In contrast to ovarian secretion, which remarkably inhibits x-ray induced ovarian tumorigenesis in mice, secretion of the testis is not similarly inhibitory. Presently Balb/c ovaries are being grafted into the spleens of intact and castrated Balb/c mice to determine the degree to which ovarian tumor development induced by transplanting an ovary into the spleen is inhibited by the testis. Adequate data on this point do not appear in the literature.

Testosterone inhibits the abnormal release of pituitary gonadotrophin after x-irradiation of female mice(4). Since neither injected (3) nor endogenously produced androgen effectively (in contrast to estrogen) inhibits ovarian tumorigenesis, in many cases exposure of the ovaries to elevated blood levels of gonadotrophins may not be essential for ovarian tumor formation induced by x-rays. It is possible that estrogenic hormone may act directly on the ovary to suppress tumor development.

Summary. When irradiated ovaries were grafted into intact as well as castrated male Balb/c mice, ovarian tumorigenesis proceeded. Functional normal ovarian grafts into previously irradiated Balb/c females inhibited tumorigenesis in the irradiated ovary. It appears that irradiation-induced ovarian tumorigenesis in this inbred strain is inhibited almost completely by ovarian, but only to a minor degree by testicular secretion.

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Presence of Erythropoietic Factor in Plasma of Normal and Hypophysectomized Rats Following Bleeding.* (22434)

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Carnat and Deflandre(1) discovered a factor in the plasma of anemic rabbits which would stimulate erythropoiesis. This factor, "erythropoietine" or "hematopoietine," has been demonstrated by many other investigators in the plasma of rabbits made anemic by repeated bleeding or by injection of phenylhydrazine(2-8). It has also been demonstrated in the plasma of rats(9,10) and in the human(11).

The influence of the endocrine glands on hemopoiesis is being actively investigated, and the anemia which follows removal of the hypophysis is well established; the present investigation was undertaken in an attempt to determine whether the anemia in the hypophysectomized animal can be related to the absence of such a plasma factor.

Methods. Adult female rats of 3 to 4 months of age and of the Sprague-Dawley strain were used in this experiment. All rats were fed Purina chow *ad libitum* supplemented once a week by lettuce. Completeness of hypophysectomies was verified by examination of the organ site at autopsy with a binocular dissecting microscope. *Donor rats,* from which plasma was obtained, were divided into 6 groups: (1) normal unbled rats; (2) and (3) bled normal rats; (4) hypophysectomized rats not yet anemic; and (5) and (6) bled hypophysectomized animals. One group of normal rats was made anemic by removing 4 cc of blood on day 1, and 2 cc on day 3, the rats being exsanguinated on day 5. At that time the hematocrit averaged 22% as compared to 43% for the normal unbled

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rats. A second group of normal rats was bled 4 cc on day 1, 2 cc on day 3, and either 2 or 3 cc on day 5. Blood was obtained on the 6th day. In spite of the fact more blood was removed from these rats than from the first group, the hematocrits averaged 25%. In the first group of bled hypophysectomized rats, 3 cc of blood were removed on day 1 only and the animals exsanguinated on day 5. The hematocrits averaged 27% compared to 41% for the unbled hypophysectomized rats. A second group of hypophysectomized rats was exposed to a more severe hemorrhage, 3 cc of blood being removed on day 1, and 2 additional cc on day 3. These animals were exsanguinated on day 5, the hematocrits averaging 21%. *Blood* from each group of rats was placed in tubes containing heparin and centrifuged. The plasma so obtained was injected into normal rats (average body weight = 193 g) in either 2 cc or 3 cc doses per day for 3 consecutive days, injections being given intravenously via tail veins. Reticulocyte counts were used as an index of erythropoietic stimulation. Blood for this study was obtained by heart puncture 24 and 48 hours after the last injection. Reticulocytes were stained with New Methylene Blue according to the method of Brecher(12).

Results. The results obtained are presented in Fig. 1. Plasma from unbled normal rats (6.0 cc total) did not produce a reticulocyte response when injected into normal recipients. The same amount of plasma from donor rats which were bled to such an extent that the hematocrit averaged 22% (normal—43%) did induce a response, the reticulocyte count 24 and 48 hours after the last injection being 1.6%, an increase of 60%. As these figures were not statistically significant, the experiment was repeated using a total dose of 9.0 cc of plasma rather than 6.0 cc. Under these circumstances the reticulocyte response was highly significant, reaching levels 367 and 483% above normal 24 and 48 hours after the last injection respectively.

When rats which had been hypophysectomized 40 days previously but which exhibited a nearly normal hematocrit of 41% served as donors (6.0 cc total), the reticulocyte

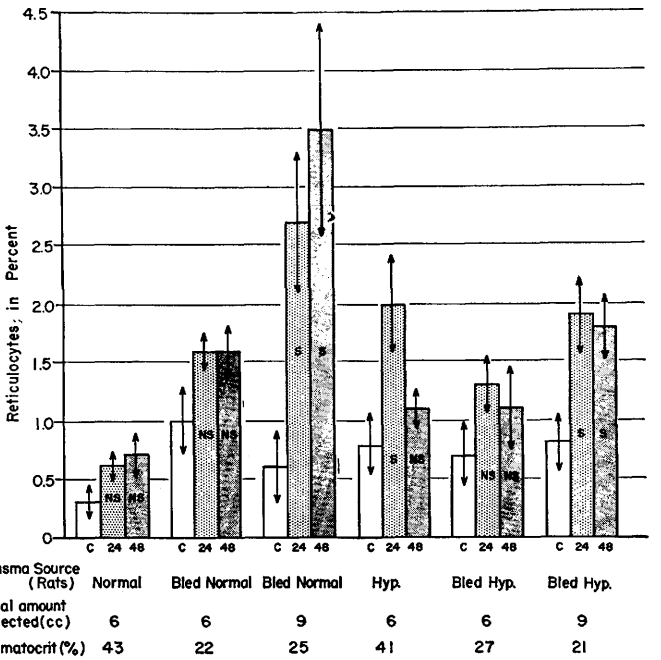

FIG. 1. Reticulocyte response to injections of 6 or 9 cc of plasma obtained from normal rats, bled normal rats, hypophysectomized rats, and bled hypophysectomized rats. Each value is an avg of counts on 5 or 6 animals. C = Control values (before inj.); 24 = 24 hr after last inj.; 48 = 48 hr after last inj. Vertical arrows = stand. error; S = significantly (Fisher's "t") different than controls; NS = not significantly different than controls.

counts were elevated in the normal recipients to 150% above normal 24 hours after the last injection. However, the number of reticulocytes had returned to normal 48 hours after injections had ceased. 6 cc of plasma from hypophysectomized rats bled to such a degree that the hematocrit reading was 27% induced increases in reticulocyte percentages in the normal recipient rats (86% above normal at 24 hours and 57% at 48 hours) which were not statistically significant. Accordingly, hypophysectomized rats were bled more severely until the hematocrit reading averaged 21% and the amount of plasma injected into normal recipient rats was increased from a total of 6 cc to 9 cc. Under these circumstances the reticulocyte percentage was significantly increased to 1.9% at 24 hours and 1.8% at 48 hours after the last injection, the 2 figures being 138% and 125% above those obtained before injections started (0.8%).

Discussion. These data support the work of others(9,10) that a plasma erythropoietic factor can be exhibited in rats, as well as rabbits, made anemic by bleeding. In addition, they also suggest that a lack of this factor is not playing a role in post-hypophysectomy anemia for it can be formed in the absence of the hypophysis.

The results obtained with plasma from unbled hypophysectomized rats are difficult to explain. There is a possibility that such an animal might be elaborating an erythropoietic factor in response to a decreased erythropoiesis in spite of the fact that the hematocrits indicated that the animals were not as yet anemic. A more thorough study of this aspect of the problem is in progress. Another point which should be noted is the relatively lesser response to plasma from bled hypophysectomized animals as compared to the response to plasma from bled normal rats.

Summary. 1. Normal adult-female rats were injected intravenously with a total of 6 or 9 cc of plasma, over a 3-day period, obtained from normal rats, bled normal rats, hypophysectomized rats, or bled hypophysectomized rats. Reticulocyte response was used as criterion of erythropoietic activity and blood was removed for reticulocyte counts 24 and 48 hours after the last plasma injection. 2. Best results were obtained with the larger dose level. Erythropoietic factor was present in the plasma of the bled normal rats, the reticulocyte count being elevated 483% above normal. The plasma factor was also found to be present in bled hypophysectomized rats, the reticulocyte count of the recipient rats being elevated 138% above normal levels. This factor was also found in the plasma of unbled hypophysectomized rats, a fact which needs further investigation.

Conclusions. This experiment indicates that erythropoietic factor can be demonstrated in normal bled rats and that hypophysectomy does not prevent its formation.

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