The Erythropoietic Effects of Anabolic Steroids.* (32268)

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Recent clinical observations suggest that certain androgenic steroids with lesser virilizing effects than testosterone are more effective than the latter in the treatment of bone marrow insufficiency. Oxymetholone (1,2), metholone, methenolone and androstanazole(3) have been found capable of inducing a remission in about 50% of adult cases with aplastic anemia, whereas poor results have been obtained with testosterone (4,5). In addition, testosterone has been ineffective in the treatment of congenital pure red cell anemia (Blackfan-Diamond syndrome)(6), a condition in which oxymetholone has been found to be ocassionally useful. Data supporting the suggestion that certain androgenic steroids have greater erythropoietic activity than testosterone are presented here.

Material and methods. Three types of experiments were performed in inbred male and female Wistar rats and female Swiss mice.

Experiments A (Table I). Normal female Wistar rats were given 5 mg of iron-dextran intramuscularly, and divided into 4 groups. One group served as controls and received 6 subcutaneous injections of 0.4 ml of sesame oil at weekly intervals; the second, third and fourth groups received 40 mg per week subcutaneously for 6 weeks of testosterone cyclopentylpropionate, metholone propionate or methenolone enanthate, respectively. Two days after the last injection, the red cell mass was measured following intravenous administration of Fe^{59} labeled erythrocytes.

Experiments B (Table II). Normal male Wistar rats received daily 5 mg subcutaneous injections of either testosterone propionate, oxymetholone or methenolone acetate daily for 4 days. The mean body weights of the 4 groups of rats employed ranged from 309 to 329 g. Accordingly the average dose was approximately 15 mg per kg. Twenty-four hours after the last injection, they were given I μc Fe^{59} intravenoulsy and, 18 hours later, the hematocrit and radioactivity in the red cells were determined on blood obtained by heart puncture. Fe^{59} incorporation was estimated assuming a blood volume of 5 ml per 100 g. Animals were kept in a fasting state throughout the experiment.

Experiments C (Table III). Normal female Swiss mice weighing 19 to 24 g were placed on a protein-free diet for 10 days. On days 2 and 3, the animals received subcutaneous injections of 2.5 mg of either testosterone propionate, oxymetholone in aqueous solution, metholone in aqueous or in oil solution, or methenolone acetate. On day 7, 1 μc of Fe^{59} was given intraperitoneally; on day 10 blood was removed by heart puncture and the hematocrit (micro) and radioactivity present in the red cells were determined. The blood volume was assumed to be 5 ml/100 g. The protein free diet used was prepared in our laboratory and consisted of the following ingredients:

	%
Starch	69.1
Dextrose	17.6
Cellulose	4.0
Corn oil	5.0
Salt mixture(7)	4.0
Vitamin mixture	0.3

Results and discussion. The results of the present study, shown in Tables I to III, confirm that low virilizing hormones, such as metholone, methenolone and oxymetholone, stimulate erythropoiesis. They are capable of inducing a polycythemic response in the normal animal (Table I) and of increasing Fe^{59} incorporation in red cells of rats and mice in which erythropoiesis had been depressed by fasting and protein-free feeding respectively (Tables II and III). The erythropoietic effect

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TABLE I. The Effects of Testosterone and Other Anabolic Hormones on Hematocrit and Total Circulating Red Cell Mass in Normal Female Rats.

Drug	No. of animals	Mean weights Initial	Final	Ht (%)	RCM (ml/100 g)	Increase RCM (%)
Controls	15	230	271	43.8	2.586 ± .065	—
Testosterone	14	274	289	48.4	2.889 ± .070	11.7
Methenolone enanthate	14	252	301	48.1	2.941 ± .062	13.7
Metholone	11	268	296	49.6	3.234 ± .210	25.0

± Standard error

Analysis of variance by Duncan's F test modified by Kramer(12)

Groups	Significant at .05 level	.01 level
Controls *vs* testosterone	yes	no
Controls *vs* methenolone	yes	no
Controls *vs* metholone	yes	yes
Testosterone *vs* methenolone	no	no
Testosterone *vs* metholone	yes	no
Methenolone *vs* metholone	no	no

is seen in both female and male animals and, in all experiments, the results in the animals injected with the anabolics differed significantly from the controls.

The results also support our hypothesis that some anabolic hormones have a higher erythropoietic activity than testosterone. Response to testosterone was constantly smaller than that to anabolics given at equal doses: a greater increase in red cell mass and/or in incorporation of iron was always obtained with oxymetholone and metholone; methenolone, the only drug tested in the 3 types of experiments, failed to show its supremacy over testosterone only in the one using male animals (Table II). In some experiments oxymetholone (Table II) and metholone (Tables I and III) had an effect significantly greater than that of testosterone.

TABLE II. Influence of Testosterone and Anabolic Hormones on Fe^{59} Incorporation in RBC of Fasted Rats.

Drug	No. of animals	Fe^{59} (%)	Increase in % over control
Controls	8	4.20 ± .17	—
Testosterone propionate	7	5.64 ± .29	34
Methenolone acetate	7	5.20 ± .48	23
Oxymetholone	7	8.62 ± .61	105

± Standard error

Analysis of variance by Duncan's F test modified by Kramer(12)

Group	Significant at .05 level	.01 level
Controls *vs* testosterone	yes	yes
Controls *vs* methenolone	yes	no
Controls *vs* oxymetholone	yes	yes
Testosterone *vs* methenolone	no	no
Testosterone *vs* oxymetholone	yes	yes
Methenolone *vs* oxymetholone	yes	yes

TABLE III. Influence of Testosterone and Anabolic Hormones on Fe^{59} Incorporation in RBC of Female Swiss Mice on a Protein-Free Diet.

Drug	No. of animals	Fe^{59} (%)	Increase in times control value
Controls	23	.178 ± .023	—
Testosterone propionate	7	2.999 ± 1.264	16.8
Oxymetholone (aqueous)	7	4.065 ± 2.046	22.8
Metholone (oil)	7	5.057 ± 1.399	28.4
Methenolone acetate	13	5.747 ± .846	32.2
Metholone (aqueous)	14	6.907 ± 1.535	38.8

± Standard error

Analysis of variance by Duncan's F test modified by Kramer(12)

Group	Significant at .05 level	.01 level
Controls *vs* testosterone	no	no
Controls *vs* oxymetholone	yes	no
Controls *vs* metholone (oil)	yes	yes
Controls *vs* methenolone	yes	yes
Controls *vs* metholone (aq.)	yes	yes
Testosterone *vs* metholone (aq.)	yes	no

All other group differences were non-significant.

Two isolated observations are in agreement with our point of view of a greater erythropoietic activity of anabolics as compared with testosterone. Gurney and Fried(8) observed a moderately greater Fe^{59} incorporation with nandrolone than with testosterone in mice, and Meineke and Crafts(9) reported that norethandrolone but not testosterone stimulates the Fe^{59} incorporation in hypophysectomized rats.

On the basis of these studies it is apparent that some androgenic steroids have more erythropoietic activity than testosterone. Our data also suggest that the erythropoietic effect of androgens may be a third type of activity of these steroids, independent of their virilizing and myotrophic activities. This possibility is suggested by the observation that compounds having less than one fourth of the virilizing effect of testosterone, like methenolone(10), are more active erythropoietically than testosterone. On the other hand, metholone which has lesser anabolic effect than testosterone, oxymetholone and methenolone (10,11) appears to be the more potent erythropoietic agent of all the 4 steroids tested.

Summary. The erythropoietic effects of testosterone, oxymetholone, methenolone and metholone were compared in normal and fasted rats as well as mice on a protein free diet. The results of these androgenic-anabolic steroid studies are as follows: 1) methenolone and metholone produced a more marked erythropoietic response than testosterone when tested in normal female rats; 2) Fe^{59} incorporation in RBC of male fasted rats was higher with oxymetholone than with testosterone; and 3) testosterone was markedly less effective than oxymetholone, methenolone and metholone in increasing Fe^{59} incorporation in RBC of mice on a protein free diet. These results suggest that the erythropoietic activities of the above steroids are independent of their virilizing and anabolic effects, since testosterone is the most virilizing of the four agents and metholone, which induced the greatest erythropoietic effects, has the least anabolic action.

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Juxtaglomerular Cells in CCl_4-Treated Experimental Hypertension.* (32269)

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Renal and endocrine (DCA) hypertensive rats have been rendered normotensive by CCl_4 injections(1,2). The possibility that alterations in renin secretion were taking

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