Lack of Hematopoiesis in Tail Bones Transposed to Abdominal Cavity of Hypophysectomized Rats.* (22793)

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Huggins and Blocksom(1) demonstrated that hematopoiesis could be stimulated in the normally inactive terminal tail bones of rats if the ends of the tails were transposed to the abdominal cavity. They had previously(2) shown that the subperiosteal temperature of the terminal vertebrae of the tail was approximately 7 to 12°C lower than the intra-abdominal temperature. They interpreted the establishment of hematopoiesis in the transposed tail bones to be the result of thermal stimulation. Hypophysectomy is known to have a deleterious effect upon peripheral blood and blood-forming organs. Hypophysectomized rats develop a slightly microcytic-hypochromic anemia with little alteration of the total white cell count. The bone marrow of hypophysectomized rats is hypoplastic showing an increase in fat, a decrease in the total number of nucleated cells per mm^3, a reduction in the total number of nucleated erythroid elements per mm^3, and, in the Wistar strain, little effect on white cell precursors (3). It has been shown that the marrow of the hypophysectomized rat is capable of responding to various stimuli similar to the normal rat. An erythropoietic response can be elicited in the marrow of hypophysectomized rats by anoxia, but the anoxic stimulus must be greater than that which will stimulate normal animals(4). The marrow of hypophysectomized rats is also capable of regenerating red blood cells following hemorrhage, but will form only enough to bring the peripheral count back to the pre-bled hypophysectomized levels(5-7). These examples show that even though the marrow is affected by hypophysectomy, a potential to respond to stimuli in a manner similar to normal remains insofar as erythropoiesis is concerned.

The present experiment was conducted to test whether or not hematopoiesis could be initiated in the yellow marrow of the terminal tail vertebrae of hypophysectomized rats, as it is in normal animals, when the tail is transposed to the abdominal cavity.

Material and methods. Adult female rats 3-4 months of age of the Wistar strain were used in the experiment. The animals were fed standard Purina chow supplemented once a week with lettuce. The procedure for transposition of the tails was as follows: A small incision was made in the lateral body wall; a circumferential cut was made in the skin of the tail about 3 inches from the end and the skin of the distal end of the tail was pulled off. The end of the tail was looped forward, inserted into the abdominal cavity through the lateral wall incision, and anchored in place by suturing the tendons of the tail to the muscle wall, and by anastomosing the skin of the tail with the skin of the abdominal wall. Hypophysectomies were performed by the parapharyngeal approach, and the completeness of the operation was determined at autopsy by examination of the organ site under a dissecting microscope. A small sample of blood was removed from the heart, heparinized and studied every 10 days throughout the experiment. Erythrocyte counts were done in duplicate, using U.S. certified blood pipettes and the improved Neubauer counting chamber. Hemoglobin determinations were made in a Klett-Summerson photoelectric colorimeter. Hematocrit determinations were made with Van Allen hematocrit tubes (no diluent) and spun for 1 hour at 2000 r.p.m. Rectal temperatures were taken at 10-day intervals. At autopsy vertebrae from within the abdominal cavity and from the outside loop were removed for study, the outside bone serving as a control along with bones from normal, unoperated rats. The bones were

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TABLE I. Peripheral Blood Picture and Body Temperatures of Normal Unoperated Control Rats, and Normal and Hypophysectomized Rats with Tails Transposed to Body Cavity.

	Initial values				Final values			
	Erythrocyte count in millions/mm³	Hematocrit in %	Hemoglobin in g/100 cc	Rectal temp., °F	Erythrocyte count in millions/mm³	Hematocrit in %	Hemoglobin in g/100 cc	Rectal temp., °F
Normal controls (6)	8.71 ± .17*	44.7 ± .52	16.1 ± .05	100.5 ± .56	9.39 ± .28	45.0 ± .57	15.9 ± .20	100.4 ± .45
With tail transposed to abdominal cavity† (41)	8.86 ± .06	45.5 ± .33	15.9 ± .10	100.0 ± .20	8.88 ± .08	45.2 ± .35	16.4 ± .10	100.2 ± .22
Hypophysectomized; tail in cavity 70 days (16)	8.74 ± .17	46.3 ± .81	16.6 ± .26	100.4 ± .14	7.53 ± .14	34.8 ± .71	12.0 ± .22	98.6 ± .22

* ± Stand. error.
† Since no significant difference was found among any of the values for the various groups of normal animals with tails transposed, the figures for the 4 groups were combined.

fixed in Helly's fixative, decalcified for 3 days in 5% formic acid, dehydrated for 2 days in graded concentrations of tertiary butyl alcohol, cleared with xylene, embedded in paraffin, cut serially at 6 micra and stained with Giemsa stain buffered to pH 4.75.

Results. The following groups of animals were studied: (I) normal controls, (II) normal rats with tails transposed for 40, 50, 60 and 70 days, (III) hypophysectomized rats with tails transposed for 70 days, and (IV) normal and hypophysectomized rats without tail transposition kept at a high environmental temperature.

Group I—normal controls—The peripheral blood values and body temperatures for these animals are tabulated in Table I. Section of the tail vertebrae revealed a typical yellow or fatty marrow as shown in Fig. 1. There is a delicate reticulum with occasional isolated hematopoietic cells identifiable in the interstices. The vessels in the marrow may or may not be filled with blood.

Group II—normal rats with transposed tail —The rats of this group can be considered as a unit because the differences in time did not manifest any significant differences in the results. Transposition of the tail had no significant effect on the peripheral blood picture even though red marrow did develop in some of the tail vertebrae (Table I). The body temperature also showed no significant change from the beginning to the end of the experiment (Table I). Vertebrae taken from the outside loop of the tail showed the typical fatty marrow seen in the normal rat (Fig. 1 and 2). The response of the vertebrae from within the abdominal cavity is tabulated in Table II. Thirty-seven of a total of 41 operated animals (90.2%) showed a positive response, a positive response being recorded when there was any accumulation of hematopoietic cells into groups within the interstices of the reticulum. This was sometimes accompanied by an obvious thickening of the reticular framework. A response that was considered "good" is illustrated in Fig. 3. It is characterized by a substantial increase in hematopoietic cells of all types. Responses equal to this were seen at all time intervals

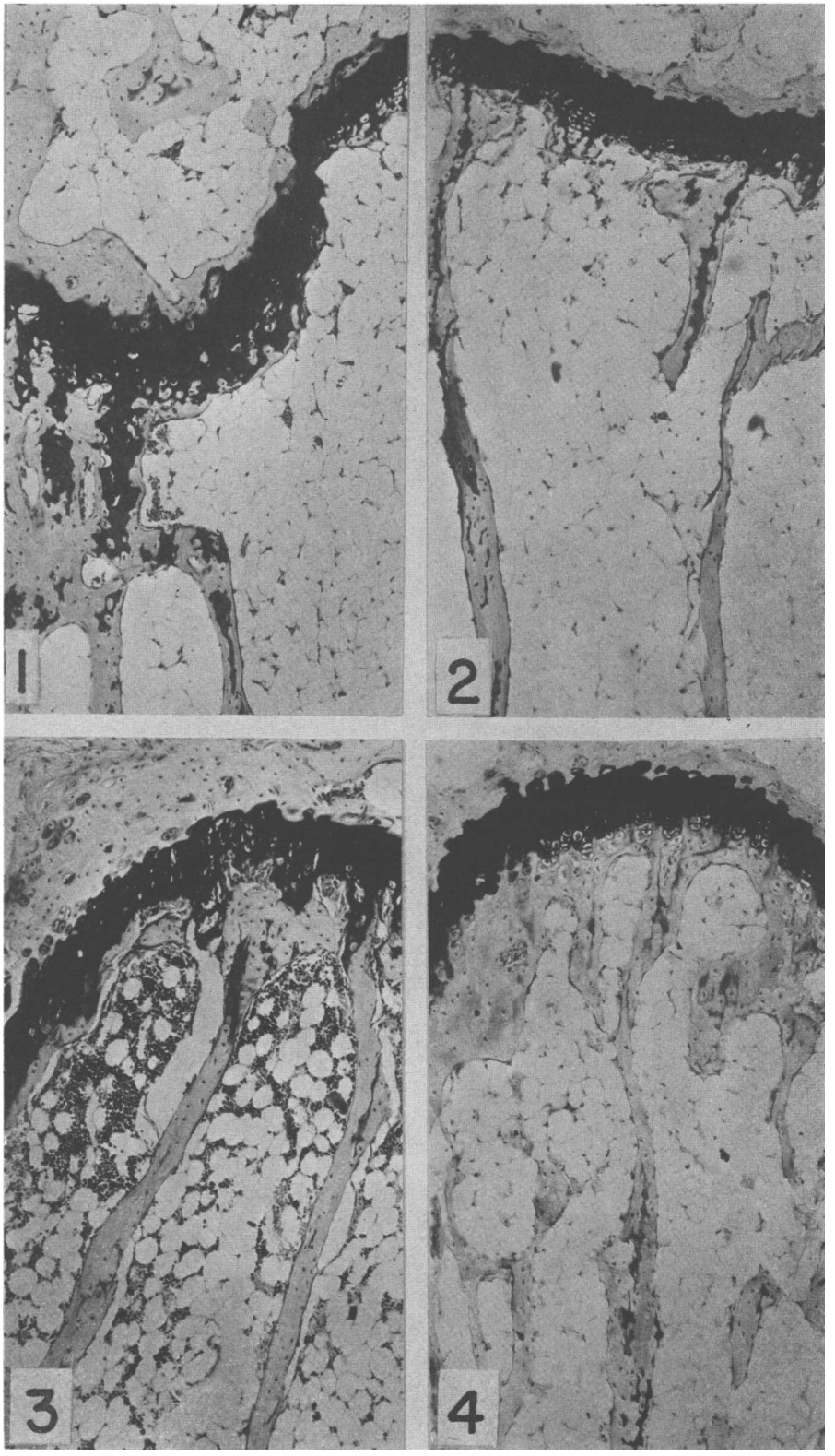
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TABLE II. Hematopoietic Response in Tail Bones Transposed to Abdominal Cavity in Normal and Hypophysectomized Rats.

	No. of rats	No. with			
		Positive response	Erythroid elements	Myeloid elements	Megakaryocytes
Tail in abdominal cavity 40 days	10	10	10	10	9
Idem 50 "	11	9	9	9	5
" 60 "	8	7	7	7	4
" 70 "	12	11	10	11	10
Hypophysectomized; tail in cavity 70 days	16	1	?	1	0

of this operated group. In every case in which there was a response the distribution of the cells was limited to the metaphysis of the bone; increases in vascularity were noted in some but not in all of the vertebrae. Table II shows that the various cell types are well represented in the different groups of operated normal animals. The response was not limited to any one cell type; erythropoiesis, myelopoiesis, as well as megakaryocytic development, was initiated. The observations also agreed with the finding of Huggins and Blocksom in that there was a tendency for a more extensive myeloid distribution than the other cell types.

Group III—hypophysectomized; tail transposed animals—Seventy days after hypophysectomy these animals showed the usual post-hypophysectomy anemia (Table I) and a significant drop (1.8°F) in body temperature. A study of the tail vertebrae from within the body cavity of these animals revealed that only 1 (6.2%) of 16 animals showed any indication of response; the response in this 1 animal was no greater than the minimal response recorded for the control group. No megakaryocytes were seen and identification of erythroid elements was questionable. The remaining animals showed marrows similar to the unoperated control group (Fig. 4).

Group IV—normal and hypophysectomized animals at elevated environmental temperatures—Huggins and Blocksom found that hematopoiesis could be stimulated in the tail bones of normal rats kept at high environmental temperatures. A small experiment was conducted to repeat this work and to extend it to the hypophysectomized animal. Five normal and 5 hypophysectomized rats were kept in an oven at 98°F for a period of 50 days. Only 1 of the 10 rats (a normal animal) showed a response.

Discussion. Huggins and Blocksom postulated that conversion of yellow marrow to red marrow could be accomplished by thermal stimulation, and that red marrow was distributed in those bones having higher environmental temperatures and yellow in those having lower temperatures. The present experiment shows that this effect cannot be shown in the hypophysectomized animal.

Although many changes occur in the hypophysectomized animal which may account for this lack of response, lowered body temperature must be considered as a possible cause. Huggins and Blocksom found that when normal, unoperated animals were placed in an oven for 21 to 70 days, at an environmental temperature of 91 to 96°F, hematopoiesis was stimulated in the tail bones of all of the animals, but the response was not as extensive as it was in the transposed vertebrae. These temperatures are well below the body temperature of the hypophysectomized rat. This suggests, since hematopoiesis can be stimulated at these lower temperatures in the normal animal, that the lowered body temperature in the hypophysectomized rat is not the

FIG. 1. Section from metaphysis of a tail vertebra from a normal rat. ×160.

FIG. 2. Similar section of a tail vertebra taken from the loop of tail lying outside of the body after the end had been transposed to within the abdominal cavity of a normal rat. ×160.

FIG. 3. Similar section of a tail vertebra taken from that portion of tail lying within abdominal cavity of a normal animal for 70 days. ×160.

FIG. 4. Same as Fig. 3 but from a hypophysectomized rat. ×160.

factor limiting stimulation. This same observation, however, was made on only 1 of 5 animals, kept at 98°F, in the present experiment. In addition, it is known that marrow in the hypophysectomized rat is less sensitive to various stimuli, *i.e.*, anoxia(4), and bleeding(5-7); therefore, until such time as hypophysectomized rats are maintained at temperatures equal to normal or even higher than normal, the lowered body temperature cannot be eliminated as possibly having had an influence on the results.

Huggins and Blocksom also showed that anemia induced by repeated bleeding or splenectomy of animals infected with Bartonella did not stimulate hematopoiesis in the tail bones of normal rats, but the accompanying anemia resulted in a more extensive hematopoietic response in bones transposed to the abdominal cavity. The anemia of hypophysectomy does not have this stimulatory effect.

It is of interest to note that in the present experiment hypophysectomy resulted in an inhibition of both erythropoiesis and myelopoiesis. This is in contrast to the results obtained by Meineke and Crafts(3) where, in femur marrow, hypophysectomy induced a depression in erythropoiesis only; there was no effect on myelopoiesis.

It is possible that hypophysectomy has removed some hormone or caused some change in the animal which is necessary for the thermal stimulus to be effective. This could be the direct effect of removal of a single hormone (such as growth hormone), a combination of hormones, or might be the result of a secondary effect on metabolism. Reparative hormonal therapy under conditions outlined in this experiment might be of value in providing information as to the mechanism involved in the initiation of hematopoiesis.

Summary. Thirty-seven of 41 normal female rats, in which the ends of the tails were transposed into the abdominal cavity, showed a stimulation of hematopoiesis in the intra-abdominal vertebrae. Developing cells of all 3 types—erythroid, myeloid and megakaryocytes—were observed in these animals. In similarly treated hypophysectomized female rats only 1 of 16 animals showed any indication of response. The experiment provides another example of the depressed hematopoietic potential of the hypophysectomized rat.

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Resistance to Bacteria in Hemorrhagic Shock. VII. Demonstration of Leucotoxin in Plasma of Shocked Rabbit.* (22794)

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In the first of this series of studies it was shown that the phagocytic index of granulocytes from the peritoneal cavity of the normal dog, immersed in plasma from the dog in hemorrhagic shock, falls more and more the longer the dog has been in shock when the

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