nor were they inhibitory to the parent strain *E. coli* K 12 which does not require biotin. When succinate or α-ketoglutarate, instead of aspartate, were added to the basal medium, adenine and its derivatives were less inhibitory and the other purines and pyrimidines did not significantly inhibit the growth of the biotinless mutant.

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Effects of Growth Hormone upon Erythropoiesis in the Hypophysectomized Rat.* (20796)

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Chronic treatment with the growth principle fails to prevent(1-5) or overcome(6) the anemia characteristic of the hypophysectomized rat. Despite this lack of influence upon the peripheral blood values, two of the above groups of investigators(1,6) have found that somatotrophin (STH) induces considerable repair of the hypoplastic marrow of the hypophysectomized rat. The present research was undertaken to determine, in more detail, the influence of STH upon erythropoiesis and to uncover the cause for the discrepancy observed between the behavior of the peripheral blood and bone marrow elements.

Materials and methods. Twelve male rats of a modified Long-Evans strain, hypophysectomized 2 months previously and weighing 150-200 g at the beginning of the experiment, were employed. They were fed a complete laboratory ration and kept at temperatures of 70-80°F. Six of the rats received daily subcutaneous injections, for 7 days, of one mg of the Raben-Westermeyer(7) STH preparation‡ dissolved in 0.2 cc water acidified with 0.1 N HCl to pH 3.0. The 6 control hypophysectomized animals were injected with 0.2 cc acid water for the same period of time. Hematologic analyses were conducted shortly before and at the termination of the 7-day period. Reticulocyte counts were made from wet mounts stained with brilliant cresyl blue. One thousand cells were counted for each determination. Quadruplicate hematocrit val-

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ues for heparinized blood, spun at 7000 RPM for 15 minutes, were obtained by a capillary micromethod. At the termination of the experiment, the rats were anesthetized with ether and exsanguinated, as fully as possible, by cardiac puncture. The volume of blood withdrawn from each animal was recorded. The femora and tibiae were dissected, cracked open and the bone marrow removed and pooled. The tissue was prepared in suspension form in homologous serum by drawing it up and down gently with a pipette. Smears were made and treated by Ralph's technic(8) using Harris' hematoxylin as a counterstain. With this method, the cytoplasm in both the mature red cells and the nucleated erythrocytes, in various stages of development, appears distinctly golden in color because of the specific staining of hemoglobin. The use of Harris' hematoxylin, as a nuclear stain, serves further to distinguish between the nucleated erythrocytes and the other marrow elements. Giemsa-stained smears were also prepared. At least 500 cells were counted for each animal to determine the percentages of nucleated erythrocytes. The humeri were fixed in Helly's fluid, decalcified by a formic acid-ion exchange resin preparation (Win-3000), sectioned in paraffin at 10 μ and stained with hematoxylin and eosin.

TABLE I. Effects of Growth Hormone upon Erythropoiesis and Organ Weights of Hypophysectomized Rats (Means ± Standard Errors).

	STH	Acid water	P*
Change in wt (g)	+21.3± 1.43	−4.8± 1.3	<.001
Reticul. (%)	3.8± .81	1.0± .14	.001
Marrow nuc. RBC (%)	18.4± 2.61	9.2± 1.1	.007
Change in hematoc. (%)	−4.2± 1.1	−1.7± 1.3	.179
Vol. "drawn blood" (ml)	5.0± .91	3.2± .52	.083
Thyroid wt (mg)†	8.7± .73	6.8± .64	.062
Adrenal " "	11.7± 1.62	9.4± .35	.244
Seminal vesic. wt (mg)	45.1± 1.76	44.9± 3.63	>.80
Thymus wt (mg)	234 ±27.7	156 ±37.1	.083
Spleen wt (mg)	688 ±61.4	409 ±29.2	.002

* P is calculated from the distribution of Fisher's t. Values less than 0.05 have been considered significant.

† One of the 6 animals treated with STH and included in the table may have been partially hypophysectomized. It possessed a larger body wt and, at the time of sacrifice, its thyroid and adrenals weighed 11 and 19 mg respectively. The remainder of its values, however, fell within the range of the other 5 STH-injected rats.

Results. Evidence is presented in Table I that STH stimulates erythropoiesis in the hypophysectomized rat. Thus significant reticulocytosis and increases in the percentages of nucleated marrow erythroid elements are apparent after a week's treatment with this agent. The marked reparative influence of STH upon the bone marrow structure of the relatively long-term hypophysectomized rat is evident from Fig. 1-4, inclusive. Despite these significant effects upon the marrow, the hematocrit values remain at the low level seen in untreated or in the acid water-injected controls.

In Table I are also indicated the significant body weight gains displayed by the animals treated with STH. It is of interest that the Raben-Westermeyer preparation, in the dosage given, does not affect significantly the weights of the thyroid, adrenals, seminal vesicles and thymus. Splenic weights, however, are elevated with this treatment.

At the termination of the experiment, it was found that consistently greater volumes of blood could be aspirated by the same investigator (GJF) from the rats injected with STH than from those given the acid water. The difference in mean values, however, is just outside the selected $P < 0.05$ limits of significance ($P = 0.08$).

Discussion. The present experiments indicate that the Raben-Westermeyer preparation of STH, possessing little or no gonadotrophic, corticotrophic or thyrotrophic hormone activity in the dosages used, exerts a significant erythropoietic effect in the long-term hypophysectomized rat. It remains to be determined whether this influence is a specific one imparted directly to the marrow or is merely a reflection of the capacity of STH to augment the food intake, thereby increasing the nutritional status of the animal.

The apparent lack of response to STH of the peripheral red cell numbers, hematocrit and hemoglobin values, as noted by others and in this report, is probably a reflection of

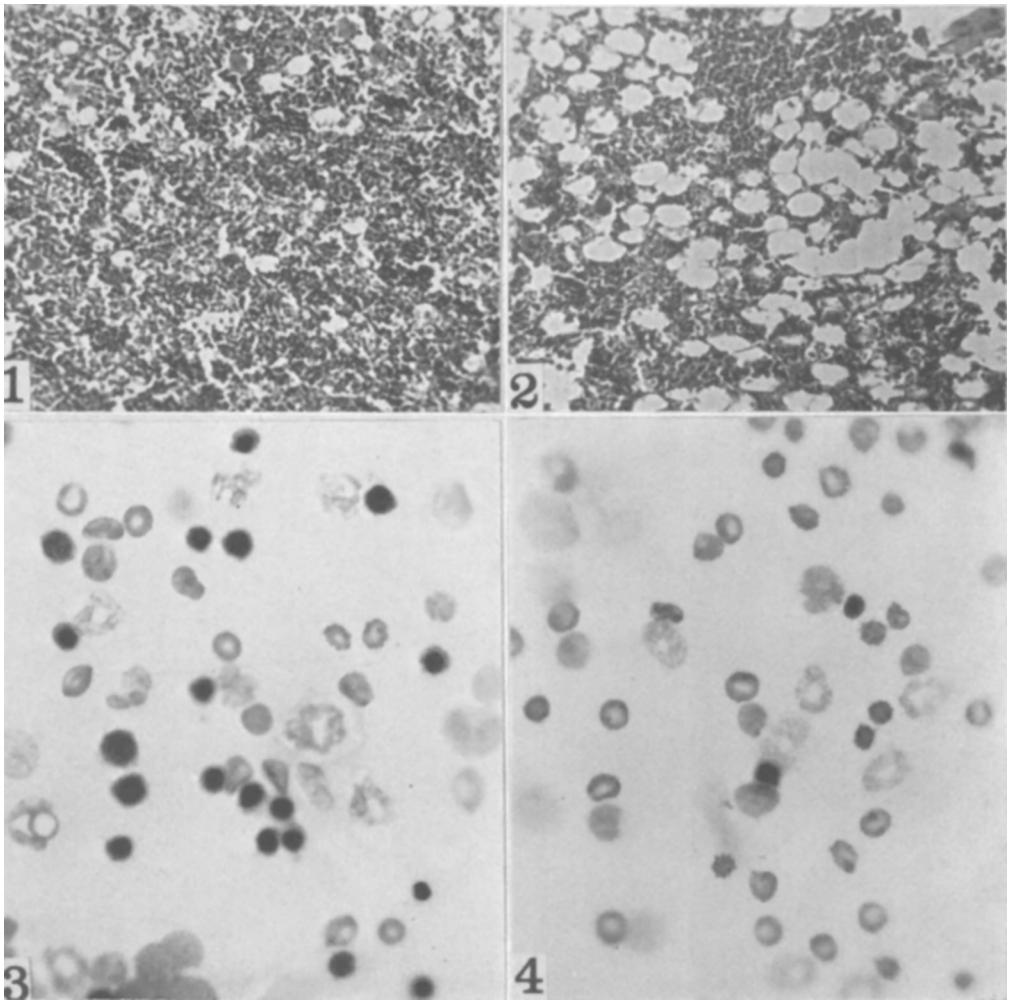

FIG. 1. Humeral bone marrow section of hypophysectomized rat treated daily for 7 days with 1 mg STH dissolved in 0.2 cc acid water. Considerable repair of post-hypophysectomy hypoplasia is observed (cf Fig. 2). Helly fixation, hematoxylin-eosin. ×140.

FIG. 2. Humeral bone marrow section of hypophysectomized rat treated daily for 7 days with 0.2 cc acid water. No effect is apparent upon the typical post-hypophysectomy hypoplasia. Helly fixation, hematoxylin-eosin. ×140.

FIG. 3. Bone marrow smear from hypophysectomized rat treated daily for 7 days with 1 mg STH dissolved in 0.2 cc acid water. Note greater concentration of nucleated erythrocytes (darkly stained) compared to Fig. 4. Benzidine, Harris' hematoxylin preparation. ×695.

FIG. 4. Bone marrow smear from hypophysectomized rat treated daily for 7 days with 0.2 cc acid water. Concentration of nucleated erythrocytes is considerably less than that observed in Fig. 3. Benzidine, Harris' hematoxylin preparation. ×695.

the ability of this hormone to augment the plasma volume, a phenomenon observed strikingly in the hypophysectomized dog(9). It appears to be of sufficient intensity in the rat to obscure the rise in peripheral red cell values that might be anticipated from the observed erythropoietic action of the hormone. In view of the strong reticulocytosis evoked by STH, it is unlikely that it acts by inhibiting the release of erythrocytes from the marrow.

Support for the contention that an increase in plasma volume has occurred in the rats receiving STH is derived from the present observations that larger quantities of blood are obtainable from the hearts of these ani-

mals at the time of sacrifice. In this connection, the experiments of Lippman(10) have indicated that "drawn blood" values are a reflection of the actual blood volume levels. Also in line with the concept of a plasma volume change is the finding that a significant rise occurs in red cell counts and hemoglobin values within a few days following cessation of STH treatment in the hypophysectomized rat(6).

Summary. Administration of STH to hypophysectomized rats results in peripheral reticulocytosis and increases in the percentages of nucleated erythroid elements within the bone marrow. Considerable repair of the hypoplastic marrow is induced by this agent. A rise in peripheral erythrocytic values fails to occur probably because of a concomitant increase in plasma volume.

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Infrared Spectroscopy of Liver Glycogen in Normal and Irradiated Adult and Fetal Rats.* (20797)

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Previous studies(1) on rabbits demonstrated infrared absorption spectra of liver for the region 1-15 μ. Two types of absorption bands were found within 2 different intervals of that region: 1. Bands below 8.1 μ, which were very similar to those of the common "protein polyamide" vibrations(2) and occur not only in liver but in all other tissues (3.04 μ NH stretching, 3.4 μ CH stretching, 6.0 μ CO stretching, the deformation vibration of NH at 6.44 μ, the deformation vibration of CH at 6.84 μ, the CH 3 vibration at 7.26 μ, and an unassigned band at 8.1 μ). 2. Bands between 8.1 μ and 11 μ which have been shown to be characteristic for liver tissue, "fingerprint bands" (double bands at 9.70-9.64 μ and 9.23 μ). It was also noted that after 2-3 days starvation followed by insulin shock, one of the characteristic double bands at 9.70-9.64 μ disappeared, while the other at 9.23 μ remained, although greatly reduced in strength.

The present paper reports differences in infrared spectroscopic findings in the liver of adult and fetal rats, definite assignment of one characteristic band to glycogen, and spectroscopic studies of the effect of ionizing irradiation on liver glycogen in adult and fetal rats.

Materials and methods. Female albino rats of about 200 g were used for all experiments. Fetal livers were obtained in the 2nd-3rd week of development. Animals were kept for about one week on a balanced diet *ad libitum*, and then examined either non-fasted, fasted for 24 hours, or after feeding various sugars by stomach tube 4 hours preceding the ex-

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