Effect of Hypophysectomy on Blood Regeneration in the Adult Female Rat.* (19309)

ALLEN SILBERGLEIT. (Introduced by William B. Atkinson.)

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It is well known that hypophysectomy induces an anemia in a variety of laboratory animals (dog, rabbit, cat), although most of the recent literature on the pituitary-blood relationship is confined to the rat. In addition, most investigators have found that an anemia accompanies panhypopituitarism in humans. Several investigators have studied hemopoietic activity in the hypophysectomized rat following subjection to anoxia, believed by some to be the fundamental erythrocytogenic stimulus. Stewart, Greep, and Meyer(1) found that hypophysectomized rats exposed to lowered barometric pressures (422 mm Hg) did not exhibit bone marrow hyperplasia, reticulocytosis, and an increased red cell count as did normal animals subjected to the same decrease in oxygen tension. In a more recent report, Feigin and Gordon(2) confirmed the finding that hypophysectomized rats do not respond to a barometric pressure of 422 mm Hg, but found that they would respond if the pressure was reduced to 320 mm Hg. Under these conditions, a blood and bone marrow reaction was evoked in hypophysectomized rats which was equivalent to that of normal rats subjected to such a pressure. The report concluded that the pituitary is not essential for an erythropoietic response to severe anoxia and that the negative response to moderate anoxia exhibited by the hypophysectomized animal was due probably to its lowered basal metabolic rate and lower oxygen requirements. Bleeding is a form of erythropoietic stimulus akin to the anoxia achieved by exposure to lowered barometric pressures. The bled rat has been used previously as a sensitive test animal for the study of hemopoietic activity following hypophysectomy. Querido and Overbeek(3) and Finkelstein and coworkers(4) noted strong reticulocytosis and recovery from hemorrhagic anemia in hypophysectomized-bled rats.

The present investigation is a highly controlled, quantitative study of blood regeneration in the hypophysectomized rat following induced hemorrhage. Its purpose is to verify and extend previous work on the subject.

Materials and methods. Female rats of the Wistar strain, 2 to 3 months of age and weighing 140-253 g at the start of the experiment, were used in this study. All rats were fed Purina Laboratory Chow, supplemented by lettuce given weekly, and tap water *ad libitum*. Hypophysectomy was performed parapharyngeally under ether anesthesia. Completeness of pituitary removal was checked at autopsy by careful examination of the pituitary capsule under a dissecting microscope. The adrenals were removed and wet weights determined. Marked atrophy of these glands in the operated rats served as additional evidence for complete removal of the pituitary. The operation was complete in all hypophysectomized rats included in this work. Hemorrhagic anemia was produced by removal of blood by means of cardiac puncture. The heart was entered with a sharpened 23-gauge hypodermic needle. Some of the rats were rendered anemic by bleeding from the tail which was immersed in a warm solution of 1.3% sodium oxalate. The heart and tail methods were equivalent for production of hemorrhagic anemia. In order to produce a similar degree of anemia from one animal to the next, the volume of blood withdrawn from each animal was determined upon the basis of the weight of the animal and the hematocrit on the day of bleeding. The number of cubic centimeters of blood to be withdrawn was calculated ac-

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cording to the following formula: cc = .02 × body wt in g × $\frac{\text{hematocrit}}{45}$. Preliminary experimentation led to formulation of this equation. It was found that bleeding 2% of body weight induced a definite hemorrhagic anemia. The hematocrit was included in the equation to compensate for the fewer red cells in the hypophysectomized rat. The 45 in the denominator was the mean hematocrit of 38 normal rats. Peripheral blood from the tail was used throughout the study for purposes of red cell count, hemoglobin, and hematocrit determinations. There was no "milking" of the tail whatsoever. The tail was clipped with scissors and the first drop of blood discarded. Four to 5 drops were placed in a small vial containing a small amount of powdered heparin. Every rat was weighed on the day of hypophysectomy and on each day that a blood sample was taken. Erythrocyte counts were made in duplicate with standard U. S. certified pipettes and the Levy-Hausser counting chamber with the improved Neubauer double ruling. Hemoglobin concentrations were determined directly in grams of hemoglobin per 100 ml of blood using a Klett-Summerson photoelectric colorimeter with a green filter having an approximate spectral range of 500-570 millimicrons. Hematocrit determinations were made with Van Allen tubes which were centrifuged for one hour at 2000 RPM. Four types of control animals were used in this study. The first group consisted of unoperated-unbled (*i.e.*, normal) rats to determine normal variation in the blood picture. The second group consisted of hypophysectomized-unbled rats to show the blood changes following hypophysectomy. The third group consisted of unoperated-bled rats to indicate blood regeneration in the normal animal following induced hemorrhagic anemia. The final control group consisted of sham-operated-bled rats to show whether the surgical procedure involved in hypophysectomy influenced blood regeneration following bleeding. The experimental or hypophysectomized-bled rats consisted of two main groups. A hemorrhagic anemia was induced 10 days post-hypophysectomy in one group and 20 days post-hypophysectomy in the other group. There were 3 small supplementary groups, consisting of 2 or 3 rats each. These small groups were bled at 5, 30, and 40 days post-hypophysectomy. Blood samples were taken at 10-day intervals in all rats. In the bled rats, blood samples were taken at 10-day intervals to the day of bleeding, at one day post bleeding, and at 10-day intervals thereafter. Initial blood samples were taken on all animals before subjection to any procedures whatever.

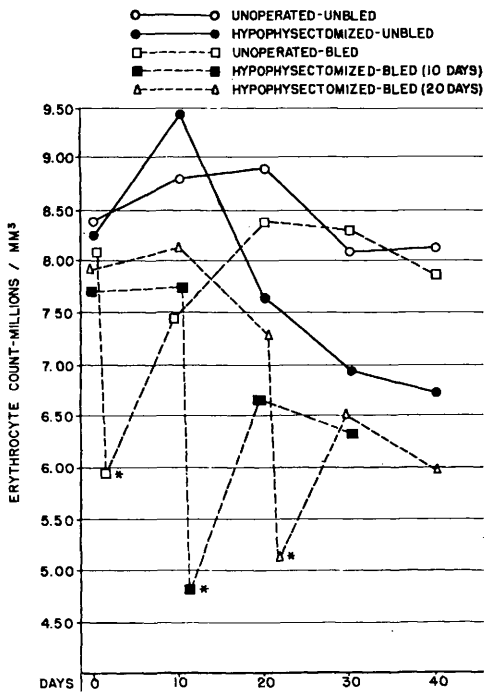

FIG. 1. Average erythrocyte counts of all groups throughout the course of the experiment. * Bled one day previous.

Results. Fig. 1, 2, and 3 give the results in graphic form.

Hypophysectomized-unbled—5 rats. The erythrocyte count, hemoglobin, and hematocrit values decreased to 81.6%, 80.5%, and 77.8% of normal, respectively, over the 40-day period following hypophysectomy.

Unoperated-bled—8 rats. The figures show that bleeding caused a 27.1% drop in erythrocyte count, a 26.2% drop in hemoglobin, and a 23.4% drop in hematocrit, *i.e.*, a drop to values 72.9%, 73.8%, and 76.6% of normal for erythrocyte count, hemoglobin, and hematocrit, respectively. Ten days post-

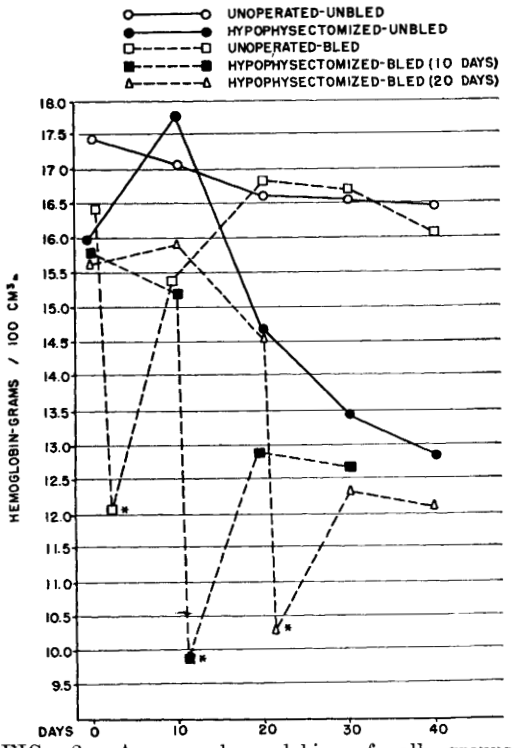

FIG. 2. Average hemoglobin of all groups throughout the course of the experiment. * Bled one day previous.

bleeding, the erythrocyte count, hemoglobin, and hematocrit had increased to values 91.9%, 93.9%, and 95.7% of normal, respectively. This is practically a complete recovery. In addition to the foregoing, 6 sham operated rats recovered completely (within 10 days) from the anemia induced by bleeding.

Hypophysectomized-bled (10 days after the operation)—8 rats. Bleeding in this group caused a decrease in erythrocyte count to a value of 61.8%, a decrease in hemoglobin to 64.7%, and a decrease in hematocrit to 65.1% of the pre-bleeding levels on the day after bleeding. On the 10th day after bleeding, the values for erythrocyte count, hemoglobin, and hematocrit had increased to values 85.8%, 84.3%, and 83.7% of pre-bleeding levels, respectively. This is incomplete regeneration. Since no further increases were noted at 20 and 30 days post-bleeding, it is apparent that maximum regeneration was reached 10 days following bleeding.

Hypophysectomized-bled (20 days after the operation)—6 rats. These rats showed a drop in erythrocyte count to 70.1%, a drop in hemoglobin to 71.0%, and a drop in hematocrit to 69.2% of pre-bleeding values on the day following bleeding. Ten days after bleeding, the values had increased to 89.1%, 84.8%, and 84.6% of pre-bleeding levels for the erythrocyte count, hemoglobin, and hematocrit, respectively. This is incomplete regeneration or incomplete recovery from bleeding. No further increase in blood values was seen at 20 days after bleeding; maximum regeneration was reached at 10 days post-bleeding. The pattern of incomplete recovery from bleeding in rats bled 20 days after the operation is similar to the incomplete recovery in rats bled 10 days after the operation. Both groups are in marked contrast to the unoperated-bled and the sham operated-bled rats which recovered completely from induced hemorrhagic anemia within 10 days. All of the foregoing data were treated statistically and the results are significant. In addition, small supplementary groups of rats (2 or 3 rats per group) were bled at 5, 30, and 40

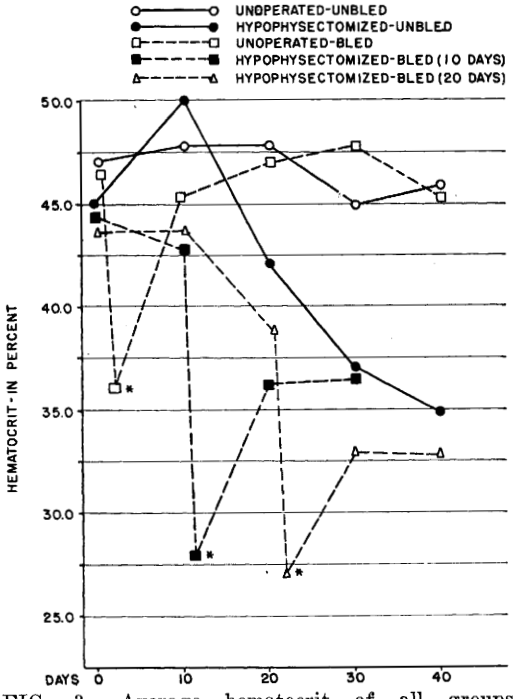

FIG. 3. Average hematocrit of all groups throughout the course of the experiment. * Bled one day previous.

days post-hypophysectomy. These animals showed the same general picture as seen in the rats bled 10 and 20 days post-hypophysectomy. Thus, while pre-bleeding values varied according to the number of days post-hypophysectomy, regeneration to the maximum level occurred in each instance within 10 days after the bleeding.

Discussion and conclusions. Within 10 days, the normal or sham operated rat recovers completely from the hemorrhagic anemia induced by bleeding to the extent of approximately 2% of body weight. The hypophysectomized rat can partially regenerate red cells lost by bleeding to the same extent. Regeneration is essentially completed during the first 10 days after bleeding. It has been shown by Crafts(5) that in the hypophysectomized rat the anemia becomes most severe at 40 days after operation and remains relatively constant thereafter. This has been called the plateau level. The present investigation has demonstrated that bleeding the hypophysectomized rat prior to the 40th day post-operative produces, as would be expected, a superimposed hemorrhagic anemia. Interestingly enough, the maximum blood level to which the hypophysectomized rat may regenerate by the 10th day after bleeding is similar to this plateau level of unbled-hypophysectomized rats. This recovery to the plateau level in hypophysectomized-bled rats occurs independent of the number of days after operation at which hemorrhage occurs.

It is likely that the incomplete response to anoxia exhibited by the hypophysectomized rat is not due directly to deprivation of any specific hemopoietic factor of the anterior pituitary. Hypophyseal control of the endocrines which regulate metabolism offers a more probable explanation. Similarly, the anemia of hypopituitarism is likely due to general metabolic upsets following hypophysectomy, since this anemia can be prevented completely by high protein diet-thyroxine-androgen therapy(5). Since incomplete response to anoxia exhibited by the hypophysectomized rat is due probably to alterations in metabolism, further investigation utilizing hypophysectomized rats treated with the high protein diet-thyroxine-androgen therapy of Crafts is in order. If such treated rats exhibit the normal polycythemic response at an atmospheric pressure of 422 mm Hg and show complete regeneration following bleeding, the metabolic explanation of post-hypophysectomy anemia will be further enhanced.

Summary. Intact normal rats and sham operated rats recovered completely from hemorrhagic anemia within 10 days. Hypophysectomized rats bled at various intervals after the operation exhibited partial regeneration of blood elements on the 10th day after bleeding. Maximal recovery was reached at that time with no further regeneration occurring subsequently. This maximum is identical with the plateau level which the hypophysectomized-unbled rat attains approximately 40 days after hypophysectomy. It is likely that the incomplete regeneration is due to the altered metabolism following hypophysectomy.

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