ent study demonstrated an absence of PTH in the oxyphil cells, as manifested by their absence of specific immunochemical staining. This study therefore presents further evidence that the oxyphil cells do not synthesize or store PTH.

Summary. The fluorescent antibody technic has been utilized to detect and to localize parathyroid hormone in the chief cells of bovine, human and rat parathyroid tissue. Immunochemical cross-reaction has been found among these species. The oxyphil cells of human parathyroid tissue did not reveal specific fluorescence. This observation is regarded as further evidence that the oxyphil cells do not normally synthesize or store parathyroid hormone.

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1. Williams, G. A., Hargis, G. K., Sidwell C. G., Yakulis, V. J., PROC. SOC. EXP. BIOL. AND MED., 1964, v115, 61.

2. Coons, A. H., Kaplan, M. H., *J. Exp. Med.*, 1950, v91, 1.

3. Lacy, P. E., Davies, J., *Diabetes*, 1957, v6, 354.

4. Baum, J., Simons, B. E., Unger, R. H., *ibid.*, 1962, v11, 371.

5. Leznoff, A., Fishman, J., Goodfriend, L., McGarry, E., Beck, J., Rose, B., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 232.

6. Leznoff, A., Fishman, J., Talbot, M., McGarry, E. E., Beck, J. C., Rose, B., *J. Clin. Invest.*, 1962, v41, 1920.

7. Emmart, E. W., Spicer, S. S., Bates, R. W., *J. Histochem. Cytochem.*, 1963, v11, 365.

8. Koffler, D., Fogel, M., PROC. SOC. EXP. BIOL. AND MED., 1964, v115, 1080.

9. Rinderknecht, H., *Nature*, 1962, v193, 167.

10. Baumstark, J. S., *Fed. Proc.*, 1964, v23, 547.

11. Sainte-Marie, G., *J. Histochem. Cytochem.*, 1962, v10, 250.

12. Chadwick, C. S., Nairn, R. C., McEntegart, M. G., *Biochem. J.*, 1959, v73, 41P.

13. Nairn, R. C., in *Fluorescent Protein Tracing*, R. C. Nairn, ed., E. & S. Livingstone Ltd., Edinburgh and London, 1962, 118.

14. Levine, M., *Anat. Rec.*, 1928, v39, 293.

15. Tashjian, A. H., Jr., Levine, L., Munson, P. L., *J. Exp. Med.*, 1964, v119, 467.

16. Berson, S. A., Yalow, R. S., Aurbach, G. D., Potts, J. T., Jr., *Proc. Nat. Acad. Sci.*, 1963, v49, 613.

17. Munger, B. L., Roth, S. I., *J. Cell Biol.*, 1963, v36, 379.

18. Roth, S. I., *Arch. Path.*, 1962, v73, 495.

19. Tremblay, G., Cartier, G. E., *Endocrinology*, 1961, v69, 658.

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Erythropoietic Effect of Testosterone in the Polycythemic Mouse. (29713)

W. FRIED, R. DE GOWIN AND C. W. GURNEY (Introduced by L. O. Jacobson)
Department of Medicine, University of Chicago, and Argonne Cancer Research Hospital, Chicago, Ill.*

Recent clinical application of testosterone to the treatment of various refractory anemias(1-3), has led us to investigate the mechanism by which testosterone effects erythropoiesis. Several investigators have demonstrated increased red blood cell counts in rats after long-term treatment with testosterone. These effects were more striking in animals with hormone levels previously altered by castration or hypophysectomy(4-6), and were at best, very slight in normal animals(7). Most early studies failed to demonstrate significant increases in hemoglobin levels or in total red cell volumes(4,5,8).

Because of the marked decrease in erythropoiesis observed in the polycythemic mouse, this animal has proved valuable in studying the mechanism of erythropoietic stimulants (9,10). In this report, we shall describe the

* Operated by Univ. of Chicago for U. S. Atomic Energy Commission.

effects of testosterone on the rate of Fe^{59} uptake in the polycythemic mouse.

Methods. In all experiments CF No. 1 female mice 6 to 8 weeks of age and weighing 25 to 30 g were used.

Mice were made polycythemic according to the method of De Gowin *et al*(10) by injecting 0.5 ml of an 80% suspension of washed mouse RBC's I.V. on 2 consecutive days. All polycythemic mice had hematocrits between 55% and 65% at termination of the experiment.

The rate of Fe^{59} uptake into newly formed RBC's was determined by injecting 0.5 μc $Fe^{59}Cl$† intravenously at the times specified after injection of the material to be assayed. Three days after injection of Fe^{59}, 0.25 ml of blood was removed by cardiac puncture. The radioactivity was determined by counting in a well type scintillation counter and calculated as percentage of the injected dose of Fe^{59} in the peripheral red cells.

Testosterone was administered subcutaneously in the form of: testosterone propionate in sesame oil‡ in concentration of 25 mg per ml, or testosterone in isotonic aqueous suspension§ in concentration of 25 mg per ml.

Results. Effects of testosterone on polycythemic mice. The following experiment demonstrates that testosterone can prevent the usual maximal transfusion-induced suppression of erythropoiesis in the normal mouse, and can stimulate erythropoiesis in a maximally suppressed animal.

Group I received 10 (Ia) or 20 (Ib) daily 2.5 mg injections of testosterone in oil. Controls (Ic) received 20 daily injections of sesame oil (0.1 ml). Fe^{59} was given on final day of injection. Polycythemia was produced by transfusing 0.5 ml RBC's on days 5 and 6 prior to injection of Fe^{59}.

Group II received 10 daily 2.5 mg injections of testosterone in oil (IIa) or an equivalent volume of sesame oil (IIb). Fe^{59} was given on the final day of injection. Polycythemia was induced by hypertransfusion 10 days prior to the first testosterone injection and again on the day of the first injection.

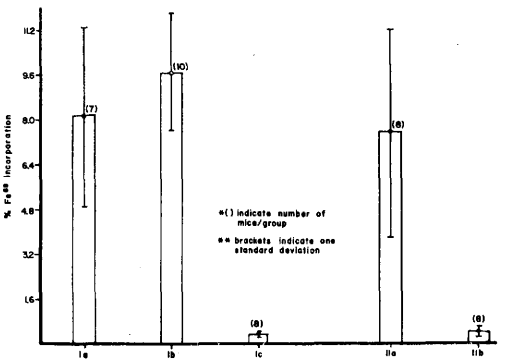

FIG. 1. Effect of testosterone on polycythemic mouse.

Fig. 1 indicates that testosterone given either before or after hypertransfusion will produce a 20- to 30-fold increase in the rate of iron incorporation as compared to controls. Twenty daily testosterone injections did not produce a significantly higher rate of Fe^{59} uptake than did 10 injections.

Duration of action of a single dose of testosterone. In this experiment the magnitude of the erythropoietic response was considered as a function of time following a single dose of 2.5 mg of testosterone. Aqueous and oil suspensions of testosterone were used in an effort to minimize any possible delay due to slow release from the injection site. All mice were injected with 0.5 ml packed RBC's 5 and 6 days prior to injection with Fe^{59}. A single 2.5 mg dose of testosterone was given at the time prior to Fe^{59} recorded on the abscissa in Fig. 2. The response in terms of Fe^{59} incorporation is recorded on the ordinate. The effect of testosterone is first detectable after 48 hours, and reaches its peak by 96 hours, after which maximum activity persists for at least 24 hours. The effect of aqueously suspended testosterone, indicated by the interrupted line in Fig. 2, does not differ from that obtained with testosterone suspended in oil.

Optimum single dose of testosterone. The following experiment was performed to determine the optimum single dose of testosterone. Mice were transfused 5 and 6 days prior to injection of Fe^{59}; a single injection

† Specific activity of the tracer was 36 mc per mg Fe.

‡ Commercially prepared by Schering Co. under trademark Oreton.

§ Commercially prepared by Organon, Inc., under trademark Organon.

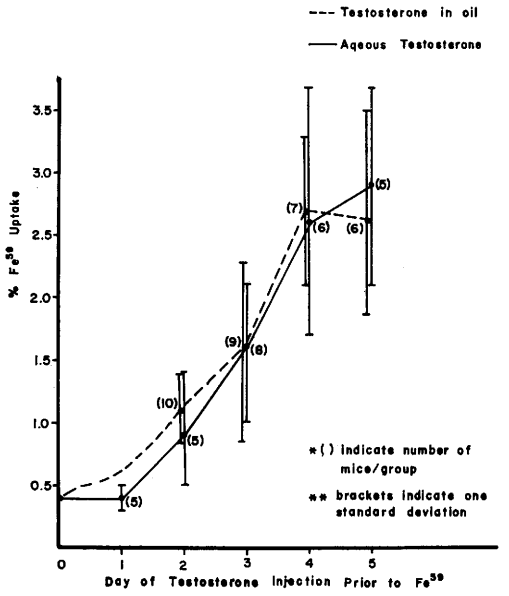

FIG. 2. Time of appearance of erythropoietic response after single dose of testosterone.

of testosterone was given 4 days prior to Fe^{59}. The dose of testosterone is indicated on the abscissa in Fig. 3. The Fe^{59} incorporation is on the ordinate. It is apparent that 2.5 or 5 mg injections of testosterone are not significantly more effective than 1 mg. If, however, less than 1 mg is given, the erythropoietic effect diminishes.

Effect of numerous daily testosterone injections on normal mice. Mice were injected daily for 45 days with 2.5 mg testosterone in 0.1 ml sesame oil or with 0.1 ml sesame oil. Several of the peripheral blood parameters as well as the rate of Fe^{59} incorporation were determined. The results are given in Table I. It is apparent that testosterone produces no detectable effect on white blood cell level or hemoglobin production and does not alter the 16 hour incorporation of Fe^{59} by newly formed erythrocytes in the normal animal.

Discussion. The experiments reported here indicate that testosterone, when given in pharmacological doses, can quite significantly increase the rate of erythropoiesis in polycythemic mice as measured by radioiron incorporation into newly formed red cells. It should, however, be emphasized that we were not able to restore erythropoiesis to anything approaching the normal rate even by repeated massive doses of testosterone, and that testosterone did not affect significantly any of the parameters of erythropoietic activity in normal mice.

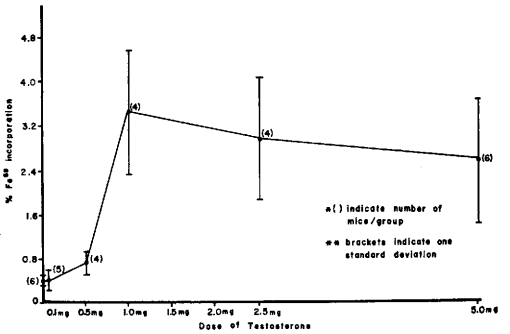

FIG. 3. Relation of dose of testosterone to Fe^{59} uptake.

The ability to stimulate erythropoiesis in the polycythemic mouse suggests that testosterone is able to lead to differentiation of a stem cell into the erythroid series, either directly or indirectly. There are a number of possible mechanisms of action to be considered. First, testosterone might increase the basal metabolic rate thereby increasing the O_2 demand sufficiently to exceed the oxygen delivery to tissues which prior to testosterone administration exceeded oxygen requirements. In such a case, endogenous production of erythropoietin would be expected. The only demonstration to date of significant increases in the BMR following testosterone has been in eunochoid humans treated with methyl testosterone(6). It is of interest that testosterone propionate, the preparation used in our experiments, had no effect on the BMR of

TABLE I. Effect of Long Range Testosterone Injection on Normal Mice.

	Hgb (13)*	Hct (13)	Retic (7)	Fe^{59} uptake (7)	WBC (7)
Testosterone	16.7 ± .9	52.2 ± 2.5	3.5 ± .5	32.6 ± 1.6	9161 ± 1900
Control	15.9 ± .3	50.3 ± 1.2	3.5 ± .3	27.8 ± 9.8	9580 ± 2400

* No. of mice/group indicated in parentheses.

eunochoid males(6). It has also been demonstrated that testosterone increases mitotic activity in the female thyroid gland(11). The effects of androgens on the basal metabolic rate of polycythemic mice are currently being investigated.

Testosterone has been shown to increase the organ weights of several non-endocrine organs, most notably the kidney(12). The epithelial cell cytoplasm is most affected, but the mechanism responsible for this change is not known. Since much evidence points to the kidney as a site of erythropoietin production(13,14), it is conceivable that erythropoietin production by the kidney might also be increased by direct action upon the renal parenchymal cell.

Testosterone might potentiate the effect of small amounts of endogenous erythropoietin elaborated in the polycythemic mouse if the small amount of iron incorporation in the unstimulated polycythemic mouse is a consequence of the continued elaboration of very slight amounts of erythropoietin. Such an effect could be a direct one, or might alternatively be indirect, by antagonization of the already demonstrated inhibitory action of estrogens on erythropoiesis(15).

Finally, testosterone might have a direct erythropoietin-like action upon the stem cells, inducing differentiation into the erythrocytic compartment. These various possibilities are currently under study.

Summary. Testosterone has been demonstrated to stimulate erythropoiesis in the polycythemic mouse. A response can be observed following a single injection of 0.5 mg of testosterone. One mg is as effective as larger single doses. A greater erythropoietic response was observed with 10 daily injections. Possible mechanisms of action of testosterone on erythropoiesis are discussed.

1. Kennedy, B. J., Gilbertson, A. S., *New Engl. J. Med.,* 1957, v256, 719.

2. Gardner, F. H., Pringle, J. C., Jr., *ibid.,* 1961, v264, 103.

3. Shahidi, U. T., Diamond, L. K., *ibid.,* 1961, v264, 953.

4. Vollmer, E. P., Gordon, A. S., Chaupper, H. A., *Endocrinology,* 1942, v31, 619.

5. Crafts, R. C., *ibid.,* 1946, v39, 401.

6. McCullough, E. P., Jones, R., *J. Clin. Endocrinol.,* 1942, v2, 243.

7. Vollmer, E. P., Gordon, A. S., *Endocrinology,* 1941, v29, 828.

8. Van Dyke, D. C., Contopolous, A. N., Williams, B. S., Simpson, M. E., Lawrence, J. H., Evans, H. M., *Acta Haematol.,* 1954, v11, 203.

9. Jacobson, L. O., Goldwasser, E., Gurney, C. W., *Ciba Symposium on Haemopoiesis,* London, J. and E. Churchill Ltd., 1960, p423.

10. De Gowin, R. L., Hofstra, D., Gurney, C. W., PROC. SOC. EXP. BIOL. AND MED., 1962, v11, 48.

11. Nathanson, I. T., Brues, A. M., Rawson, R. W., *ibid.,* 1940, v43, 737.

12. Selye, H., *J. Urology,* 1939, v42, 637.

13. Jacobson, L. O., Goldwasser, E., Fried, W., Plzak, L., *Trans. Assn. Am. Physicians,* 1957, v70, 305.

14. Naets, J. P., *Nature,* 1958, v181, 1134.

15. Dukes, P. P., Goldwasser, E., *Endocrinology,* 1961, v69, 21.

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Specificity of Immunofluorescence in Experimental and Human Renal Diseases.* (29714)

GERHARD TRESER, INGE SAGEL AND KURT LANGE

Renal Service and Laboratory, Departments of Medicine and Pediatrics, New York Medical College, New York City

Immunofluorescent staining for gamma globulin and complement and its components has come into wide use for the localization of immune processes in tissues and especially in the kidney. The objection has been made, that positive immune staining for gamma globulin and even for complement and its components may be due to a mere transuda-

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