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Inhibition of Erythropoietic Stimulation by Testosterone in Polycythemic Mice Receiving Anti-Erythropoietin.* (31146)

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Erythropoiesis in the normal animal can be stimulated by erythropoietin and various other hormones. In the mouse made polycythemic by transfusion, erythropoiesis is virtually abolished. A single injection of erythropoietin into such mice initiates an orderly wave of erythropoiesis in the hematopoietic tissue which can be detected easily 3 days later either by measurement of the blood reticulocyte level or the incorporation of Fe^{59} into the red blood cells(1,2). In the polycythemic mouse, in contrast to the normal mouse, the injection of ACTH, thyroxin(3), triiodothyronine, or testosterone(4,5) does not initiate a wave of erythropoiesis comparable to that produced by erythropoietin. The injection of testosterone, however, is followed by a wave of erythropoietic activity in the polycythemic mouse observable several days later than that caused by injection of erythropoietin(6). It has been further demonstrated by several groups of investigators(7-10) that plasma taken from several animal species after injections of testosterone stimulates erythropoiesis in the polycythemic mouse. This stimulation has been generally assumed to be due to the presence of erythropoietin in the plasma taken from the testosterone injected animals; however, Fried and Gurney (9) have stated that it is equally "possible that this erythropoietic activity is due to a metabolic product of testosterone which is slow to be formed, reaches high concentrations in the plasma in 4 days, and is active in much smaller titers than testosterone alone."

The present paper demonstrates that the stimulation of erythropoiesis in polycythemic mice following testosterone does not occur in the presence of an immune serum against erythropoietin.

Methods. Eight- to 10-week-old C_3H female mice weighing about 25 g were transfused by intraperitoneal injection of isologous red blood cells on 2 consecutive days. The red blood cells were washed 3 times with saline before use.

On the fifth and sixth day after the last transfusion 25 mg of testosterone propionate in 0.1 ml of sesame oil was injected subcutaneously. Control mice received similar injections of sesame oil alone. Ninety-six or 56 hours after the second testosterone injection, 0.5 μc of Fe^{59} as iron citrate (specific activity approximately 10 $\mu c/\mu g$) was injected intravenously and 72 hours later the incorporation of the injected Fe^{59} in the circulating blood was determined. The blood sample was washed once with saline before counting. The blood volume of the polycythemic mice was assumed to be 7% of the body weight. Mice having hematocrits below 55% at the time of sampling were discarded.

A group of mice receiving testosterone injections also received intraperitoneal injections of 0.1 ml of immune serum capable of neutralizing the erythropoietic activity of erythropoietin. The immune serum was injected daily commencing on the day of the second testosterone injection and continuing until the day before the Fe^{59} injection. The preparation and properties of the immune

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TABLE I

	No. of animals	72 hr Fe^{59} uptake
Sesame oil	7	.07 ± .01*
Testosterone propionate in sesame oil	11	1.64 ± .30
Testosterone propionate in sesame oil plus anti-erythropoietin immune serum	13	.03 ± .01

* Standard error of mean.

serum have been previously described(3).

Results. With an interval of 96 hours between the last testosterone injection and the Fe^{59} injection, a small but highly significant stimulation of erythropoiesis occurred ($P<0.001$). This stimulation was completely abolished by the injection of immune serum. The results are shown in Table I. Stimulation by testosterone did not occur when the interval between the last injection of testosterone and Fe^{59} was only 56 hours; the value of 0.09 ± 0.01 was indistinguishable from the control value of 0.10 ± 0.02.

Discussion. Erythropoiesis is stimulated in polycythemic mice by the injection of testosterone. This stimulation can be detected if the interval between the injection of the hormone and the injection of Fe^{59} is at least 96 hours. The response seen at this time was not, however, as great as that observed by Fried *et al*(6) for the same dose of testosterone. If the interval between the injection of the hormone and Fe^{59} is decreased to 56 hours, the time interval frequently used in the assay for erythropoietin, a significant stimulation of erythropoiesis, as measured by Fe^{59} uptake in the peripheral blood, does not occur. These findings are similar to those of Naets and Wittek(5) and Fried *et al*(6), but a direct comparison is not possible because of some differences in experimental detail, such as length of time after the production of polycythemia before the testosterone was injected and whether a 72- or 24-hour Fe^{59} incorporation was made.

The finding that the injection of immune serum capable of neutralizing erythropoietin in polycythemic mice receiving testosterone completely prevents the development of an erythropoietic response, suggests that the stimulus for erythropoiesis following testosterone administration is due to an increased erythropoietin production. It would follow that the increased erythropoietic activity in the blood of animals injected with testosterone found by Mirand *et al*(10) and Fried and Gurney(9) is due to the presence of erythropoietin and not some metabolic product of the injected testosterone. There is no evidence at the present time indicating that any hormone or agent stimulates erythropoiesis without the mediation of erythropoietin. The mechanism of testosterone stimulation of erythropoietin production in polycythemic mice remains unknown.

Summary. The injection of testosterone into polycythemic mice produces a small but significant stimulation of erythropoiesis, as measured by Fe^{59} uptake into the peripheral blood, if the interval between the injection of the hormone and Fe^{59} is 96 hours. This stimulation of erythropoiesis is completely abolished by the injection of immune serum against the hormone erythropoietin.

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