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Haematological responses of the African giant rat (*Cricetomis gambianus*) to castration and androgen replacement

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ABSTRACT

Six African giant rats (AGR) (*Cricetomis gambianus*) were castrated and two weeks later were given androgen (depo-testosterone) replacement. The effects of these were studied on the red blood cell (RBC) count, packed cell volume (PCV), haemoglobin (Hb) and erythrocyte fragility and white blood cell (WBC) of these animals. Castration resulted in reduced values of RBC, Hb, and PCV, but did not affect the WBC count. Castration also affected the integrity of the RBC through its osmotic fragility.

Key words: haematology, castration, androgen replacement, African giant rat, *Cricetomis gambianus*

Introduction

It has been shown repeatedly that adult men have higher values than women for red blood cell (RBC) count, haemoglobin (Hb) concentration and packed cell volume (PCV) (WILIAMSON, 1916; HAWKINS et al., 1954). These differences are not solely due to iron deficiency anaemia, pregnancy

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or menstrual blood loss (VAHLQUIST, 1950), but may take their root from the suggestion that erythropoiesis and elaboration of sex hormones are intimately related (HAWKINS et. al., 1954).

This phenomenon has also been reported in many animal species. For instance, removal of many endocrine glands induced anaemia which disappears following replacement of the lacking hormone (ROOT, 1954). Furthermore, castration in male animals reduced haemoglobin and red blood cell count, but increased these values in females of the same species (STEINGLASS et al., 1941). In rats, recovery from haemorrhage is hastened by testosterone and depressed by oestrogen. DE BIASS (1951) has suggested that testosterone directly stimulates bone marrow erythropoiesis, while thyroid hormones are important for the transfer of erythroid cells into the peripheral circulation. Clinically, it has been shown that the anaemia in the hypogonadal male is extremely responsive to testosterone (McCULLAGH and JONES, 1941).

Androgens have also been administered to patients with myxedema and hypopituitarism to correct secondary anaemia in these disorders (GLASS, 1945; WATKINSON et al., 1947).

The literature contains only very few studies on the African giant rat, especially its biology and physiology (AJAYI, 1974; OLOWOOKORUN, 1974; DUROTOYE and OKE, 1990).

The objective of the present study was to examine the influence, if any, of testosterone on the haematological indices of the African giant rat.

Materials and methods

Animal housing and feeding. Each cubicle was made of solid metal sheet wall and measured 15x15x15 cm. The floor was made of wire netting to allow urine and faeces to drop freely to the ground. Each cage stood on 1.5-foot-high wooden feet. Each cubicle was fitted with a hinged door on its upper side which allowed it to open outwards. The cages were placed inside the well-ventilated animal house of the Department of Veterinary Physiology and Pharmacology of the University of Ibadan, Ibadan, Nigeria.

Each animal was fed *ad libitum* with the kind of feed they normally consume in the wild. This included cassava tubers, palm kernel fruits, etc.

The animals also received a supplementation of commercially prepared mouse cubes (Ladokun Feeds, Nigeria Ltd.). These contained 21% protein, 3.3% fibre, 6% Ca^{2+}, 0.8% phosphate. Unrestricted fresh water was also made available.

Castration. The testes of the giant rats (6) were bilaterally removed surgically under 50 mg/kg ketamine hydrochloride anaesthesia (Astrapin, Germany) given intramuscularly. After surgery, each animal also was given an injection of 10 mg/kg oxytetracycline (Alfantan International, B. V. Woerden, Netherlands). Sutanol 250 (150 mg/kg, depo-testosterone, Organon, Netherlands) was also given *i.m.* at two and 4 weeks after castration. One injection is effective at a release rate of 250 mg for 3 weeks (Organon, Netherlands).

Blood collection. Blood was collected from each rat before surgery (control), 2 and 4 weeks after surgery and 2 weeks after replacement.

Blood (1-2 ml) was collected from each animal from the orbital sinus of the eye, using heparinised capillary tubes, into a bijou bottle containing EDTA. The blood was used to determine RBC count, PCV, Hb, erythrocyte fragility, WBC and differential WBC count.

Determination of haematological indices. Packed cell volume was determined using the microhaematological method. Red blood cell and white blood cell counts were determined using the haemocytometer method. The cyanmethaemoglobin method was used to determine haemoglobin concentration. MCV, MCH and MCHC were calculated as described by SCHALM et al. (1975).

Osmotic Fragility. This was done on each blood sample at room temperature (28 °C) by the method of (SUESS et al., 1948) as described by SCHALM et al. (1975).

Differential WBC count. Two blood smears were made from each sample and stained with Leishman stain. Manual differential count was done on each. At least 100 white blood cells were counted on each slide and the average of the two taken.

Analysis or result. Means (± sd) were determined for each parameter measured and the analysis of variance (ANOVA) was used to compare the

means for the various sampling period (groups). AP level of 0.05 was taken as statistically significant.

Results

Red blood cell (RBC) count. Mean RBC count in the control AGR was 5.43 ± 79 $\times 10^{12}$/L. Two weeks after castration this value decreased to 4.27 ± 0.22 × 10^{12}/L (P<0.01), and was maintained around this value up until the 4th week post-castration. Mean value of red blood cell count returned towards the pre-castration value following two weeks of androgen replacement (Table 1).

Table 1. Mean ± sd of some erythrocyte indices in the African giant rat (AGR) before and after castration and after androgen replacement.

Erythrocyte indices	Control	2 weeks AC	4 weeks AC	2 weeks PAR
RBC Count	5.43 ± 0.79	4.27 ± 0.22	4.37 ^{c} ± 0.14	5.13 ± 0.58
Hb (g/dl)	15.0 ± 1.60	10.80 ^{d} ± 1.67	11.50 ^{d} ± 1.07	13.20 ^{a} ± 0.97
PCV(l/I)	46.83 ± 5.51	40.17 ^{b} ± 1.57	35.50 ^{e} ± 2.22	39.33 ^{c} ± 1.11
MCV(fL)	87.50 ± 12.41	95.90 ± 6.78	81.80 ± 6.02	77.80 ± 10.47
MCH (pg)	28.45 ± 6.22	25.78 ± 4.03	32.45 ± 2.99	33.53 ^{a} ± 2.49
MCHC (g/Dl)	32.70 ± 6.59	26.80 ± 3.25	26.33 ^{a} ± 2.32	25.93 ^{a} ± 2.49
Max.Fragility (%NaCl)	0.14 ± 0.11	0.33 ^{b} ± 0.10	0.35 ^{c} ± 0.09	0.31 ^{a} ± 0.12
Mean Corp Fragility (%NaCl)	0.46 ± 0.07	0.52 ± 0.03	0.51 ± 0.03	0.50 ± 0.04
Min. Fragility (NaCl)	0.71 ± 0.08	0.63 ± 0.06	0.61 ^{b} ± 0.03	0.72 ± 0.10

AC = after castration; PAR = post androgen replacement
^{a} = P<0.05; ^{b} = P<0.02; ^{c} = P<0.01; ^{d} = P<0.002; ^{e} = P<0.001

Haematocrit. Two weeks after castration, mean value for PCV decreased from the pre-castration value of 46.83 ± 5.52% to 40.17 ± 1.57% (14% reduction). This is a statistically significant difference (P<0.05). A further decline of 10% in mean PCV value was observed two weeks later, bringing the mean haematocrit value to 35.5% four weeks after castration. These variations, compared to the pre-castration levels, are respectively statistically significant (P<0.02, P<0.001). Two weeks of androgen replacement caused a rise in the PCV value. However, this new value is

still lower than the pre-castration value, but higher than the post-castration value (Table 1).

Variation in mean Hb value following castration and androgen replacement are similar to those observed for the PCV (Table 1). A 28% decline in the Hb value was observed two weeks after castration (15.00 ± 1.66 versus 10.80 ± 1.67 mg/dL). This is a statistically significant difference (P<0.002). Two weeks of androgen replacement only raised the Hb value to 13.20 ± 0.79 mg/dL. Although this value is still lower than the pre-castration level, it is significantly higher (P<0.02) than the post-castration value (Fig. 1.)

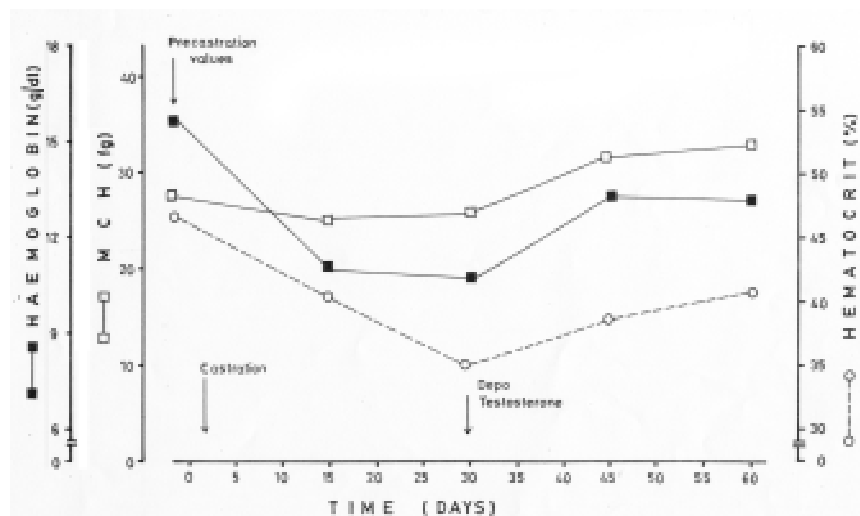

Fig. l. Mean (n = 6) haemoglobin concentration (Hb, solid square), mean corpuscular haemoglobin (MCH, open square) and haematocrit (PCV, open circle) during the period of study of African giant rats

Erythrocyte indices (MCV, MCH and MCHC). Although variations were observed in the mean values of MCV and MCH, 2 and 4 weeks after castration and hormone replacement, MCH value increased significantly (P<0.05) to 33.53 ± 2.45 from the control value of 28.45 ± 6.22 pg. These

variations were both statistically significant (Table 1). However, mean MCHC value decreased by 18%, 19.4% and 20.7%, respectively, at 2 and 4 weeks post-castration and 2 weeks post hormone replacement. The difference between the control value and values at 4 weeks post castration and 2 weeks post replacement is statistically significant ($P<0.05$). Mean MCHC value did not return towards pre-castration value after two weeks, but 4 weeks after hormone replacement.

Erythrocyte osmotic fragility. Minimum erythrocyte osmotic fragility (10% haemolysis) did not differ from the control value two weeks after castration. However, 4 weeks after castration minimum fragility (10% haemolysis) occurred in 0.61% saline compared to the 0.71% saline of the pre-castrated AGR.

This difference is statistically significant ($P<0.02$). Two weeks of hormone replacement returned this value to 0.72% (Table 1).

Maximum fragility (>90% haemolysis) occurred in the control AGR at 0.14% saline. This is significantly lower ($P<0.02$) than the corresponding value at 2 and 4 weeks post castration, as well as 2 weeks post hormone replacement (Figures 2 and 3). No significant variation was observed in the mean corpuscular fragility of erythrocytes of the AGR following castration and hormone replacement (Table 1).

Table 2. Mean ± sd of WBC count lymphocytes, neutrophils, eosinophils, monocytes and basophils in the African giant rat (AGR) before, two weeks after, four weeks after castration, and two weeks after androgen replacement.

Indices	Control (n = 6)	2 weeks AC (n = 6)	4 weeks AC (n = 6)	2 weeks PAR (n = 6)
WBC count × 10^9/L	5.30 ± 0.56	6.66 ± 1.03[a]	6.83 ± 1.06[a]	6.75 ± 1.14[a]
Lymphocytes × 10^9/L	2.11 ± 0.32	2.19 ± 0.25	2.23 ± 0.20	2.31 ± 0.38
Neutrophils × 10^9/L	2.77 ± 0.54	3.83 ± 0.88[a]	3.39 ± 0.95[a]	3.76 ± 0.88[a]
Eosinophils ×10^9/L	0.18 ± 0.07	0.22 ± 0.08	0.22 ± 0.07	0.26 ± 0.11
Monocytes × 10^9/L	0.33 ± 0.07 0.	40 ± 0.06	0.39 ± 0.09	0.41 ± 0.08
Basophils × 10^9/L	NIL	NIL	NIL	NIL

n = number of animals used; AC = after castration; AR = post androgen replacement
a = $P < 0.05$

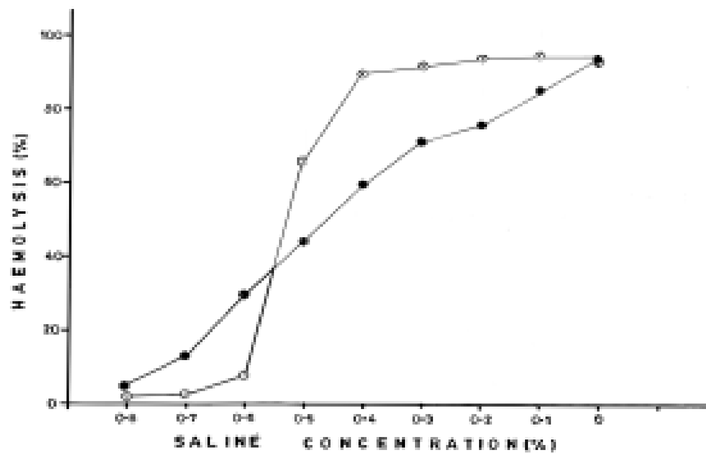

Fig. 2. The deformability (fragility) in graded concentrations of normal saline of the erythrocytes of African giant rat (n = 6) before castration (control, solid circles) and 4 weeks (n = 6) after castration (open circles)

Difference leukocyte (WBC) count. Two weeks following castration absolute WBC count increased from 5.39 ± 0.56 10^9/L to 6.66 ± 1.03 × 10^9/L. This is a statistically significant difference (P<0.05). This value further increased to 6.83 ± 1.08 × 10^9/L. four weeks post castration. Even after two weeks of androgen replacement the value of WBC was still significantly higher (P<0.05) than the control value. Castration and hormone treatment did not result in any significant difference in the value of lymphocyte, eosinophils and basophils (Table 2). However, the proportion of (2.77 ± 0.54%) before castration increased slightly to 3.83 0.88% two weeks after castration, and later decreased to 3.39 ± 0.95%. It later rose 3.76 ± 0.88%. These values are respectively significantly higher (P<0.05) than the control value (Table 2).

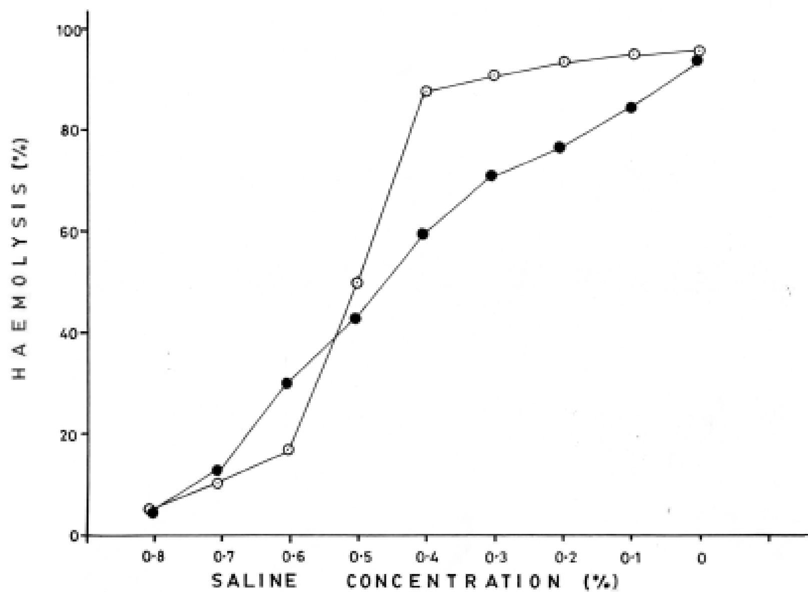

Fig. 3. The percentage haemolysis of the erythrocytes of African giant rats (n = 6) in graded concentrations of normal saline before castration (control, solid circle) and 2 weeks after androgen replacement (open circle)

Discussion

The control values reported here for RBC count, Hb concentration, PCV in the AGR agreed with corresponding values reported previously by OLOWOOKORUN (1974) and DUROTOYE and OKE (1990).

Results of the current study show that castration in the AGR reduces the RBC count, an effect which is reversed by androgen replacement. This finding therefore corroborates similar findings reported earlier in laboratory rats (CRAFT, 1946; KENNEDY and GILBERTSEN, 1957). The observed effect of castration and androgen replacement on Hb concentration in this study is similar to results of earlier studies in which GARDNER and PRINGLE (1961) showed that administration of testosterone enanthate to hypogonadal males increased haemoglobin concentration. Variations in the haematocrit value

observed in this study followed a pattern similar to that of RBC and Hb concentration. A 36% rise in PCV has been reported previously in hypogonadal males treated with testosterone enanthate (GARDNER and PRINGLE, 1961).

Four weeks after castration in the present study, minimum osmotic fragility (below 10% haemolysis) occurred in 0.61% saline. This indicates that the RBC of AGR became more osmotically stable (less fragile) in higher physiological saline. Maximum fragility (above 90% haemolysis) did not seem to differ between the RBCs of the two groups of AGR. Hence, it is probable that testosterone has a role to play in the behaviour of erythrocyte of the AGR in physiological saline. Testosterone withdrawal in the castrated AGR appeared to improve the integrity of the RBCs to osmotic fragility. The mechanism by which testosterone affects RBC osmotic fragility is presently somewhat unclear. However, according to WEED, 1970, testosterone may affect certain factors which predispose RBC membranes to osmoticc lysis. Such factors include reduction in red blood cell ATP concentration, which invariably affects the ATP-motivated Na^{+} - K^{+} pump of the erythrocyte membrane, reduction in the enzymes involved in phosphate dehydrogenase and changes in the RBC membrane lipid content. All of these invariably affected the density, and subsequently the osmotic fragility, of the RBC membrane. MARCH et al. (1966) found no effect of testosterone on erythrocyte osmotic fragility in birds. This suggests a species factor in the response of the vertebrate erythrocyte membrane to testosterone.

Castration in the present study resulted in an increase in the absolute values of WBC count, which was accounted for mainly by changes in the number of neutrophils, and was not reversed by androgen replacement. Hence, this change is not caused by testicular androgen but may be due to some kind of tissue reaction, or to other factors yet to be identified.

Conclusions

In the present study it has been shown that gonadal androgen testosterone helps to maintain the values of RBC, Hb and PCV in the AGR. After castration, values of all these decreased but were resumed

during androgen replacement. Castration in the AGR also affects the integrity of RBC. However, white blood cells were affected to any real degree by castration and androgen replacement.

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SAŽETAK

Šest divovskih afričkih štakora (*Cricetomis gambianus*) bilo je kastrirano te im je dva tjedna nakon toga primijenjen testosteron. Njegov učinak promatran je na razini hematoloških promjena tj. broju eritrocita, ukupnog volumena stanica, hemoglobina te krhkosti eritrocita i broju leukocita. Nakon kastracije ustanovljen je smanjeni broj eritrocita i smanjena količina hemoglobina, a broj leukocita ostao je nepromijenjen. Dokazana je i veća osmotska krhkost eritrocita.

Ključne riječi: hematologija, kastracija, zamjena androgenima, afrički divovski štakor (*Cricetomis gambianus*)