of feeding deterrent properties and insecticidal action of these compounds is suggested.

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Combined Effect of Cobalt and Testosterone on Erythropoiesis. (30558)

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It has long been known that cobalt increases erythropoiesis in man and experimental animals(1,2,3,4), and that it achieves its erythropoietic effect by initiating erythropoietin formation(5,6). The clinical effectiveness of testosterone therapy in various refractory anemias has led to investigations which suggest that one method by which this hormone promotes erythropoiesis is also by stimulating the elaboration of erythropoietin(7). In this report we shall describe the combined effects of cobalt and testosterone on erythropoiesis in transfusion-induced polycythemic mice, and on plasma erythropoietin levels in normal mice.

Methods. All experiments were carried out on CF No. 1 female mice, 6 to 10 weeks of age and weighing 20-30 g.

Polycythemia was induced according to the method of DeGowin *et al*(8) by intravenous injection of an 80% suspension of washed mouse RBC's on 2 consecutive days. All polycythemic mice had hematocrits between 55-75% at the end of the experiment.

Testosterone was administered subcutaneously in the form of testosterone propionate in sesame oil in a concentration of 25 mg/ml. Cobalt was administered subcutaneously in the form of cobaltous chloride in normal saline from stock solution in a concentration of 25 μmoles/ml.

The rate of Fe^{59} uptake into newly formed RBC's was determined as follows: One-half (0.5) μc of Fe^{59} chloride was given intravenously 60 hours after cobalt injection. Three days after injection of Fe^{59}, the animals were killed and 0.25 ml of blood was removed by cardiac puncture. Radioactivity was measured in a well-type scintillation counter, and the percentage of the injected dose of Fe^{59} appearing in the peripheral red cells was calculated from these counts(8).

Plasma erythropoietin titers were determined using the polycythemic mouse assay described by DeGowin *et al*(8).

Results. Erythropoietic effect of testosterone and varied doses of cobalt: The following experiment was performed to determine the combined effects of testosterone and various doses of cobalt on erythropoiesis in polycythemic mice. Fig. 1 illustrates the results.

On day 1 three groups of mice received single injections of 2.5 mg of testosterone in oil, while the appropriate controls received single injections of 0.1 ml of sesame oil. Sixty hours later, on day 3, single injections of either 1.0 or 2.5 μmoles of cobalt or nor-

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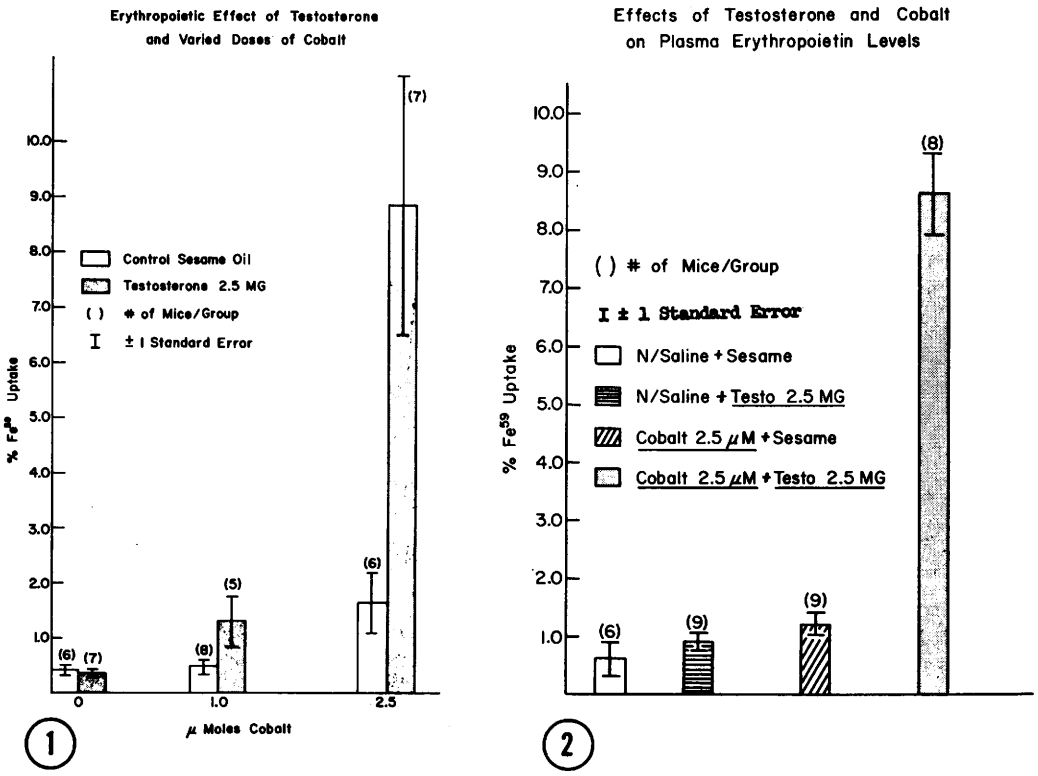

FIG. 1 and 2

mal saline were given to one group each of experimental and control animals, as indicated in Fig. 1. Fe^{59} was given 60 hours after the injection of saline or cobalt. Polycythemia was induced by transfusing 0.7 ml RBC's on 2 consecutive days. The first injection was given on the same day as testosterone or sesame oil administration.

Fig. 1 shows that 2.5 μmoles of cobalt given alone will increase the rate of iron incorporation to 4 times that observed in the saline controls. Two and five-tenths milligrams of testosterone, given 60 hours before either 1.0 or 2.5 μmoles of cobalt, will increase the rate of iron incorporation to 3 and 5 times, respectively, the rate observed in the corresponding sesame oil-cobalt controls.

Erythropoietic effect of cobalt and testosterone given at varied time intervals: In this experiment, the magnitude of the erythropoietic response to cobalt and testosterone was considered as a function of the time elapsing between their injection into polycythemic mice. Table I illustrates the results.

Mice in groups IA and IB to VA and VB received single injections of 2.5 mg of testosterone on days 1 to 5. Control groups IX and IY to VX and VY received single injections of 0.1 ml of sesame oil on the same days. Subgroups IA to VA and IX to VX received single injections of 2.5 μmoles of cobalt in normal saline on day 5, while subgroups IB to VB and IY to VY received single injections of 0.2 ml of normal saline on this day. Fe^{59} was given 60 hours later on day 8. Polycythemia was induced by transfusion with 0.55 ml RBC's 5 and 6 days before injections of Fe^{59}.

Table I shows that when testosterone is given 0 to 4 days before cobalt, it exerts a synergistic effect on the rate of iron incorporation as compared to the control rate. Peak synergism is observed when testosterone is given 2 days or 3 days before cobalt.

Effect of testosterone and cobalt on plasma erythropoietin levels: The following experiment was performed to determine the combined effect of cobalt and testosterone on

TABLE I. Erythropoietic Effect of Cobalt and Testosterone Given at Varied Time Intervals in Transfusion-Induced Polycythemic CF No. 1 Female Mice.

Group		No. of mice per group			% Fe^{59} uptake (S.E.)
I.	A.	5	Testosterone, 2.5 mg (−4 days)	Cobalt, 2.5 μM	1.6 ± .4
	B.	8	" " "	Normal saline	.5 ± .1
	X.	7	Sesame oil	Cobalt, 2.5 μM	.4 ± .1
	Y.	7	" "	Normal saline	.3 ± .1
II.	A.	8	Testosterone, 2.5 mg (−3 days)	Cobalt, 2.5 μM	6.8 ±1.4
	B.	7	" " "	Normal saline	.9 ± .3
	X.	7	Sesame oil	Cobalt, 2.5 μM	.7 ± .2
	Y.	7	" "	Normal saline	.4 ± .0
III.	A.	7	Testosterone, 2.5 mg (−2 days)	Cobalt, 2.5 μM	7.0 ±2.0
	B.	5	" " "	Normal saline	1.0 ± .5
	X.	7	Sesame oil	Cobalt, 2.5 μM	.5 ± .1
	Y.	5	" "	Normal saline	.4 ± .1
IV.	A.	7	Testosterone, 2.5 mg (−1 day)	Cobalt, 2.5 μM	4.5 ±1.7
	B.	6	" " "	Normal saline	1.2 ± .4
	X.	7	Sesame oil	Cobalt, 2.5 μM	.7 ± .2
	Y.	7	" "	Normal saline	.5 ± .0
V.	A.	7	Testosterone, 2.5 mg (0)	Cobalt, 2.5 μM	3.2 ±1.0
	B.	7	" " "	Normal saline	.5 ± .1
	X.	7	Sesame oil	Cobalt, 2.5 μM	.6 ± .1
	Y.	6	" "	Normal saline	.3 ± .1

( ) Days prior to cobalt injections.

erythropoietin levels in the plasma of normal mice. The results are recorded in Fig. 2.

Two groups of mice received single injections of 2.5 mg of testosterone in oil, and their appropriate controls received single injections of 0.1 ml of sesame oil on day 1. On day 3, single injections of 2.5 μmoles of cobalt or normal saline were given to one of the 2 groups of experimental animals and to one of the two groups of controls, as indicated in Fig. 2. Twelve hours later, on day 4, all the animals were killed, their plasma was harvested and assayed for erythropoietin activity.

Fig. 2 shows that plasma from normal mice receiving either 2.5 mg of testosterone or 2.5 μmoles of cobalt alone will produce respectively, 1½- and 2-fold increments in the rate of iron incorporation compared to plasma from mice receiving saline and sesame oil. Plasma from mice receiving both testosterone and cobalt produces a 14-fold increase in the rate of iron incorporation over control values, a figure that is substantially greater than would be expected from the summed effects of testosterone or cobalt given separately.

Discussion. Cobaltous chloride, given orally or parenterally for a period of weeks, induces and maintains a polycythemia in man and experimental animals(2,3,4). It has been shown that this erythropoietic effect is exerted through increased production of erythropoietin(5,6). Testosterone increases erythropoiesis in various experimental animals(9,10,11) and has been used effectively in some previously refractory anemias(12,13, 14). Evidence has been presented suggesting that one way in which testosterone effects erythropoiesis is also by stimulating the elaboration of erythropoietin(7).

In the experiments reported here, 2.5 mg of testosterone produced a minimal or no erythropoietic effect. The lack of response to testosterone alone in the first experiment is probably attributable to the fact that larger volumes of blood (and hence, greater red cell masses) were used than in subsequent experiments. It has been demonstrated previously that excessive plethora dampens

the erythropoietic effect of testosterone(15). In the first experiment it was noted that 2.5 μmoles of cobalt alone produced an increased response. This dose was therefore used in the succeeding experiments.

The first and second experiments show that cobalt and testosterone in combination act synergistically in increasing the rate of iron incorporation into the red cells of polycythemic mice. This finding was interpreted as demonstrating that cobalt and testosterone in combination potentiate erythropoiesis. That peak synergism occurs when testosterone is given 2 days or 3 days before cobalt is consistent with previous findings that peak erythropoietin formation is reached 12 hours after injection of cobalt and 3 days after injection of testosterone(6,7).

The third experiment indicates that cobalt and testosterone in combination act synergistically to increase erythropoietin levels in the plasma of normal mice. It is of interest to note that the increase in erythropoietin levels observed in Exp. 3 is of the same order of magnitude as the increase in erythropoiesis measured in Exp. 1 and 2. The third experiment reconfirms the hypothesis that both agents stimulate erythropoiesis *via* increased production of erythropoietin. However, the synergistic interaction demonstrated in these experiments suggests that the fundamental mechanisms by which cobalt and testosterone stimulate elaboration of erythropoietin are quite different. Investigation of these mechanisms awaits the elucidation of the biosynthetic pathway of erythropoietin action.

The clinical use of cobalt in cases of refractory anemias is limited by its toxic effects. The use of testosterone is also limited by its side effects, especially in female patients. These experiments suggest that greater clinical effectiveness, with fewer side effects, might be attained by combining lower doses of both agents, than can be obtained with either medication alone.

Summary. It has been demonstrated that a combination of cobalt and testosterone serves to potentiate erythropoiesis in the polycythemic mouse, and acts synergistically to increase erythropoietin levels in the plasma of the normal mouse. When these 2 substances are used together, the erythropoietic response in plethoric animals and the amount of erythropoietin elaborated in normal animals are substantially greater than the sum of the responses produced by each agent separately.

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