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Effect of Various Hormones on Red Cell Production in Mice*

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The influence of some hormones on erythropoiesis is well documented.[1-5] Recently, it has been shown that certain steroid metabolites stimulates red blood cell production.[6]

Erythropoietin (erythrocyte stimulating factor, ESF) is the major factor governing erythropoiesis.[7-9] It appears that some of the hormones influence blood cell production via an effect on erythropoietin levels.[5 10 11]

However divergent opinions still exist regarding the erythropoietic effectiveness of some hormones-perhaps due to the variety of animals, techniques, dosages and assay methods used. In most cases, only one or few hormones were evaluated by a single group of workers. There appears to be on recent study of the effect of a large number of hormones on red cell and erythropoietin production.

In this study, the erythropoietic effect of a large number of hormones, as determined by 48 hours Fe59 incorporation into circulating red cells of protein depiver CFI female mice, is presented. Doses of hormones used were based on doses employed clinically so that results obtained would be more meaningful when applied to man. In the first part of the study the effect of daily injections of each hormone on Fe59 incorporation by red blood cells of mice was determind. In the second part, the effect of 7 daily injections was studied. Finally, in order to determine

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whether the influence of hormones was mediated via a stimulation of ESF production or release, the effect of anti-ESF administered in conjunction with hormones was studied.

Materials and Methods

CFI adult female mice weighing approximately 20-25 grams were employed as assay animals according to a modified erythropoietin bioassay described by us previously. [12] [13]

In the first part of the study groups of 4-6 mice were placed on a protein free diet* for 4 days to reduce endogenous erythropoietin production of the mice. From the 5th day through the 8th day (the day animals were sacrificed) they were fed standard laboratory chow.** On day 4 and 5 mice were injected with the hormone being studied. On the 6th day they were injected with 0.5 microcuries of radioactive ferrous sulfate*** (Fe59) in 0.5 ml of saline. 48 hours later (8th day) animals were briefly anesthetized with ether and sacrificed by exanguination. The radioactivity of 0.5 ml of whole blood was then determined in a well type scintillation counter. Erythropoietic activity was expressed as per cent incorporation of injected Fe59 (standard solution) into circulating red blood cells of the mice in 48 hours, using the following equation:

$$\% \text{ Fe59 incorporation} = \frac{\text{Total radioactivity in circulating blood x 100}}{\text{Radioactivity of injected Fe59}}$$

Total circulating blood of the mouse on 4 days protein deprivation diet was calculated to be 5.5 % of the body weight, based on determinations mode by exanguination.[12]

Dilutions of all materials were made in physiological saline. Groups of 4-6 animals were used for each study and averages taken. Saline controls were kept throughout. All injections were made intraperitoneally. Doses of hormones employed were comparable to those used clinically. Calculations were based on those made by Freireich. [14] In some cases, doses 10 times greater and 10 times less were also studied.

In the second part of the study, animals were injected on day 1 through 7 to determine sustained effects of hormones.

. * Nutritional Biochemical Corporation, Cleveland, Ohio.

** Purina Lab Chow, Ralston Purian Co., Checkerboard Square, St. Louis, Missouri.

*** Ferrutope (Fe59), Mallincrodt Nuclear, St. Louis, Missouri.

In the third part of the study, anti-ESF (Wright)* was injected on day 4 and 5, in addition to the injections of hormones or saline, to determine whether erythropoietic effect of these hormones was due to the stimulation of erythropoietin production or not.

Results

The majority of the hormones studied, significantly stimulated the incorporation of intraperitoneally injected Fe59 into circulating red blood cells of mice (Table I). However, androgens, thyroid stimulating hormone (TSH), and pitressin appeared to be most effective. The short term (2 days) stimulatory influence of various hormones was proportional to the injected dose. At equivalent doses, Deca-Durabolin was less effective than testosterone in stimulating erythropoiesis.

TABLE I

EFFECT OF HORMONES ON RED CELL PRODUCTION

Hormones with Significant Stimulatory Effect ($P < 0.01$)			
Hormones	Dosage I. P. 2 Days	No. of Mice	% Fe59 Incorporation Into Rbc of Mice (mean)
Testosterone	5 mg.	9	12.61 ± 5.44*
Testosterone	0.5 mg.	8	10.78 ± 2.22
Testosterone	0.05 mg.	6	9.09 ± 1.09
Deca Durabolin	2.5 mg.	3	8.93 ± 1
TSH	0.4 u	6	11.96 ± 1.6
TSH	0.04 u	12	7.99 ± 3.42
TSH	0.004 u	5	7.91 ± 2.06
Pitressin	100 m. u	9	10.51 ± 2.80
Parathormone	0.2 u	6	8.70 ± 4.01
Adrenalin	0.05 mg.	4	7.00 ± 1.16
Adrenalin	0.005 mg.	3	7.02 ± 1.70
Adrenalin	0.0005 mg.	5	5.81 ± 2.78
Oxytocin	100 m. u	5	6.48 ± 2.8
Progesterone	0.4 mg	3	6.21 ± 4.51
Growth Hormone (Bovine)	0.2 mg	5	5.62 ± 0.74
Glucagon	0.05 mg	4	5.16 ± 0.31
Prolactin (Bovine)	0.2 mg	5	4.40 ± 1.1
Saline Control	0.2 ml	20	2.79 ± 1.6

*Standard Deviation of the Mean

* Rabbit anti-human erythropoietin was obtained through the courtesy of C. S. Wright, M. D., at the Medical College of Georgia, School of Medicine, Augusta, Georgia.

TABLE II

EFFECT OF HORMONES ON RED CELL PRODUCTION

Hormones without significant stimulatory effect ($P > 0.05$)

Hormones	Dosage 1. P., 2 Days	No. of Mice	% Fe59 Incorporation Into Rbc of Mice (Mean)
Insulin	0.04 u	3	4.98 ± 3.4*
Glucagon	0.005 mg	6	3.78 ± 1.5
ACTH	0.16 u	5	2.75 ± 1.7
Thyroxin	1 ug	6	2.65 ± 1
Hydrocortisone	0.1 mg	5	2.34 ± 1
Premarin	0.1 mg	5	1.49 ± 0.3
Saline Control	6.2 ml	20	2.79 ± 1.6

* Standard Deviation of the Mean

While some hormones had no effect, estrogens (premarin) actually depressed the rate of red cell production in mice in 48 hours (Table II).

Injections of hormones daily for a longer period of time (7 days) gave results similar to those obtained by two day injections. Hormones most effective in stimulating erythropoiesis in 2 day injections significantly increased Fe59 incorporation into red cells of the mouse compared to saline controls (Table III). Adrenalin, however, appeared to be significantly inhibitory when injected for a longer period of time (7 days) compared to saline control ($P < 0.02$), while pitressin and progesterone became ineffective in stimulating erythropoiesis.

The mechanism of the erythropoietic effect of hormones was investigated by simultaneous injections of hormones and anti-ESF. The simultaneous use of anti-ESF resulted in further suppression of endogenous erythropoietin of the mouse in saline injected controls and completely abolished erythropoietic effect of TSH, Testosterone and pitressin (Table IV). These neutralising effects of anti-ESF were significant.

Discussion

Our results confirm the stimulatory and inhibitory effect of androgen and estrogen on red cell production reported previously.[2] [15-19] The clinical use of androgens to treat certain anemias[20] [21] is thus supported by this study. Furthermore, since erythropoietic effect of androgen appears to be through stimulation of erythropoietin, shown here (Table IV) and by others, [10] [11] [26] the use of androgen in treatment of anemias characterized by low level of circulating erythropoietin may also be

TABLE III

EFFECT OF HORMONES ON RED CELL PRODUCTION OF MICE

Hormones	Daily Injections for Seven Days Dosage (I. P.)	No. of Mice	% Fe59 Inc. Into Rbc (mean)	Degree of Significance
TSH	0.04 u	12	8.21 ± 1.44*	P<0.0005
Testosterone	0.5 mg	5	6.80 ± 0.9	P<0.0005
Growth Hormone (Bovine)	0.1 mg	5	6.16 ± 0.85	P<0.002
Prolactin (Bovine)	0.1 mg	6	5.33 ± 1.4	P<0.01
Pitressin	100 Um	5	4.0 ± 1	P>0.15
Progresterone	0.4 mg	5	3.65 ± 1.06	P>0.35
Adrenalin	0.005 mg	5	1.74 ± 0.81	
Saline Control	0.2 ml	5	3.34 ± 1.03	

* Standard Deviation of the Mean

TABLE IV

EFFECT OF SIMULTANEOUS INJECTION OF HORMONES AND ANTI-ESF ON RED CELL PRODUCTION

Hormones	Dosage I. P., 2 Days	Anti-ESF	No of Mice	% Fe59 Inc. Into Rbc (Mean)	Significance of Suppression
TSH	0.04 U	–	12	7.99 ± 3.42*	
TSH	0.04 U	0.05 ml**	6	1.62 ± 0.84	P<0.0005
Testosterone	0.5 mg	–	5	6.80 ± 1	
Testosterone	0.5 mg	0.05 ml	4	1.0 ± 0.3	P<0.0005
Pitressin	100 mU	–	4	4.07 ± 1.45	
Pitressin	100 mU	0.05 ml	5	1.69 ± 0.47	P<0.01
Saline Control	0.2 ml	–	20	2.79 ± 1.6	
Saline Control	0.2 ml	0.05 ml	4	0.46 ± 0.26	P<0.005

* Standard Deviation of the Mean

**Rabbit anti- human erythropoietin, 0.01 ml. capable of neutralising 1 international unit of erythropoietin.

beneficial. In view of the general anabolic effect of androgens, a direct effect on bone marrow stem cells aside from an effect on ESF levels by androgen therapy should also be considered, since in most instances where androgen therapy was reported to be beneficial, the anemia was characterized by a generalized bone marrow hypoplasia or aplasia despite normal or increased erythropoietin levels.[12 20 21] Dose and treatment schedules may account for differences in clinical results. Dosages and injection schedules appeared to influence the results in our experiments (Table I and III). Perhaps the accumulative effect of high doses of androgen in the group receiving daily injections for 7 days is responsible for the lower erythropoietic effect.

The adverse effect of daily treatments was also observed with other hormones. Prolonged treatment of mice with growth hormone and prolactin did not increase the moderate stimulatory effect above that observed after two days treatment (Tables I and III). Adrenalin injections for 7 days actually resulted in depressed erythropoiesis, while treatment for 2 days enhanced erythropoiesis. Pitressin and progesterone became ineffective in stimulating red cell production when given for 7 days. It appears that certain hormones may stimulate or depress erythropoiesis depending on the dose and duration of treatment in mice.

Thyroid Stimulating Hormone (TSH) appears to be almost as effective as androgen in stimulating erythropoiesis (Table I). This effect is probably due to the increased production of erythropoietin following since it is completely abolished when anti-ESF is injected simultaneously (Table IV). Although thyroxin is also reported to stimulate erythropoiesis,[4 23] we could not substantiate erythropoietic effect of thyroxin administered for short time. It may however increase the metabolic demand for oxygen, and thus erythrocyte production when administered for a longer period of time.

Erythropoietic effect of prolaction, oxytocin and pitressin was previously reported by Jepson.[24-26] Our findings confirm their results (Table I). However, pitressin appeared to be a significantly more potent stimulant to erythropoiesis when injected for 2 days only, its effect being mediated via stimulation of erythropoietin[26] (Tables I, III and IV).

In contrast to the results published by Van Dyke[27] and Garcia[28] hydrocortisone and ACTH did not stimulate Fe59 incorporation into red blood cells of the mouse (Table II).

Although some stimulatory effect of glucagon, and progesterone was observed, their erythropoietic effect was of lesser magnitude.

The mechanism of stimulation of red cell production by various hormones other than erythropoietin is poorly understood. If it is mediated through increased erythropoietin production or release, this may be a transient phenomenon at least for some of the known stimulatory hormones. It is conceivable that the initial increase of circulating erythropoietin level induces feed back inhibition or is counteracted by anti–erythropoietic factors when a certain plateau is reached. [12] Other alternatives would be inhibitory effect of cumulative hormones or increased number of newly released red cells in circulation. However the latter, if important, is too small to detect by the routine hematocrit determinations we have performed and in such short term experiments. Finally, peculiarity of physiological responses inherent to species and initial erythropoietin stimulation by non–specific factors which can be detected by the assay method used have also to be considered. [13] These are in accordance with our results of 7 days injection experiments and with the fact that the polycythemia is not a common finding in clinical syndromes characterised by increased production of certain hormones, known to stimulate erythropoiesis in animal experiments. Direct effect on maturation or release of red cell precursors in the bone marrow has been postulated for testosterone but not for other hormones.

Summary

The effect of various hormones on red cell production in mice has been studied using protein deprived CFI adult female mice. Fe59 incorporation into circulating red cells was used as an index of the erythropoiesis.

The majority of the hormones had a stimulatory effect on red cell production of mice when injected for 2 days. However only androgens, TSH, growth hormone and prolactin demonstrated a sustained effect when injected daily for longer period of time. Their erythropoietic effects appeared to be mediated via stimulation of erythropoietin production.

REFERENCES

1. Crafts, R.: The Effects of Endocrines on the Formed Elements of the Blood, Endocrinology 29: 596, 1941.
2. Steinglass, R., Gordon, A., and Charipper, H.: Effect of Castration and Sex Hormones on Blood of the Rat, Proc. Soc. Exptl. Biol. Med. 48: 169, 1941.
3. Crafts, R.: Effects of Iron, Copper, and Thyroxine on Anemia Induced by Hypophysectomy in Adult Female Rat, Am. J. Anat. 79: 267, 1946.
4. Crafts, R., and Meineke, H.: The Anemia of Hypophysectomized Animals, Ann. N. Y. Acad. Sci. 77: 501, 1959.

5. Krantz, S., and Jacobson, L.: Other Humoral and Non-Humoral Influences on Erythropoiesis, In: Erythropoietin and the Regulation of Erythropoiesis, The University of Chicago Press, Chicago, Illinois, 1970, p. 151.

6. Levere, R., Gordon, A., Zanjani, E., Kappas, A.: Stimulation of Mammalian Erythropoiesis by Metabolites of Steroid Hormones, Transact. Assoc. Amer. Physicians 83: 150, 1970.

7. Gordon, A., Piliero, S., Kleinberg, W., and Freedman, H.: A Plasma Extract with Erythropoietic Activity, Proc. Soc. Expt. Biol. Med., 86: 255, 1954.

8. Gordon, A.: Hemopoietine, Physiol. Rev. 39: 1, 1959.

9. Gurney, C.: Erythropoietin, Adv. in Metabolic Disorders, Vol. 3, p. 279., Academic Press Inc., New York, N. Y., 1968.

10. Fried, W., and Gurney, C.: The Erythrocyte-Stimulating Effects of Androgens, Ann. N. Y. Acad. Sci., 149: 356, 1968.

11. Fisher, J., Roh, B., and Halvorsen, S.: Inhibition of Erythropoietin Effects of Hormones by Erythropoietin Antisera in Mildly Plethoric Mice, Proc. Soc. Expt. Biol. Med., 126: 97, 1967.

12. Fırat, D., and Banzon, J.: Erythropoietic Effect of Plasma from Patients with Advanced Cancer, Cancer Research 31: 1353, 1971.

13. Fırat, D., and Haber, C.: A Modified Erythropoietin Bioassay using Protein Deprived Mice, Turkish Journal of Pediatrics, 14: 52, 1972.

14. Freireich, E. J. et al: Quantitative Comparison of Toxicity of Anticancer Agents in Mouse, Rat, Hamster, Dog, Monkey and Man, Cancer Chem. Rep. 50: 219, 1966.

15. Finkelstein, G., Gordon, A., and Charipper, H.: The Effect of Sex Hormones on the Anemia Induced by Hemorrhage in the Rat, Endocrinology 35: 267, 1944.

16. Van Dyke, D., Contopoulos, A., Williams, B., Simpson, M., Lawrence, J., and Evans, M.: Hormonal Factors Influencing Erythropoiesis, Acta Haematol. 11: 203, 1954.

17. Dukes, P., and Goldwasser, E.: Inhibition of Erythropoiesis by Estrogens, Endocrinology 69: 21, 1961.

18. Piliero, S., Medici, P., and Haber, C.: The Interrelationships of the Endocrine and Erythropoietic Systems in the Rat with Special Reference to the Mechanism of Action of Estradiol and Testosterone, Ann. N. Y. Acad. Sci. 149: 336, 1968.

19. Medici, P., Haber, C., and Piliero, S.: Effects of Estradiol and Testosterone on Erythropoiesis, Am. Zool. 4: 343, 1944.

20. Kennedy, B., and Gilbertson, A.: Increased Erythropoiesis Induced by Androgenic Hormone Therapy, New Eng. J. Med. 256: 719, 1957.

21. Shahidi, N., and Diamond, L.: Testosterone-Induced Remission in Aplastic Anemia, Amer. J. Dis. Child. 98: 293, 1959.

22. Fisher, J., and Crook, J.: Influence of Several Hormones on Erythropoiesis and Oxygen Consumption in the Hypophysectomized Rat, Blood 19: 557, 1962.

23. Evans, E., Rosenberg, L., and Simpson, M.: Erythropoietic Response to Calorigenic Hormones, Endocrinology 68: 517, 1961.

24. Jepson, J., and Lowenstein, L.: Effect of Prolactin on Erythropoiesis in the Mouse, Blood 24: 726, 1964.

25. Jepson, J., and Lowenstein, L.: The Effect of Testosterone, Adrenal Steroids and Prolactin on Erythropoiesis, Acta Haematol. 38: 292, 1967.

26. Jepson, J., McGarry, E., and Lowenstein, L.: Erythropoietin Excretion in a Hypopituitary Patient-Effects of Testosterone and Vasopressin, Arch. Intern. Med. 122: 265, 1968.

27. Van Dyke, D., Garcia, J., Simpson, M., Huff, R., Contopoulos, A., and Evans, H.: Maintenance of Circulating Red Cell Volume in Rats after Removal of the Posterior and Intermediate Lobes of the Pituitary, Blood 7: 1017, 1952.

28. Garcia, J., Van Dyke, D., Huff, R., Elmlinger, P., and Oda, J.: Increase in Circulating Red Cell Volume of Normal and Hypophysectomized Rats after Treatment with ACTH, Proc. Soc. Exptl. Biol. Med., 76: 707, 1951.