Effect of Combined Thyroxin-Cortisone-Growth Hormone Therapy on Hematopoiesis in Hypophysectomized Rats.* (23397)

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It has been well established that an anemia follows hypophysectomy. The role played by thyroid and adrenal glands in the etiology of this anemia has been the subject of several investigations. Combined thyroidectomy and adrenalectomy induced changes in the peripheral blood similar to those following hypophysectomy(1). In addition, combined thyroxin-cortisone therapy in hypophysectomized rats prevented occurrence of an anemia(2). Van Dyke, *et al.*(3), however, found that anemia following hypophysectomy was more severe than that following combined thyroidectomy, adrenalectomy and gonadectomy and proposed that the "erythropoietic hormone" from the pituitary was the explanation for this finding. This prompted a reinvestigation of this problem by the present authors (4); whereas the peripheral anemias in hypophysectomized and thyroidectomized-adrenalectomized rats were similar, the marrows were not identical. In addition, although we found (5) combined thyroxin-cortisone therapy would eliminate post-hypophysectomy anemia, the bone marrow was severely hypoplastic. When the whole picture was analyzed it was apparent that the pituitary gland was in some way playing a role in hematopoiesis exclusive of its effects on thyroid and adrenal glands.

The present investigation was performed to determine whether growth hormone could be this additional pituitary factor. Although several studies(6-11) have shown that growth hormone therapy has no effect on post-hypophysectomy anemia, both Fruhman and Gordon(10) and Meineke and Crafts(11) reported that growth hormone increased the number of erythroid elements in bone marrow. It seemed possible that a lack of growth hormone was involved in post-hypophysectomy anemia in addition to the hypothyroidism and hypoadrenocorticalism which inevitably follow removal of the pituitary gland. Since we have postulated that post-hypophysectomy anemia is a reflection of decreased oxygen need(12), oxygen consumption was also measured and correlated with blood and marrow findings.

Materials and methods. Female rats, 3 to 4 months of age, of the Sprague-Dawley strain were used and were fed standard Purina chow *ad lib.* supplemented once a week with lettuce. Completeness of hypophysectomy was checked at autopsy by examining the organ site with a dissecting microscope. Blood samples were obtained via heart puncture with the rats under ether anesthesia. The blood was transferred to heparinized vials. Erythrocyte and white cell 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 one hour at 2,000 rpm. Blood volume measurements were made by the Evans Blue dye method as described by Metcoff and Favour (13). Total number of nucleated cells/mm^3 of marrow and actual number of myeloid and erythroid elements/mm^3 of marrow were determined by a method already published(11). The method used for determining oxygen consumption has been described(12).

Procedure and results. The rats in this experiment were divided into 3 groups: (1) normal controls, (2) hypophysectomized—no treatment, and (3) hypophysectomized for 4 months followed by daily injections of 0.005 mg thyroxin, 0.6 mg cortisone acetate† and

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† Cortisone Acetate generously supplied by Merck and Co., Inc.

TABLE I. Peripheral Blood Picture in 3 Groups of Adult Female Rats: (1) Normal Controls, (2) Hypophysectomized for 7 Months, and (3) Hypophysectomized for 4 Months Followed by Daily Injections of 0.005 mg Thyroxin, 0.6 mg Cortisone Acetate, and Gradually Increasing Amounts (0.2-0.8 mg) of Growth Hormone for 3 Months. ± = stand. error.

	Normal controls	Hyp., no treatment		Hyp., treated	
	8 rats	7 rats	% of normal	11 rats	% of normal
Body wt, g	293 ± 7.97	187 ± 8.28	64	302 ± 8.59	103
Erythrocytes, millions/mm³	8.27± .17	7.71± .21	93	9.22± .19	111
Hematocrit, %	45.6 ± .7	38.1 ± .7	84	47.0 ± .4	103
Hemoglobin, g/100 cc	15.5 ± .2	12.6 ± .2	81	16.0 ± .1	103
Reticulocytes, %	1.1 ± .1	.9 ± .2	82	1.3 ± .2	118
Mean corpuscular vol, μ^3	55.1	49.4	90	51.0	93
" " hemoglobin, $\mu\mu$g	18.7	16.3	87	17.3	93
" " " conc., %	34.0	33.0	97	34.0	100
Total cellular vol, cc/100 g body wt	2.33± .06	1.73± .04	74	2.39± .08	103
Total white cell count, thousands/mm³	10.3 ± .6	12.9 ± 1.0	125	12.6 ± .4	122

increasing amounts (0.2 mg increment every 10 days until 0.8 mg was being given) of growth hormone‡ for 3 months. The results are divided into 3 categories, *i.e.*, the effect (1) on peripheral blood picture (2) bone marrow, and (3) oxygen consumption.

Results of the peripheral blood studies are tabulated in Table I. Hypophysectomy induced the usual anemia which was, because of hemoconcentration, more severe than the peripheral blood picture indicated; total cellular volume/100 g body weight exhibited a 26% decrease. This anemia was accompanied by a slight increase in total white cell count and a lowered body weight. The 3 hormone therapy completely repaired the anemia with the total erythrocyte, hematocrit, hemoglobin, total cellular volume, body weight and total white cell values all at normal or slightly elevated levels. There were no significant differences found in the reticulocyte counts although there was a tendency for them to be lower after hypophysectomy and slightly elevated after treatment.

Histologic study of bone marrows (Fig. 1, 2, 3) showed that they were hypoplastic following hypophysectomy and indistinguishable from normal after hormone therapy. The data obtained from bone marrow counts are presented in Table II. In agreement with the histologic picture, there was a reduction in total number of nucleated cells/mm³ of marrow tissue following hypophysectomy, and an elevation to normal following the 3 hormone therapy. The total nucleated cell count following hypophysectomy was not statistically different from normal; the trend indicated, however, is consistent with our past experience and the changes noted are believed to reflect biologically significant alterations in the marrow. Hypophysectomy induced a de-

TABLE II. Bone Marrow Picture in 3 Groups of Adult Female Rats: (1) Normal Controls, (2) Hypophysectomized for 7 Months, and (3) Hypophysectomized for 4 Months Followed by Daily Injections of 0.005 mg Thyroxin, 0.6 mg Cortisone Acetate, and Gradually Increasing Amounts (0.2-0.8 mg) of Growth Hormone for 3 Months. ± = stand. error.

	Normal controls	Hyp., no treatment		Hyp., treated	
	6 rats	6 rats	% of normal	10 rats	% of normal
Total nucleated cells, millions/mm³ of marrow tissue	1.748± .06	1.597± .13	91	1.724± .06	99
Total erythroid elements, thousands/mm³ of marrow tissue	866.4 ±107.4	633.8 ±54.8	73	709.1 ±49.0	82
Total myeloid elements, thousands/mm³ of marrow tissue	698.4 ±101.4	724.6 ±59.4	104	784.4 ±44.9	112

‡ Growth Hormone Lot R377237 and Somar Lot M208 generously provided by Armour Co.

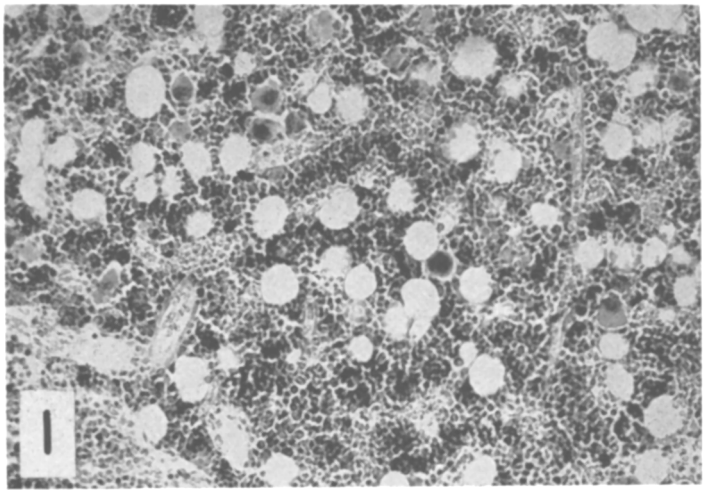

FIG. 1. Femur marrow from normal rat ×160. Note small amt of fat and dark islands of cells representing erythroid elements.

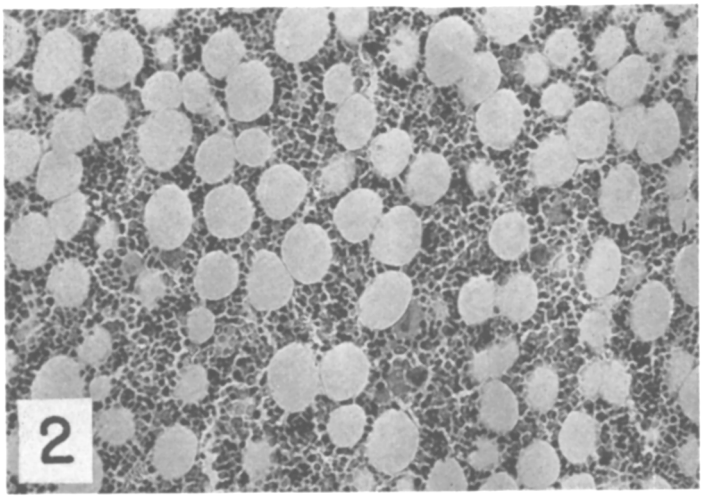

FIG. 2. Femur marrow from hypophysectomized rat 7 mo after surgery × 160. Note increase in amt of fat and that erythroid islands are small and indistinct.

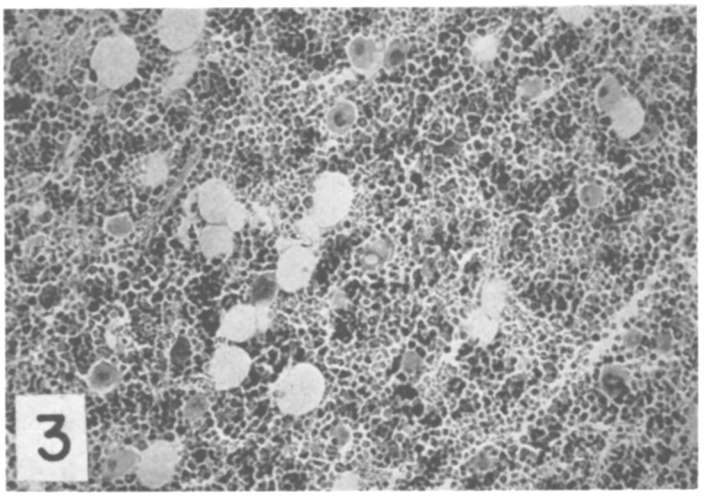

FIG. 3. Femur marrow from rat hypophysectomized for 4 mo followed by daily inj. of 0.005 mg thyroxin, 0.6 mg cortisone acetate, and gradually increasing amounts (0.2 to 0.8 mg) of growth hormone for 3 mo × 160. Note small amt of fat and normal appearance of erythroid islands.

TABLE III. Oxygen Consumption in 3 Groups of Adult Female Rats: (1) Normal Controls, (2) Hypophysectomized for 7 Months, and (3) Hypophysectomized for 4 Months Followed by Daily Injections of 0.005 mg Thyroxin, 0.6 mg Cortisone Acetate, and Gradually Increasing Amounts (0.2-0.8 mg) of Growth Hormone for 3 Months. ± = stand. error.

	Normal controls	Hyp., no treatment		Hyp., treated	
	8 rats	7 rats	% of normal	11 rats	% of normal
Oxygen consumption, l/m^2 body surface/hr	8.20 ± .24	6.98 ± .27	85	7.76 ± .25	95

crease in erythroid elements in the marrow to 73% of normal but induced no significant changes in number of myeloid elements. The 3 hormone treatment elevated myeloid elements to 12% above normal; the count for erythroid elements was 82% of normal.

Oxygen consumption by the 3 groups of rats studied is presented in Table III. Hypophysectomy induced a decrease in oxygen consumption which was returned to 95% of normal by the 3 hormone therapy.

Discussion. Previous work(3,4) has shown that blood and bone marrow pictures following combined thyroidectomy-adrenalectomy are not identical with those following hypophysectomy. This suggests that removal of some pituitary factor, in addition to those regulating the thyroid and adrenal cortex, is needed to explain the peripheral blood and bone marrow picture following hypophysectomy.

Insofar as the peripheral erythrocyte picture is concerned, the results in the present experiment show that the anemia is completely repaired by a combined thyroxin-cortisone-growth hormone therapy. This repair to the peripheral erythrocyte number was not different from that obtained with thyroxin-cortisone treatment alone(2,5) but the bone marrow studies demonstrate differences worthy of some discussion.

In degree of cellularity the marrows of the animals treated with all 3 hormones were indistinguishable from normal, which is in contrast to the hypoplastic marrow of thyroxin-cortisone treated rats(5) or the hyperplastic marrow of rats treated with growth hormone(11). This histological picture is paralleled by total nucleated cell counts of the marrows; the number of cells is normal in rats treated with the 3 hormones, 69% of normal in thyroxin-cortisone treated animals (5) and 128% of normal in those treated with growth hormone(11). These results, however, do not indicate whether changes were in the erythroid or myeloid series of cells.

Treatment with thyroxin-cortisone alone (5) induced a marked depression of the total number of *myeloid* elements in the marrow (52% of normal) which was reflected in the peripheral blood by a reduced total white cell count (58% of normal). Growth hormone alone, in contrast, resulted in elevated counts of both the marrow myeloid elements and the peripheral white cell count(11). Combining all 3 hormones, as shown in the present experiment, prevents these changes; total number of marrow myeloid elements and peripheral white cells were closer to normal levels. It should be noted that changes in marrow myeloid elements were reflected in similar changes in numbers of peripheral white cells. (The part lymphocytes are playing in the total white cell changes has not been studied other than to note that, in general, there is an increase in marrow lymphocytes after hypophysectomy which is returned to near normal levels by all therapies mentioned.)

Changes in marrow *erythroid* elements are more difficult to interpret because changes in marrow and peripheral blood do not always parallel one another; there is a difference between production of erythrocytes in the marrow and delivery of these cells to the peripheral blood. Hypophysectomy induced a decrease in erythroid elements to 73% of normal which was reflected in a peripheral reduction of total red cell volume/100 g body weight to 74% of normal. Previous work(5) has shown that combined thyroxin-cortisone therapy in hypophysectomized rats maintained a normal peripheral erythrocyte level

but did so with a marrow which was gradually being exhausted; number of erythroid elements was 83% of normal. Growth hormone(11), in contrast, increased the marrow erythroid elements to a level 49% above normal; in spite of this increased production, these cells were not delivered to the peripheral blood—the anemia persisted. Combining all 3 hormones induced changes in marrow erythroid elements and in total cellular volume of peripheral erythrocytes/100 g of body weight similar to those obtained with combined thyroxin and cortisone therapy.

This lack of an effect of growth hormone on the erythroid elements when combined with thyroxin and cortisone is difficult to explain when it has been shown that growth hormone has a profound effect on these elements when given alone(10,11). We feel that this finding is a matter of rate of production and release of erythroid elements and is a result of imbalance in hormone dosage. This conclusion agrees with the work of Fruhman and Gordon(10) who reported that growth hormone and cortisone were antagonistic to one another in their effects on development of both erythrocytes and white cells, and that the number of erythroid elements in bone marrow of rats treated with cortisone and growth hormone was intermediate between rats treated with either hormone alone. Evidence in the present experiment shows that growth hormone, when given in conjunction with thyroxin and cortisone, is not completely inactive; it has prevented the marked loss of myeloid elements and peripheral white blood cells seen in thyroxin-cortisone treated hypophysectomized rats to such an extent that the number of cells/mm^3 of marrow is normal as is the peripheral white cell number.

Is the loss of growth hormone a factor, in addition to thyrotropic and adrenocorticotropic hormones, which is responsible for hematological changes found in hypophysectomized rats? It seems clear that growth hormone has a stimulating effect on both myeloid and erythroid elements in bone marrow when given alone(10,11), although this may be a general effect of growth hormone injections rather than any specific one on marrow cells. The present experiment, however, allows the conclusion that growth hormone has an effect on myeloid elements, counteracting effects of thyroxin-cortisone injections; if it had an effect on erythropoiesis in the present experiment, it was masked by the reverse effects of cortisone.

Previous work(12) has shown that oxygen consumption is returned to normal levels by thyroxin-cortisone therapy but is not affected by growth hormone alone. It was postulated that decreased oxygen need is a causative factor in the anemia following hypophysectomy. The present experiment provides another example of repair of oxygen consumption with a parallel alleviation of the peripheral anemia.

Summary. Previous work has shown that a combined thyroxin-cortisone therapy in hypophysectomized rats will eliminate the anemia and induce a marked decrease in peripheral white cells; this was accompanied by severe hypoplasia of the bone marrow. It has also been shown that growth hormone increases peripheral white cell count in hypophysectomized rats but has no effect on peripheral anemia; the bone marrow was hyperplastic. Adding growth hormone to the thyroxin-cortisone therapy resulted in repair of post-hypophysectomy anemia, in normal histological appearance of bone marrow, in normal total number of cells/mm^3 of marrow tissue, and in values for myeloid elements/mm^3 of marrow and peripheral white cells slightly above normal. The values for erythroid elements/mm^3 of marrow averaged 82% of normal. These hematological findings were accompanied by a normal oxygen consumption.

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Effect of *Candida Guilliermondi* Polysaccharide on Transplantable Mouse Sarcoma 37. (23398)

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Previous experiments conducted in this laboratory(1) indicated that several species of *Candida* and *Saccharomyces* exerted a lytic action on Sarcoma 37 ascites and Ehrlich ascites cells *in vitro,* either when incubated together in isotonic glucose solution, or when grown simultaneously in tissue culture; though normal mouse tissues, similarly treated, remained unharmed. These results confirmed and extended those of several earlier workers, already reviewed(1). When we injected mice bearing solid transplantable Sarcoma 37 with living and killed *Candida* species by various routes, sloughing of tumors occurred in varying percentages (approximately 90% with living *C. Guilliermondi* injected intravenously(2,3). The fate of the organisms in tumor and host tissues of intravenously injected mice was followed, and the selectively destructive action of the organisms, administered in non-toxic but tumor-necrotizing doses, was confirmed(4). Heat- and chemically-killed cultures were active, though to a lesser degree(3) and it was therefore decided to test whether some effective agent could be isolated by chemical means from killed or lyophilized cultures.

Our first attempt was to separate a polysaccharide fraction by the procedure of Pillemer *et al.*(5). Since *Candida Guilliermondi* was the most suitable organism for use in the living state because of its high tumor-necrotizing properties with respect to Sarcoma 37, and its nonpathogenicity to mice, cultures of this organism were chosen as our starting material.

Methods. The *Candida* cells were grown in 12 liter flasks on glucose-peptone medium (1% peptone, 2% glucose) and were aerated at room temperature. After 3 days these cultures were centrifuged in a Westphalia apparatus. The yield was, on the average, 300 g of wet yeast cells. These cells were treated according to Pillemer's method for extraction of zymosan, as follows:

Three hundred grams of yeast cells were suspended in 1200 ml of 0.5 *M* sodium monohydrophosphate solution and boiled for 3 hours. To this solution was added 0.17 g of trypsin (Wilson 1:250) on the 1st, 3rd, 6th, and 10th day of incubation at 37°C. Two-tenths ml of toluene was also added on the 1st, 5th, 8th and 11th days. Incubation was continued for 16 days at the same temperature, with occasional shaking, and the pH of the liquor of incubation was adjusted daily to 7.8-8.0 by careful addition of 2 *N* NaOH. The liquor was then centrifuged, the supernate discarded, and the precipitate stirred with 1200 ml of water at room temperature for 1 hour and again separated by centrifugation. The resulting precipitate was suspended with vigorous stirring in 1200 ml of water, maintained at 95-100°C for 1 hour, and recentrifuged while hot. This procedure was repeated twice more. No trace of glucose was found in the final supernate upon testing with Fehling's solution. The precipitate was stirred vigorously with 2400 ml of absolute