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Effect of Androgens on the Red Cell 2, 3 Diphosphoglycerate Hemoglobin Oxygen Affinity and Red Cell Mass in Mammals[1] (38400)

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The erythropoietic capacity of androgenic steroids is now well recognized. Classically, the most accepted mechanism of action has been via the stimulation of erythropoietin (EP) production (1). Recently, evidence has been brought forth to support an additional effect of androgenic steroids, *i.e.*, to increase stem cell responsiveness to EP (2–7).

The following report is a study of the effect of an androgenic steroid on the red blood cell (RBC) volume in the squirrel monkeys, the RBC 2, 3 diphosphoglycerate (2, 3 DPG) and the position of the oxygen hemoglobin equilibrium curve [the partial pressure of oxygen at which the hemoglobin is 50% oxygenated (P_{50})] in the squirrel monkey and in mice. The possible relationship of the presented data is discussed.

Materials and Methods. A. *Animals*. Sixteen female squirrel monkeys (*Saimiri sciureus*), 8–10 mo old with an average weight of 655 g, were individually caged and randomized equally into treated and control groups. The animals were given Roakland primate diet (Tekland, Inc., Monmouth, IL), supplemented with fruits and water, *ad libitum*. Three weeks before the studies were begun, all monkeys received 5 mg of iron-dextran (Imferon, Lakeside Laboratories Inc., Milwaukee, WI) im to ensure adequate iron stores. The cage areas were maintained on a 12-hr on, 12-hr off light cycle and at an average temperature of 25°. Sodium pentothal (2 mg ip) was used to anesthetize the monkey while blood-letting. The femoral vein was always used to obtain blood or for iv administration. Complete blood counts were done using standard techniques.

The treated group was given 19-nortestosterone decanoate (19-ND) (obtained from Dr. H. Strade, Organon, West Orange, NJ) dissolved in propylene glycol (PG) (Fisher Scientific Co., Fairlawn, NJ) at 50 mg/kg body wt, im; control group received the diluent PG only. The monkeys were given five weekly injections of the steroid or the diluent.

Blood volume determinations. A modification of the method of Read (8) was used as previously described (9). The RBC 2, 3 DPG and P_{50} were measured as previously described (10).

B. Mice. Swiss Webster white virgin female mice (West Jersey Biological Company, Wenonah, NJ), randomized in groups of ten, were used and supplied with Purina Rodent Chow and water, *ad libitum*. The mice received 1–2 mg of iron as iron-dextran solution (Imferon, Lakeside Laboratories, Inc., Milwaukee, WI) intraperitoneal to ensure adequate iron stores.

Post hypoxic plethora. Erythropoietic activity in plasma of mice at ambient pressure was determined one hour after the administration of a single injection of 2.5 mg of the androgen and compared to the activity 3 days later as previously described (11).

Results. Radioiron incorporation into RBC of post hypoxic polycythemic mice, following administration of plasma obtained from mice maintained in normal conditions and injected with 19-ND 1 hr or 3 days before collection of plasma, were 1.4 ± 0.151 and 22.3 ± 3.86 (M ± SEM) indicating that 19-ND is a potent stimulant of EP production.

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TABLE I. Results of Total RCM in Response to PG or 19-ND Administration.

	Total RCM in ml/animal[a]
Control groups	
Before PG administration	14.1 ± 0.5
After PG administration	13.0 ± 0.4
Treated groups	
Before 19-ND administration	13.7 ± 0.4
After 19-ND administration	16.2 ± 0.5

[a] Mean ± SEM.

The results of the total red cell mass (RCM) data are given in Table I, clearly indicating an average increase of 18% ($P < 0.05$) following androgen therapy. There was no appreciable change in the RCM in animals receiving PG.

The RBC 2,3 DPG and the P_{50} data are given in Table II, clearly indicating a significantly increased level of RBC 2, 3 DPG and a "right shift" of the P_{50} following androgen administration, without an appreciable change in these values in the PG treated control group. The correlation coefficient (R) for the RBC 2, 3 DPG and the P_{50} was 0.828.

Discussion. Although these observations are clear in demonstrating an increase in RCM and RBC 2, 3 DPG and a "right shift" of the P_{50}, the common denominator for these data is not fully explained.

The mechanism by which androgenic steroids stimulate erythropoiesis has been studied extensively. Their capacity to stimulate EP production has been documented (1, 12). It has also been suggested that, in addition, androgens are capable of augmenting stem cell responsiveness to EP (4, 7, 13). Some data suggest that the latter effect is related to the capacity of androgens to trigger resting stem cells into cycle (2, 3). Thus, one possible explanation for the current observation is that the androgenic compound released a population of young red cells to the circulation (as shown by the increased RCM). The younger mean red cell age may explain a modest increase in RBC 2, 3 DPG (14).

The mechanism by which EP production is stimulated is not clear. The studies reported herein clearly demonstrate an increase of 2, 4 and 1,3 m/ml in RBC 2,3 DPG of the monkey and mice, and a "right shift" of 4.4 and 2 mm Hg in the P_{50} of the monkey and the mice accordingly. A direct effect of the androgens on the red cell metabolism resulting in increase in RBC 2,3 DPG has been reported (15). The observed increase in EP level and RCM as reported here following androgen administration mitigate against this concept since we expect a primary rise in RBC 2,3 DPG to facilitate release of the oxygen to the tissue, and therefore, be associated with a diminution of EP production and no increase in RCM. It is of interest that similar increase in RBC 2,3 DPG and a "right shift" of the P_{50} has been reported following thyroid administration (16) presumably related to an increase in the tissue metabolic rate induced by the

TABLE II. Red Blood Cell 2,3 DPG and P_{50} in Response to PG or 19-ND Administration.[a]

	Monkeys		Mice	
	RBC 2,3 DPG[b]	P_{50}[c]	RBC 2,3 DPG[b]	P_{50}[c]
Control group				
Before PG administration	5.9 ± 0.5	35.4 ± 0.4		
After PG administration	5.8 ± 0.5	35 ± 0.4		
P				
19-ND group				
Before 19-ND administration	5.9 ± 0.4	35.1 ± 0.3	8.9 ± 0.1	40.5 ± 0.25
After 19-ND administration	8.3 ± 0.5	39.5 ± 0.3	10.3 ± 0.2	42.5 ± 0.25
P	< 0.005	< 0.005	< 0.05	< 0.05

[a] Expressed as Mean ± SEM.
[b] In μM/ml RBC.
[c] In mmHg at pH 7.4.

hormone. It is plausible that alterations in tissue metabolic rate are related to androgenic administration.

Summary. The effect of androgens on the RCM in the squirrel monkey and the RBC 2,3 DPG and hemoglobin oxygen affinity in the squirrel monkey and in mice was studied. It was found that administration of 19-ND resulted in an increase in RCM, and elevation of the RBC 2,3 DPG and a "right shift" of the oxygen hemoglobin equilibrium curve. The possible relationship of these phenomena is discussed. It is suggested that the increase in RBC 2,3 DPG is not a result of a direct effect of the androgen on the metabolism of the RBC.

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