The Role of Growth Hormone and Thyroxine in Erythropoietic Restoration in Hypophysectomized Monkeys (35910)

L. VITALE, N. T. SHAHIDI, G. E. KERR, R. C. WOLF, D. R. KORST, AND R. K. MEYER

University of Wisconsin Medical Center and Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706

Hypophysectomy results in a reduction of red cell volume (RCV) in laboratory animals (1, 2) and in man (3). This reduction is associated with a decrease in the number of erythroid elements in the bone marrow (4, 5). The alteration of plasma volume (PV) following hypophysectomy has not been consistent. In rodents hypophysectomy results in peripheral anemia (1, 4). In hypophysectomized dogs and man there is a parallel decrease in PV so that the hemoglobin concentration and the hematocrit remain constant (2,3). The exact reasons for the decrease in RCV and PV following hypophysectomy are not clear and the role of corticosteroids, thyroxine (T^4) and human growth hormone (HGH) on the restoration of these abnormalities has not been fully evaluated in primates. The present study was designed to evaluate the effect of the above hormones, separately and in combination, on PV, RCV, nucleated erythron in the bone marrow, and plasma erythropoietin activity (PEP) in hypophysectomized monkeys.

Methods. The laboratory material comprised six normal and three hypophysectomized female rhesus monkeys (*Macacca mulatta*). All monkeys were on adequate diet and received iron supplementation. The hemograms were done by standard hematological methods. The RCV and PV were performed by a combined closed technique (6) using ^{131}I plasma and ^{51}Cr labeled RBC, according to the methods described by Crispell *et al.* (7) and Sterling and Gray (8), respectively. PEP activity was assayed in polycythemic mice by measuring erythrocyte ^{59}Fe uptake and reticulocyte percentage (fluorescent method) as described before (9). All medications were given intramuscularly except for T^4 which was given orally.

Results. Table I illustrates the hematological and biochemical values in normal and hypophysectomized monkeys before any hormonal therapy. As shown, the values for RCV, PV, nucleated erythron, and PEP in hypophysectomized monkeys were markedly lower than those obtained in normal animals. As expected, there was no significant difference in hematocrit and hemoglobin concentrations between the two groups.

Monkey I, who was in the adolescent age group, had an RCV of 14 ml/kg and a PV of 31.6 ml/kg of body weight 6 weeks after hypophysectomy. Corticosteroids and T^4, given alone or in combination, had no beneficial effect on this monkey's RCV or PV. This monkey died 18 weeks following hypophysectomy. The RCV determined 3 days prior to death was found to be 7.5 ml/kg of body weight.

Monkey II (Fig. 1), with an initial RCV of 11.9 ml/kg and a PV of 28 ml/kg, showed likewise no response to either corticosteroids alone or to combined T^4 and corticosteroids. However, when HGH was added to T^4 and corticosteroids there was a rise in both RCV and PV levels to 27 and 49 ml/kg of body weight, respectively. The percentage of nucleated erythron in the bone marrow at this time was 45.5%. After 6 weeks of therapy, HGH was discontinued, while the other two hormones were maintained. Studies 6 weeks after discontinuation of HGH revealed a marked decrease in RCV and nucleated erythrons in the bone marrow. These values fell to pretreatment levels 12 weeks after the discontinuation of HGH. Resumption of HGH immediately after the discontinuation of T^4 and corticosteroids produced again a rise in nucleated erythron in the bone marrow and restored the PV and

TABLE I. Hematological and Biochemical Values in Normal and Hypophysectomized Monkeys Prior to Hormonal Therapy.

	Mean values and 1 SD for healthy adult monkeys, 6	Values for hypophysectomized monkeys		
		I	II	III
Hematocrit (%)	40.3 ± 4.1	38.0	39.5	40.0
Hemoglobin (g/100 ml)	13.0 ± 1.1	13.1	13.2	14.2
Plasma protein (g/100 ml)	7.8 ± 0.2	7.7	8.1	7.8
Reticulocytes (%)	0.6 ± 0.3	0.2	0.2	0.8
Bone marrow erythroid percentage	21.0 ± 4.0	15.0	10.5	10.0
Plasma erythropoietin activity (units/ml)	5.0 ± 2.0	0.3	0.0	0.8
Red cell vol (ml/kg)	22.3 ± 1.0	14.0	11.9	12.9
Plasma vol (ml/kg)	40.8 ± 1.1	31.6	28.0	28.1
Serum iron (μg/100 ml)	106.0 ± 22.9	86.0	90.0	82.0
Protein bound iodine (μg/100 ml)	4.3 ± 0.3	2.2	1.8	2.2

RCV to normal. However, when HGH was given alone at the same dose 12 weeks after the discontinuation of all hormones it resulted only in a moderate increase in PV and RCV. The addition of T^4 to HGH restored again the values to normal.

Monkey III (Fig. 2) who had an initial RCV and PV value of 12.9 and 28 ml/kg of body weight, respectively, showed similar response. As shown, corticosteroids and com-

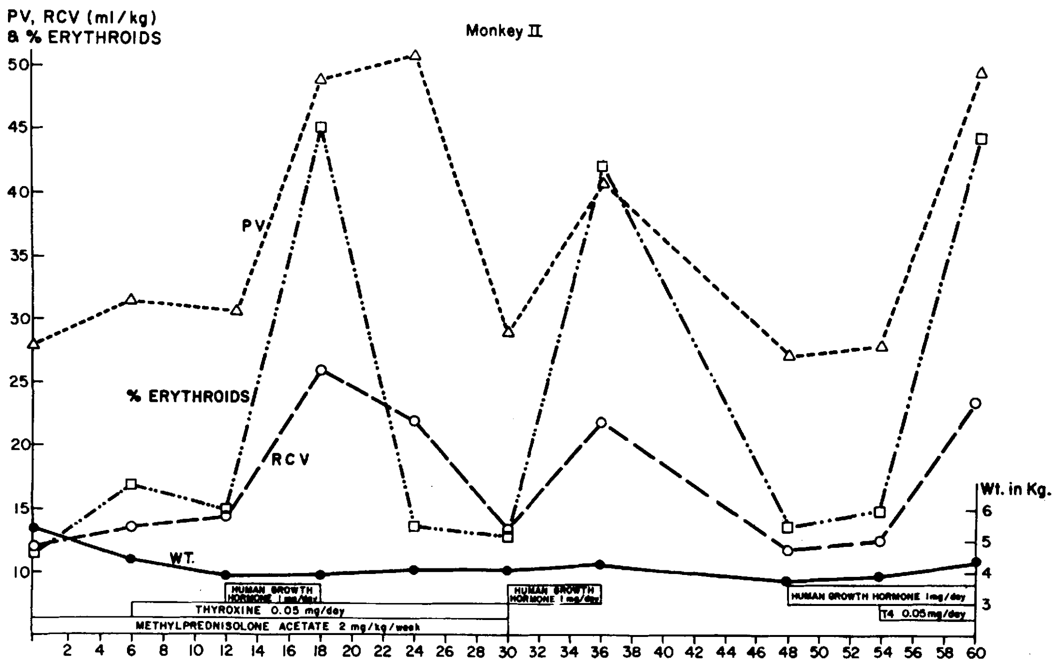

FIG. 1. The effect of methylprednisolone acetate, thyroxine (T^4) and human growth hormone (HGH) on the nucleated erythron, red cell volume (RCV), and plasma volume (PV) in hypophysectomized monkey no. II. Note the dramatic effect of combined HGH and T^4 on nucleated erythron, RCV, and PV.

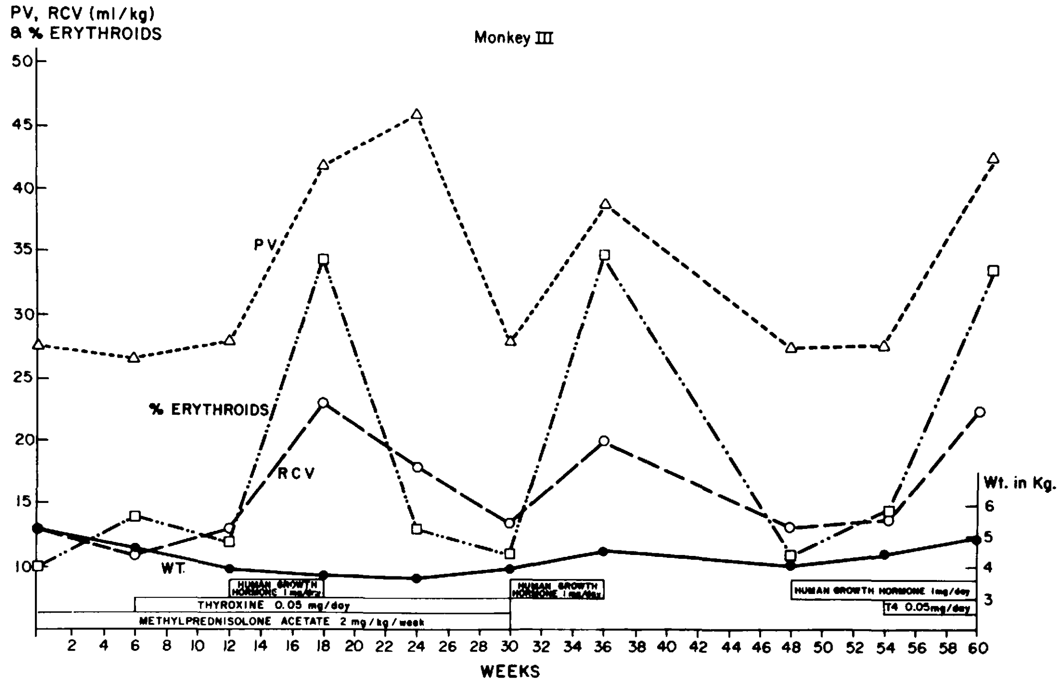

FIG. 2. The effect of methylprednisolone acetate, thyroxine (T^4) and human growth hormone (HGH) on the nucleated erythron, red cell volume (RCV), and plasma volume (PV) in hypophysectomized monkey no. III.

bined corticosteroid–T^4 therapy had no significant effect on RCV, PV, or nucleated erythron. The addition of HGH resulted in an increase in nucleated erythron and an increase in the RCV and PV to normal values. After discontinuation of HGH, the values decreased to pretreatment levels. The administration of HGH alone immediately after the discontinuation of T^4 and corticosteroids again produced increased erythroid activity in the bone marrow and restoration of RCV and PV to normal. However, as in the second monkey, when HGH was given 12 weeks after discontinuation of all hormones it failed to significantly increase the RCV or PV. However, as in the second monkey, the combined HGH and T^4 therapy restored the RCV and PV to normal.

The PEP activity in all hypophysectomized monkeys increased following combined T^4–corticosteroid therapy and reached peaks of 3, 6, and 1.8 units in monkeys I, II, and III, respectively. However, this was not associated with any erythropoietic response in the bone marrow or peripheral blood. Following the administration of HGH to monkeys II and III, there was further rise in PEP levels (12 and 3.1 units, respectively) which was accompanied by dramatic increases in the erythroid percentage in the bone marrow and in the volume of the circulating red cell. After discontinuation of HGH, the PEP fell to pretreatment levels.

Discussion. The above findings indicate that in hypophysectomized monkeys combined T^4 and HGH can restore the RCV and PV to normal. Such a restoration is most likely the result of the synergetic effect of HGH and T^4. Indeed, the synergetic effect of these two hormones on calorigenesis has been repeatedly demonstrated by Evans and associates (10, 11), using other parameters. The initial increase in RCV following HGH given alone immediately after the discontinuation of corticosteroid–T^4 therapy was most likely due to the slow degradation of T^4 (12) and, consequently, the persistence of this hormone in adequate concentration for several weeks after its discontinuation. The mechanism whereby growth hormone–T^4

therapy stimulates erythropoiesis is not certain. The marked increase in PEP activity during combined HGH–T^4 therapy suggests that the erythropoietic effect of these hormones is mediated through the action of erythropoietin. It should be noted, however, that PEP activity increased also, although to a lesser extent, during combined T^4–corticosteroid treatment without significant increase in erythropoiesis. This finding suggests either a direct effect of growth hormone on the bone marrow as reported by Fisher and associates (13) or that the magnitude of PEP activity during corticosteroid–T^4 therapy was not sufficient to enhance erythropoiesis.

Summary. Studies in three hypophysectomized monkeys revealed markedly reduced red cell volume, plasma volume, percentage of nucleated erythron in the bone marrow, and plasma erythropoietin activity. Corticosteroids and thyroxine, given alone or in combination, had no significant effect on these abnormalities; the combined growth hormone and thyroxine therapy, however, restored all the abnormalities to normal.

We thank the National Pituitary Agency for providing us with human growth hormone. This study was supported in part by NIH grants AM-5313 and FR-00167.

1. Berlin, N. I., Van Dyke, D. C., Siri, W. E., and Williams, C. P., Endocrinology **47**, 429 (1950).

2. Bozzini, C. E., Houssay, A. B., Varela, J., Rendo, M. E. B., Ugarte, C. A. A., and Wagner, A., Acta Physiol. Lat. Amer. **14**, 12 (1964).

3. Falkheden, T., Sjögren, B., and Westling, H., Acta Endocrinol. **42**, 552 (1963).

4. Crafts, R. C., and Meineke, H. A., Ann. N.Y. Acad. Sci. **77**, 501 (1959).

5. Bozzini, C. E., Endocrinology **77**, 977 (1965).

6. Shahidi, N. T., and Clatanoff, D. V., Brit. J. Haemat. **17**, 335 (1969).

7. Crispell, K. R., Porter, B., and Nieset, R. T., J. Clin. Invest. **29**, 513 (1950).

8. Sterling, K., and Gray, S. J., J. Clin. Invest. **29**, 1614 (1950).

9. Korst, D. R., and Quirk, J., Ann. N.Y. Acad. Sci. **149**, 236 (1968).

10. Evans, E. S., Contopoulos, A. N., and Simpson, M. E., Endocrinology **60**, 403 (1957).

11. Evans, E. S., Simpson, M. E., and Evans, H. M., Endocrinology **63**, 836 (1958).

12. Sterling, K., and Chodos, R. B., J. Clin. Invest. **35**, 806 (1956).

13. Fisher, J. W., Roh, B. L., Couch, C., and Nightingale, W. O., Blood **23**, 87 (1964).

Received May 7, 1971. P.S.E.B.M., 1971, Vol 138.