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Effect of Castration and Sex Hormones on Blood of the Rat.

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A sex difference in red cell count has been observed in normal rats,[1] mice,[2] rabbits,[3] cats,[4] dogs,[5] sheep,[6] horses,[7] fowl,[8] and, as is well known, in man.[9] The count, in general, has been found to be higher in the male than in the female, although results contradictory to these have been reported.[10] Castration has been claimed to produce an increase in RBC in female fowl,[11] rabbits,[12] and dogs[13] and a decrease in the male rabbit[14] but such effects have also been denied.[6]

In view of the contradictory nature of the above results and since no detailed experiments have been made on the effects of castration and sex hormones on the blood picture of rodents, it was decided to subject this problem to a more complete investigation in rats.

Materials and Methods. To determine the effects of castration, 10 male and 10 female adult rats (200-255 g) of a highly inbred strain were used. Several preliminary counts were made over a 7-week period preceding the operation. Experimental treatment in the form of daily injections of 10-20 R.U. estradiol† into 6 castrated females and 1-2 mg testosterone† into 6 castrated males was administered for 4 weeks.

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† We are indebted to Dr. Erwin Schwenk, Schering Corporation, for supplies of testosterone propionate and estradiol benzoate.

All determinations were made once or twice weekly from tail blood. Red cell counts, made in duplicate, were required to agree within ±4% and white cell counts to within ±10%. Reticulocytes, stained with brilliant cresyl blue, were counted on wet smears. Differential white cell counts were made on dried smears stained with Wright's. Hemoglobin estimations were performed with a Hellige hemometer. Histological examinations of the bone marrow were made on material obtained from right femurs.

Results. The normal RBC in 50 normal females of our colony

FIGS. 1-4. Graphs showing the mean red cell counts, hemoglobin contents and reticulocyte percentages of 4 groups of rats. Figs. 1 and 2 give the values for 10 animals, Figs. 3 and 4 for 6 animals.

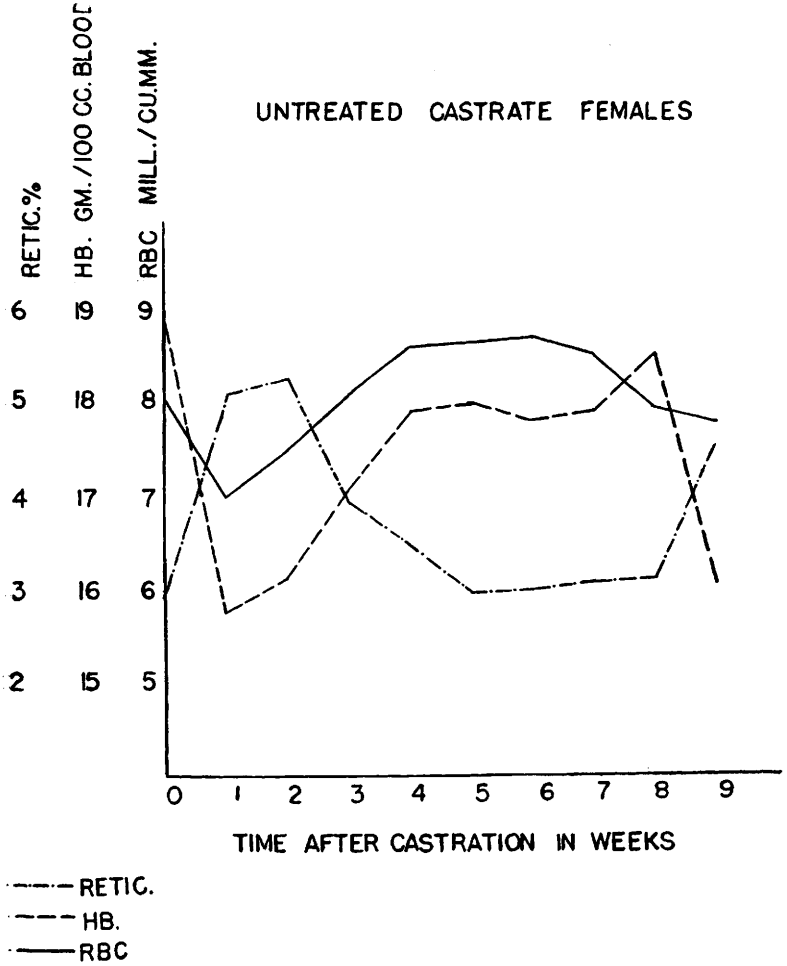

FIG. 1

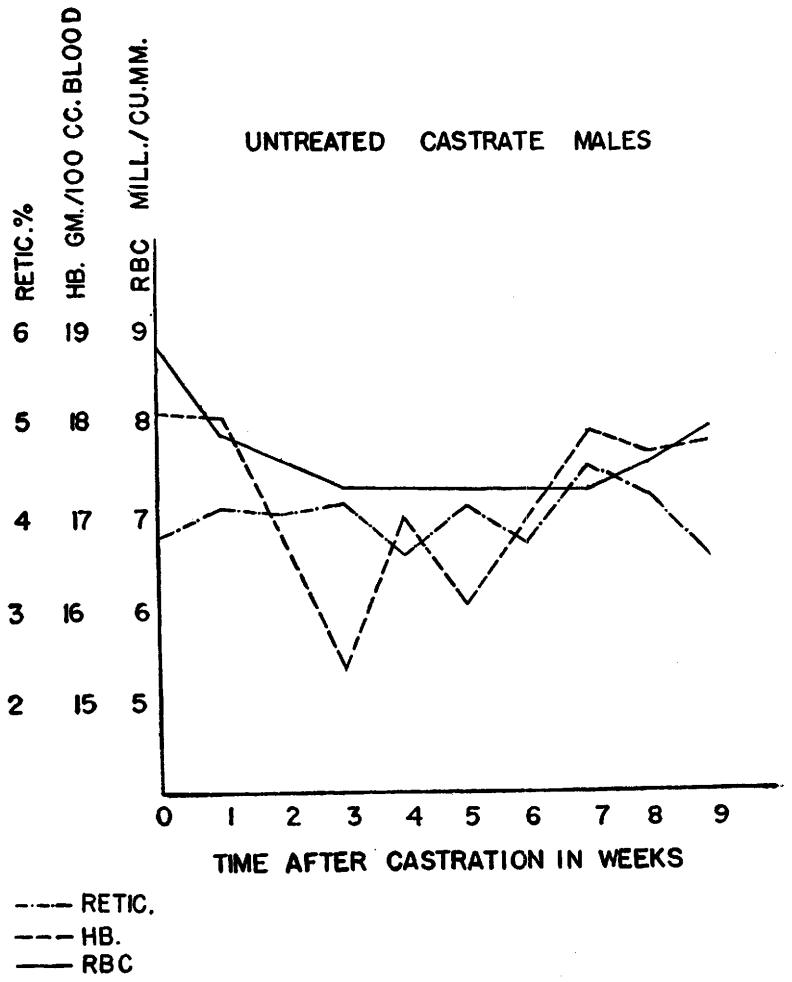

FIG. 2

has been found to be 8.2 ± 0.1 million per mm³ and that of 25 normal males 8.6 ± 0.1 million per mm³ As may be seen from Fig. 1, the RBC in the untreated castrated females dropped the first week after the operation and then rose steadily attaining a maximum of 8.8±0.2 million per mm³ at the end of 6 weeks. Following this, the count tended to drop until the normal count was once again attained at the end of 9 weeks. Reticulocytes rose from 2.9% to a maximum of 5.4% at the end of 2 weeks. There now occurred a steady decrease towards normal values and then a sudden increase to 4.6% at a time corresponding to the fall in RBC.

In the untreated castrated males (Fig. 2), the RBC dropped to 7.4 ± 0.2 million per mm³ by the end of the 3rd week and remained at this level until the 7th week, after which it tended to rise slightly. Reticulocytes, in males, were unaffected throughout the period of observation.

Injections of estradiol into females, castrated 6 weeks previously, resulted in a drop of RBC from 8.9 ± 0.2 million per mm³ to 6.7 ± 0.4 million per mm³ attained at the end of 4 weeks of treatment. Reticulocytes showed a slight increase during this time. In

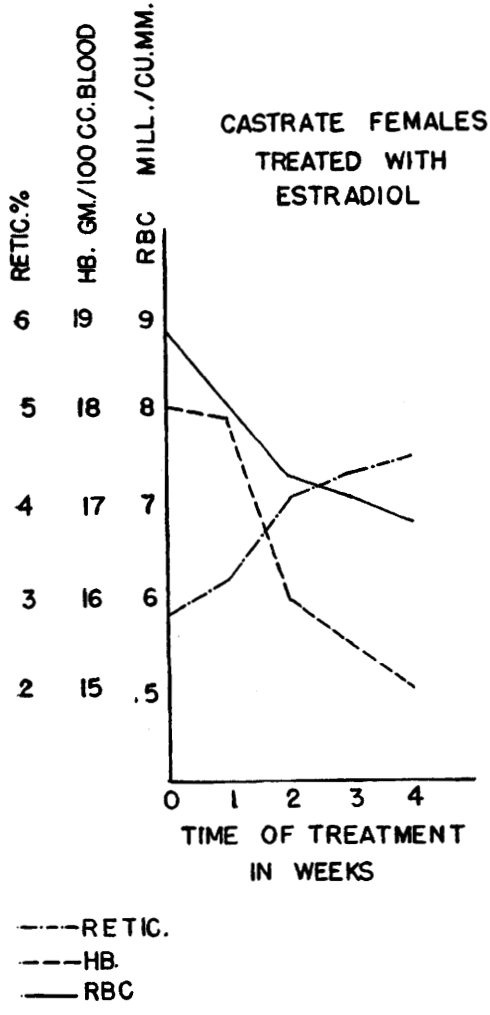

FIG. 3

males, castrated 6 weeks previously, treatment with testosterone caused an increase in RBC from 7.4 ± 0.3 million per mm^3 to an average of 9.1 ± 0.3 million per mm^3 at the end of 4 weeks of injection. Reticulocytes were not affected by this treatment.

A study of the bone marrows indicates a hyperplasia and increased erythrogenic activity in animals injected with testosterone (Fig. 7) or in females not influenced by estrogens—*i. e.*, castrated females (Fig. 6). An erythroid hypoplasia accompanied, in some cases, by

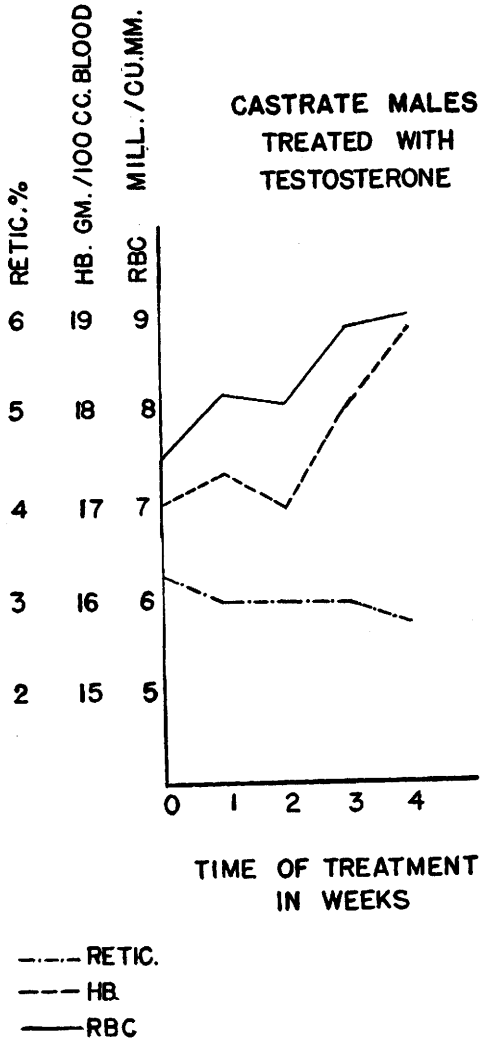

FIG. 4

an increase in the numbers of myeloid areas occurs in the marrows of estrogen-treated animals (Fig. 8) or in those not influenced by androgens—*i. e.,* castrated males (Fig. 5).

Hemoglobin values, in practically every case, seemed to parallel, but were not as marked, as the red cell changes. Variations in white cell counts and differential white cell determinations were too inconstant to warrant any definite conclusions.

Discussion. Normal male rats of our laboratory possess a significantly higher RBC than females. Our data also indicate that the situation is temporarily reversed following castration; the count in the male drops while that in the female rises so that a month after removal of the gonads, the RBC in the male is lower than in the female. It is seen also that the normal sex difference in red cell count may be accentuated with the appropriate sex hormone. Such results may be explained by assuming that androgens stimulate and estrogens depress erythrogenic activity.

FIGS. 5-8. Cross sections of rat femoral bone marrow, fixed in Bouin's and stained with Harris' Hematoxylin-eosin; × 400. FIG. 5—Section from untreated male, 2 months after castration. Note general hypoplasia, diminution in numbers of erythroid elements. FIG. 6—Section from untreated female, 3 months after castration. Some hyperplasia of erythroblastic elements observed. Little vacuolization. FIG. 7—Section from castrated male injected daily for 4 weeks with 1-2 mg testosterone. Intense erythrogenic activity observed. Hypoplasia noted in untreated castrate male (Fig. 5) is repaired. FIG. 8—Section from castrated female injected daily for 4 weeks with 10-20 R.U. estradiol. Vacuolization is marked. Erythrogenic activity is partially inhibited.

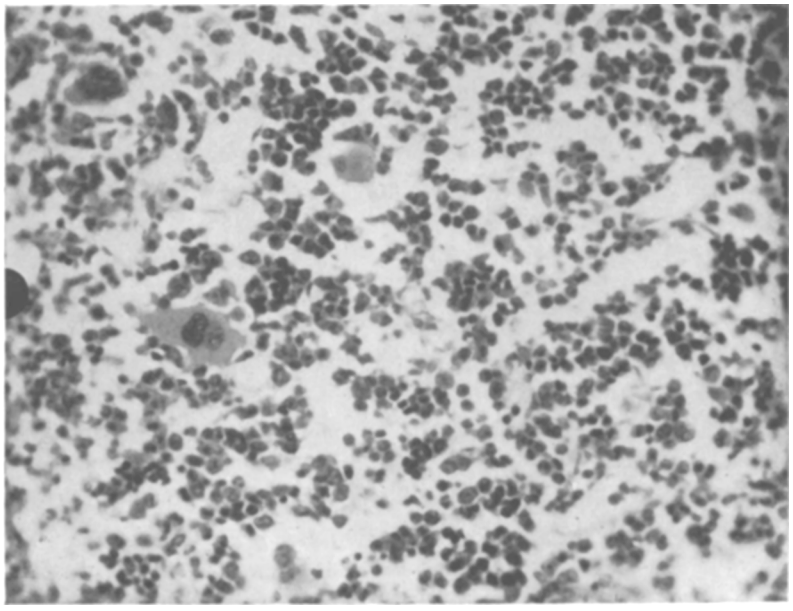

FIG. 5.

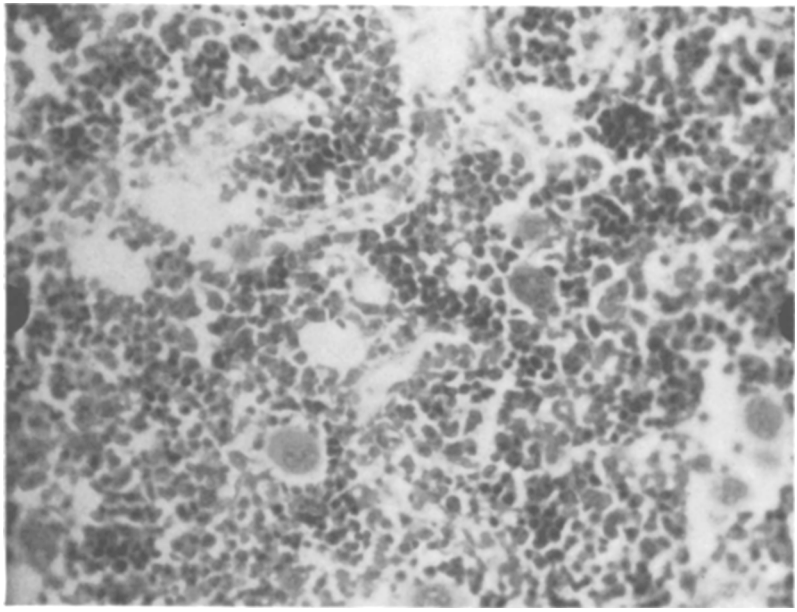

FIG. 6.

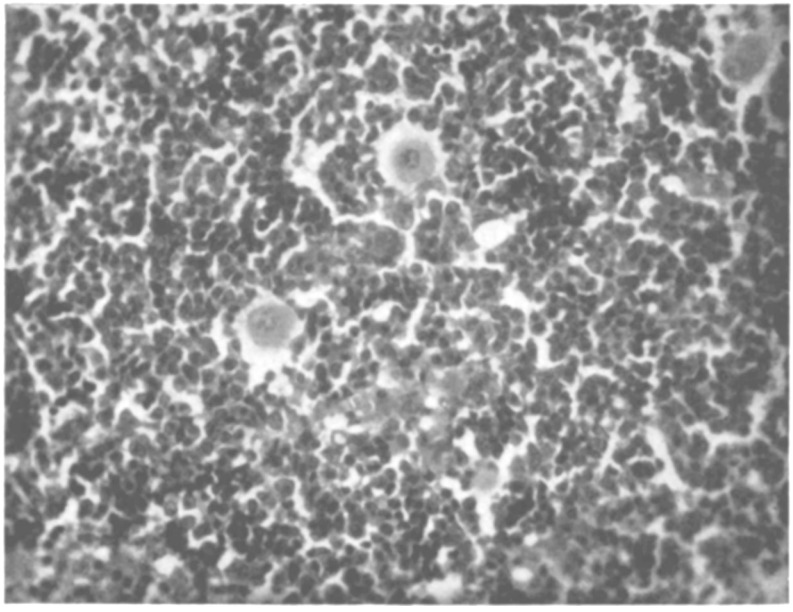

FIG. 7.

That the increase in RBC and hemoglobin in the castrated female rat is due, to some extent, to an increased rate of red cell proliferation may be seen from the behavior of the reticulocytes and the bone

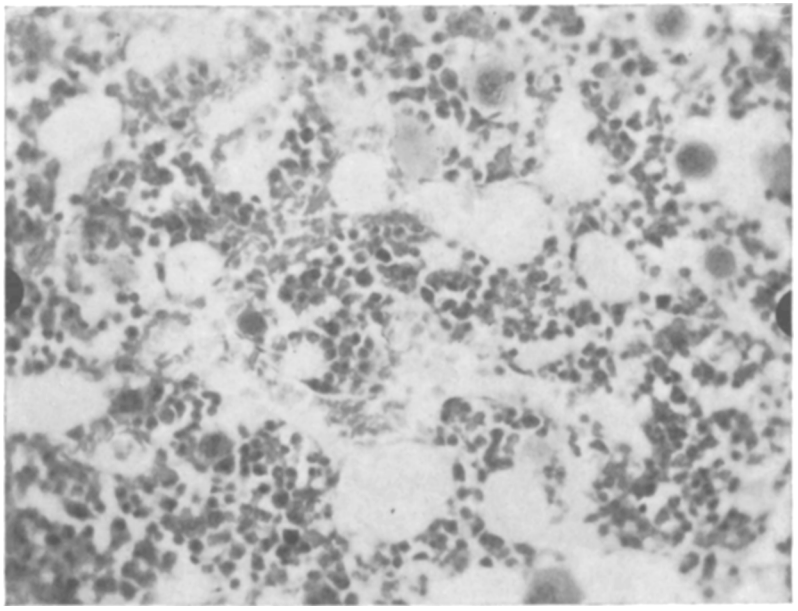

FIG. 8.

marrow appearance. The reticulocytes increase in number soon after the operation, which is to be expected, since, with the removal of the ovaries, estrogenic hormone, which inhibits red cell formation, has been withdrawn. The marrows of untreated castrated females likewise reveal evidence of considerable erythrogenic activity. Moreover, estrogen treatment in castrated females results in marrow hypoplasia, associated with the drop in RBC.

Conversely, castration in the male is attended, for a time, by a diminution in RBC. This is due, most likely, to reduced marrow activity caused, presumably, by withdrawal of testicular hormone. The marrows of untreated castrated male rats show, in fact, a surprising decrease in erythroid areas. The reticulocyte counts remain at relatively low levels in the castrated male which, again, is in accord with the hypothesis set forth above. Although the reticulocyte response to testosterone is not as marked as that observed in the hypophysectomized rat,[15] the influence on the marrow is striking. Myeloid areas become replaced by active red cell progenitors and the general picture is one of erythroid hyperplasia.

The mechanism for the sex hormone influence on marrow activity is not as yet clear. It is unlikely that the sex hormones produce

[15] Vollmer, E. P., Gordon, A. S., Levenstein, I., and Charipper, H. A., *Anat. Rec.*, 1940, **78**, 91.

their effects in castrates by operating through the pituitary. One would have to assume, under these circumstances, that estrogens and androgens affect this organ in opposite directions and this is contrary to general evidence. It is possible that the sex hormones influence the blood picture through their well known actions on metabolism and general somatic growth. Estrogens, for example, depress the thyroid which has been shown to be concerned in erythropoiesis.[16] If, however, the thyroid is the only organ concerned, to explain the results, it would have to be assumed that androgens stimulate the production of thyroid secretion, for which there is no direct evidence.

Summary. 1. The RBC and hemoglobin content of normal rats show higher values than those of normal females. 2. Following castration, the RBC and hemoglobin contents of the males drop, while those in the females rise. 3. Injections of estradiol into castrated females, and testosterone into castrated males produce, respectively, a drop and rise in RBC and hemoglobin. 4. Bone marrow studies indicate that androgens stimulate and estrogens inhibit erythrocytopoiesis. 5. Additional support is thus given to the sex hormone explanation of the normal sex difference in RBC reported for many species of animals.

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A New Strain of Virus of Influenza B Isolated During an Epidemic in California.*

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Isolation of strains of influenza virus not antigenically related to the virus of influenza A[1] has been reported by Francis[2] and by Magill.[3] Francis designated his strain influenza B. The virus iso-

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