-pregnant value. It has been stated that "some hormonal influence may affect the blood volume changes observed in pregnant women" (9).

Although the transplanted ovarian tumor undoubtedly produces a substance with an estrogenic effect, as indicated by the changes in the uteri and the vaginal smears, it seems unlikely that the condition of hypervolemia could be attributed to estrogen. There is no evidence that estrogen alone or in combination with progesterone significantly alters blood volume.

The hormonal effect noted is not entirely due to estrogen as enlargement of the seminal vesicles also occurs. The changes are not due to the crude compound as tumor bearing animals which did not





FIGS. 1-3

receive any hormonal treatment developed the same changes and animals without tumors receiving the crude compound did not.

It is unlikely that these changes are due to infection as reversion in weight occurs in those animals operated upon with removal of the tumor and also by the fact that these changes do not occur in a twin of a parabion having a transplanted tumor (unpublished data).

SUMMARY

1. Mice bearing a transplanted granulosa cell tumor have hypervolemia and associated sinusoidal dilatation in the liver, spleen, and adrenal glands.

2. An estrogenic effect, as evidenced by vaginal smears and enlargement of the uteri, was noted in castrated female animals bearing this tumor.

3. Enlargement of the seminal vesicles and prostate was observed in castrated, tumor bearing males.

REFERENCES

1. BROOKSBY, J. B., and NEWTON, W. H. Effect of the Placenta on the Body Weight of the Mouse. *J. Physiol.*, **92**:136-150, 1938.
2. CLIFFTON, E., and PAN, S. The Effect of a Progesterone Compound on the Growth of a Transplanted Granulosa Cell Tumor. *Proc. Soc. Exper. Biol. and Med.*, **69**:516-518, 1948.
3. FURTH, J. Transplantation of Induced Granulosa Cell Tumors and Luteoma in Mice. Secondary Effects of These Growths. *Proc. Soc. Exper. Biol. and Med.*, **61**:212-214, 1946.
4. FURTH, J., and BOON, M. C. Liver Changes Associated with a Transplanted Granulosa Cell Carcinoma in Mice. *Proc. Soc. Exper. Biol. and Med.*, **58**:112-114, 1945.
5. FURTH, J., and SOBEL, H. Hypervolemia Secondary to Grafted Granulosa Cell Tumors. *J. Nat. Cancer Inst.*, **7**:103-113, 1946.
6. FURTH, J., and SOBEL, H. Neoplastic Transformation of Granulosa Cells in Grafts of Normal Ovaries into Spleens of Gonadectomized Mice. *J. Nat. Cancer Inst.*, **8**:7-16, 1947.
7. NEWTON, W. H., and LITS, F. J. Pseudo-parturition in the Mouse and the Relation of the Placenta to Post-partum Oestrus. *J. Physiol.*, **84**:196-207, 1935.
8. NEWTON, W. H., and LITS, F. J. Criteria of Placental Endocrine Activity in the Mouse. *Anat. Rec.*, **72**:333-349, 1938.
9. THOMPSON, K. J., HIRSHEIMER, A., GIBSON, J. G., and EVANS, W. A. Studies on the Circulation in Pregnancy. *Am. J. Obst. and Gynec.*, **36**:48-59, 1938.

FIG. 1.—Section of liver of tumor bearing animal No. 106 C57 showing early sinusoidal dilatation. Mag. 16 mm. objective and 10× ocular.

FIG. 2.—Section of liver of tumor bearing animal B-1C57

showing marked sinusoidal dilatation. Mag. 16 mm. objective and 10× ocular.

FIG. 3.—Section of adrenal gland of tumor bearing animal B-1 C57 showing sinusoidal dilatation. Mag. 16 mm. objective and 10× ocular.