THE HAEMATOLOGICAL PROFILE OF THE SPRAGUE-DAWLEY OUTBRED ALBINO RAT IN NSUKKA, NIGERIA

[1]IHEDIOHA, John Ikechukwu., [1]OKAFOR, Chika and [2]IHEDIOHA, Thelma Ebele

[1] Clinical Pathology (Haematology and Clinical Chemistry) Unit, Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, P. O. Box 3236 Nsukka, Nigeria

[2] Biomedical Research Support Unit, Foundation for Education and Research on Health, Nsukka, Nigeria.

Corresponding author: IHEDIOHA, John Ikechukwu. Clinical Pathology (Haematology and Clinical Chemistry) Unit, Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, P. O. Box 3236 Nsukka, Nigeria. E-mail: jiiferh@yahoo.com Phone: 234-(0)8035387156

ABSTRACT

This study determined the haematological profiles of Sprague-Dawley (SD) outbred albino rats of both sexes and different age sets bred and maintained at the Faculty of Veterinary Medicine Laboratory Animal Unit, University of Nigeria, Nsukka, Nigeria. Erythrocyte counts (EC), packed cell volume (PCV), haemoglobin concentration (Hb), erythrocyte sedimentation rate (ESR), total leukocyte counts (TLC), and differential leukocyte counts (DLC), were carried out following standard procedures on blood samples collected from 543 rats (267 males and 276 unbred females) during a 14-month study period. Results of the determinations for each of the haematological characteristics were compared with standard reference values generated in temperate countries for specific age sets and sexes of the rats. Findings from our study showed that there were significant differences in the normal values of some of the indices between the sexes and age sets of rats studied; also there were significant differences for some indices in some age sets and sexes between the results obtained in Nsukka Nigeria and the ones generated in temperate climatic conditions - means of the PCV, Hb, mean corpuscular volume, mean corpuscular haemoglobin and absolute numbers of the different leukocytic cellular elements of the rats studied were found to significantly differ from comparable standard reference values generated in temperate locations for specific age sets and sexes, but the means of the EC and TLC were not found to significantly differ from the temperate values. The results of the study were discussed in relation to climatic and geographical locational factors (especially temperature) as they affect the normal reference haematological values, and the relevance of haematology in model animal experimentation and biomedical research.

Keywords: Haematology, Rat, Sprague-Dawley strain, Nsukka, Nigeria.

INTRODUCTION

Laboratory or model animals are recognised as important tools for investigating and understanding human and animal diseases and other biological processes (NIH, 1999a; MI, 2002). Experimentation with laboratory animals forms the backbone of biomedical, bio-agricultural and bio-industrial researches. It enables the development and selection of new medical and veterinary pharmaceuticals, their toxicity testing, development and improvement of surgical materials and procedures, investigation of experimental diseases and pathology, development and production of antisera and vaccines, and development of medical and veterinary diagnostic techniques amongst other applications (Uzoukwu, 1981; NIH, 1999a; MI, 2002; Gallagher, 2003). Worldwide, rodents are the major and most commonly used animals for biomedical research; the use of other animals is small in comparison (MI, 2002). The rat is the most widely studied experimental animal as demonstrated by the number of publications on studies using rats in the last decade – nearly 500,000 PubMed publications (NIH, 1999b). The rat model with its enormous strengths and

versatility of application has been found to be the most appropriate experimental model for the study of human diseases (NIH, 1999b), and it remains the dominant animal model for risk assessment of virtually all forms of therapeutics and chemical toxicities (Jelovsek *et al.*, 1989; Berkowitz and Katzung, 2001). The Sprague-Dawley (SD) rat, an albino strain of the Norwegian rat (*Rattus norvegicus*), is a widely accepted and dependable general purpose research model used in virtually all disciplines of biomedical research; it is the commonly available rat for experimental studies worldwide (TTL, 1998a), and in Nsukka, Nigeria.

Haematological studies are important in animals and humans because the blood is the major transport system of the body and both the input and output substances of almost all the body's metabolic processes and any deviations from normal are detectable in the blood profile. An evaluation of the haematological profile usually furnishes vital information on the response of the body to injury, deprivation and/or stress. Such an evaluation is indispensably important in arriving at a diagnosis, making a prognosis, assessment of the efficacy of therapy and toxicity of drugs and chemical substances. The erythrocytic and leukocytic profiles of greatest importance include erythrocyte counts, packed cell volume (haematocrit), haemoglobin concentration, erythrocyte sedimentation rate, mean corpuscular values, total leukocyte counts and differential leukocyte counts (Schalm *et al.*, 1975).

The standard reference values for the erythrocytic and leukocytic profiles are influenced by certain physiological factors such as age, sex, breed, species and physiological activity status, and climatic/geographical locational factors such as temperature, humidity, altitude and day length. This implies that for an animal to be used for study in any specific location standard reference values for these haematological indices must be established for that geographical location (Schalm *et al.*, 1975; Coles, 1986). Till date, standard reference haematological values of the SD albino rat, which is commonly used for research and experimentation in the University of Nigeria's Life Sciences and Biomedical departments and other biomedical research centres located in Nsukka, has not been determined. Rather, published standard reference values established for the temperate regions of the world are often used by our researchers as the only available alternative.

The present study reports the results of determinations of the haematological profile (erythrocytic and leukocytic indices) of the SD albino rat bred and maintained at the Faculty of Veterinary Medicine Laboratory Animal Unit, University of Nigeria, Nsukka, Nigeria.

MATERIALS AND METHODS

Study Area: Nsukka is situated within the derived savannah belt of Eastern Nigeria between latitudes $5^{o}50'$ and $7^{o}00'$ north and longitude $6^{o}52'$ and $7^{o}54'$ east, at an average elevation of approximately 500 metres above sea level (FMANR, 1999). It is an area of fairly high temperature with a yearly minimum and maximum of 21.17 ^{o}C and 29.67 ^{o}C with a mean of 25.42 ^{o}C (FMANR, 1999). The angle of the sun's rays over Nsukka is near vertical; the difference between the longest and shortest days in the year is only 48 minutes (FMANR, 1999). There is rainy season from March to October and dry season from November to February with a yearly average rainfall of 119.5 mm; the relative humidity in Nsukka is about 70 % during rainy season and falls to about 20 % during the dry season (FMANR, 1999). These climatic factors are capable of influencing the haematological profiles of SD rats bred and currently used in Nsukka research laboratories.

The Faculty of Veterinary Medicine Laboratory Animal Unit, University of Nigeria, Nsukka is the principal source of laboratory animals for the university community's biomedical researchers, independent research centres in the Nsukka and Enugu town and other universities and research centres in Eastern Nigeria.

Rats: The rats used for the study were 543 conventional grade Sprague-Dawley outbred rats bred and maintained at the Faculty of Veterinary Medicine Laboratory Animal Unit, University of Nigeria, Nsukka between February 2002 and April 2003. The 543 rats comprised of 267 males and 276 unbred females of age range varying from 3 to 72 weeks. The rats were kept in groups according to their ages and sexes in clean cages in a screened animal house. They were fed on standard rat diet composed of 16 % crude protein, which was formulated to meet their nutritional requirements (NAS, 1972). They were also provided with clean drinking water *ad libitum*.

Blood Sample Collection: The blood samples for the haematological study were collected

between the hours of 8.00am and 10.00am each day of the study from the ophthalmic venous plexus located in the orbital sinus of the rats using a micro-capillary pipette (Stone, 1954), as modified by Riley (1960). About 1ml of blood was collected from each rat into a labelled clean sample bottle containing 1 mg of Na-EDTA powder as anticoagulant. Blood was collected from each rat only once. For each age set and specific sex, blood samples were collected from at least 30 rats made up of three batches of rats bred at various times within the experimental period. The relevant haematological determinations were carried out on the blood samples immediately upon collection.

Haematological Procedures: Standard procedures were followed in all the haematological determinations – erythrocyte counts and total leukocyte counts were carried out by the haemocytometer method using an improved Neubauer counting chamber (Hawksley, England); packed cell volume was determined by the microhaematocrit method; the haemoglobin concentration was determined using a standard haemometer (Marienfeld, Germany); and the erythrocyte sedimentation rate was determined by the Wintrobe method (Schalm *et al.*, 1975; Cole, 1986). The mean corpuscular values were computed using the standard formulae. Smears for differential leukocyte counts were prepared and stained by the Leishman technique and the different cells of the leukocytic series were enumerated by the longitudinal counting method (Coles, 1986).

Statistical Analysis: Results generated were collated and presented as means with standard deviation of the specific haematological values for the different age sets and sexes of the rats and tested for significance using ANOVA and Student's *t* test as appropriate; difference was accepted at the probability level of $p < 0.05$. Results of the present study were further compared with the most widely used comprehensive haematological profile of the SD rat compiled by Schalm *et al*. (1975) using a Student's *t*-test. Other published haematological profiles of the SD rat (TCRBL, 1973; TTL, 1998b; Saito *et al.*, 2003) were not used for comparison because these studies did not comprehensively detail the variations usually associated with the different age sets and sexes.

RESULTS AND DISCUSSION

Erythrocytic indices such as erythrocyte counts (EC), packed cell volume (PCV) and haemoglobin concentration (Hb) are important indicators of the functional state of the erythron (Schalm *et al.*, 1975). Erythrocyte counts reflect the total number of red blood cells per unit volume of circulating blood while Hb determinations indicate the oxygen carrying capacity of blood, and PCV determinations show the proportion of blood that is made up of cellular elements and the proportion that is plasma (Coles, 1986). Results of the EC, PCV and Hb determinations for both sexes (Table 1) showed that for both males and females these indices were lowest at weaning (3 - 4 weeks of age) and increased successively with age up till maturity and then declined at old age (60 - 72 weeks of age). The EC obtained ranged from a mean of 4.99 and 5.03 million cells per microlitre of blood in males and females respectively at 3 - 4 weeks of age to as high as 7.87 million cells in 8 - 22 week old males and 7.61 million cells in 15-16 week old females (Table 1). This trend of EC results compared favourably with and was in agreement with the findings of Schalm *et al.* (1975) who reported that mean erythrocyte numbers of SD rats increased with age from an average of 5.25 million cells per microlitre of blood during the first month of life in both sexes to peak of 8.5 million cells in males and 7.5 million cells in females at 6 months of age and older. Results of the PCV determinations showed no significant differences between males and females for the specific age sets; the age trend was an increase in the mean PCV values from 37.60 % in males and 38.00 % in females at 3-4 week of age when it was lowest to its peak of 45.67 % in males and 45.01 % in females at 15 - 16 weeks of age without significant declines as the rats aged (43.80 % and 43.24 % in males and females respectively at 60-72 weeks of age) [Table 1]. This reported trend agrees with the findings of Schalm *et al*. (1975), though after 3-4 weeks of age the mean PCVs reported by Schalm *et al.* (1975) were consistently significantly higher than the one being reported for this study for each age set and sex compared. The significantly higher PCVs reported by Schalm *et al*. (1975) is believed to be due to the relatively colder environmental temperatures of the temperate climates, as studies by Olsen (1973) showed that exposure of animals to cold environmental temperatures lead to increase of about 2 - 5 % of their PCV.

Table 1: The erythrocytic profile of Sprague-Dawley outbred rats of different ages and sexes at Nsukka, Nigeria

Age (weeks)	*Erythrocyte counts (10^6 cells/ul of blood)*		*Packed cell volume (%)*		*Haemoglobin concentration (g/dl)*		*Mean corpuscular volume (fl)*		*Mean corpuscular haemoglobin (pg)*		*Mean corpuscular haemoglobin concentration (g/dl)*		*Erythrocyte sedimentation rate (mm/hr)*		*Number of rats sampled*	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
3 –4	4.99 (0.74)	5.03 (0.84)	37.60 (1.68)	38.00 (1.31)	11.80[a] (0.39)	12.18[b] (0.30)	75.35 (4.20)	75.55 (4.56)	23.65 (1.80)	24.21 (2.02)	31.38 (1.28)	32.05 (1.67)	1.24[a] (0.30)	0.50[b] (0.11)	30	30
6 –7	6.38 (0.53)	6.41 (0.18)	41.00 (3.00)	42.00 (2.54)	13.28[a] (0.43)	12.48[b] (0.38)	64.26 (5.36)	65.52 (3.80)	20.82 (2.14)	19.47 (1.82)	32.39[a] (1.08)	29.71[b] (1.42)	1.14[a] (0.29)	0.62[b] (0.17)	30	30
9 – 10	7.06[a] (0.72)	6.68[b] (0.38)	43.77 (4.74)	43.90 (1.06)	14.09[a] (0.58)	13.18[b] (0.58)	62.00[a] (4.01)	65.72[b] (3.96)	19.96 (1.86)	19.73 (1.76)	32.19[a] (1.12)	30.02[b] (1.08)	1.04[a] (0.39)	0.59[b] (0.25)	45	45
12 – 13	6.71[a] (0.98)	7.60[b] (0.76)	43.20 (1.21)	45.01 (2.46)	13.28[a] (0.08)	13.95[b] (0.54)	64.38[a] (3.34)	59.22[b] (3.68)	19.79[a] (1.72)	18.36[b] (2.11)	30.74 (0.96)	30.99 (1.13)	0.84 (0.15)	0.88 (0.23)	30	36
15 – 16	7.61 (0.50)	7.61 (0.49)	45.67 (2.09)	44.70 (2.48)	13.64[a] (0.22)	13.26[b] (0.33)	60.01 (3.43)	58.74 (4.07)	17.92 (1.76)	17.42 (1.83)	29.87 (1.24)	29.66 (1.32)	0.93[a] (0.27)	1.12[b] (0.22)	36	30
18 – 22	7.87[a] (0.35)	6.93[b] (0.64)	44.60 (1.92)	44.30 (2.53)	13.94[a] (0.08)	13.43[b] (0.46)	56.67[a] (3.92)	63.92[b] (3.74)	17.71[a] (1.80)	19.38[b] (1.02)	31.68[a] (1.32)	30.32[b] (1.30)	0.76[a] (0.19)	1.39[b] (0.40)	30	45
24 – 26	7.72[a] (0.90)	7.01[b] (0.72)	44.20 (1.52)	43.96 (2.66)	14.28[a] (0.62)	13.67[b] (0.52)	57.25[a] (3.46)	62.71[b] (4.01)	18.50[a] (1.56)	19.50[b] (1.12)	32.31[a] (1.40)	31.10[b] (1.14)	0.80[a] (0.22)	1.26[b] (0.31)	30	30
60 – 72	7.27[a] (0.57)	6.72[b] (0.63)	43.80 (1.21)	43.24 (2.13)	13.58 (0.33)	13.76 (0.65)	60.24[a] (3.28)	64.35[b] (3.43)	18.68[a] (1.94)	20.48[b] (1.06)	31.00[a] (1.23)	31.82[b] (1.07)	0.56 (0.17)	0.57 (0.15)	36	30

** Results are presented as means with standard deviation in brackets; M = Males, F = Females. .[a b] Different superscripts in an age set row indicate significant differences between the mean values of the parameters between males and females: ab = $p < 0.01$*

The Hb recorded in this study was also lowest in rats of 3 - 4 weeks of age (11.80 g/dl for males and 12.18 g/dl in females) with only slight increases as the rats reached maturity, and without any significant declines as the rats aged (Table 1). The mean Hb recorded at 3 - 4 weeks of age for both sexes were slightly higher than that reported by Schalm *et al.* (1975), but from 6 - 7 weeks of age upwards the mean Hb values recorded were significantly lower than that reported by Schalm *et al.* (1975). These significant differences are believed to be temperature related (Olsen, 1973).

The mean corpuscular values [mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and the mean corpuscular haemoglobin concentration (MCHC)] computed from the EC, PCV and Hb are usually useful in elucidating and classifying anaemia morphologically; they represent an estimation of the alterations in size and haemoglobin content of individual red blood cells (Coles, 1986; TTL, 1998b). Results of this study showed that the MCV and MCH were highest at 3 - 4 weeks of age with an MCV of 75.35 fl for males and 75.55 fl for females, and MCH of 23.65 pg in males and 24.21 pg in females; the MCV and MCH values decreased consistently with age in both sexes without any specified pattern in the variation between the sexes (Table 1). The recorded results compared effectively with that reported by Schalm *et al.* (1975) with significant differences between results of the two studies only at 3 - 4 weeks of age and 60 - 72 weeks of age. The MCHC of both males and females were not found to vary significantly between the age sets (Table 1), and the results reported for this study compared favourably with that of Schalm *et al.,* (1975) and were not found to be significantly different from it.

Erythrocyte sedimentation rate (ESR) is an indicator of the suspension stability of the erythrocyte; changes in the ESR reflect changes in the physicochemical properties of the erythrocyte surface and the plasma (Coles, 1986). The ESR is an important index for evaluating the response of an animal or human body to inflammatory and necrotic processes (Meyer & Harvey, 1998). The ESR results generated from the study showed spectacular age-related trend pattern differences between males and females; the mean ESR of males was highest (1.24 mm/hr) at 3 - 4 weeks of age and progressively decreased with age to 0.56 mm/hr at 60 - 72 weeks of age, in contrast that of females was lowest (0.50 mm/hr) at 3 - 4 weeks of age and progressively increased with age, reached a peak of 1.39 mm/hr at 18 - 22 weeks of age and then declined to 0.57 mm/hr at 60 - 72 weeks of age (Table 1). There was no published comprehensive ESR results that detailed differences in sex and age that could be used to compare the ESR results recorded in our study. The only report in literature on sex differences in ESR of adult rats by TCRBL (1973) only presented an average ESR of 0.7 mm/hr for adult males and 1.8 mm/hr for adult females.

Total leukocyte counts (TLC) and differential leukocyte counts (DLC) reflect the systemic status of an animal in relation to its response and adjustment to injurious agents, stress and/or deprivation; the indices are of value in confirming or eliminating a tentative diagnosis, in making a prognosis and guiding therapy (Coles, 1986). The TLC and DLC could further provide information on the severity of an injurious agent, the virulence of an infecting organism, the susceptibility of a host, and the nature, severity and duration of a disease process (Meyer & Harvey, 1998). The TLC of the rats studied was found to be lowest at 3 - 4 weeks of age (7.18×10^3 cells per microlitre of blood in males and 7.61×10^3 cells per microlitre of blood in females) and increased significantly with age up until 12 - 13 weeks of age, and then started declining progressively though in the oldest rats (60 - 72 weeks of age) the TLC was found to be at its highest in both sexes (Table 2). This trend did not significantly differ from that reported by Schalm *et al.*, (1975) except in the results of 60 - 72 week old females, which was found to be significantly different (the TLC reported by Schalm *et al.*, (1975) was found to be significantly lower).

Results of the differential leukocyte counts (Table 2) showed that the absolute lymphocyte counts (ALC) was lowest at 3 - 4 weeks of age (4.07×10^3 cells per microlitre of blood in males and 4.76×10^3 cells per microlitre of blood in females) and was found to increase up till maturity and then declined though the values for 60 - 72 week old rats was high. The changes in absolute neutrophil and absolute monocyte counts recorded in the study for both sexes and different ages was not found to follow any definite pattern, though males had higher counts than females for most age sets (Table 2). Absolute eosinophil counts were lowest at 3 - 4 weeks of age for both sexes and increased progressively with age up till old age (60 - 72 weeks of age), with males having a higher eosinophil count than females for all age sets except in 6 - 7 week old rats where absolute eosinophil counts of females was found to be higher than that of males (Table 2).

Table 2: The leukocytic profile of Sprague-Dawley outbred rats of different ages and sexes at Nsukka, Nigeria

Age (weeks)	Total Leukocyte counts		Absolute Lymphocyte counts		Absolute Neutrophil counts		Absolute Monocyte counts		Absolute Eosinophil counts		Absolute Basophil counts		Number of rats sampled	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
3 –4	7.18 (1.80)	7.61 (1.36)	4.07 (1.71)	4.76 (1.72)	2.19[a] (0.56)	1.74[c] (0.84)	0.76 (0.10)	0.83 (0.21)	0.23 (0.13)	0.21 (0.14)	0.01 (0.03)	0.00	30	30
6 –7	8.67[a] (1.87)	10.27[b] (2.50)	5.89[a] (1.32)	6.93[b] (1.22)	1.20 (0.26)	1.06 (0.07)	0.60[a] (0.35)	1.37[b] (0.60)	0.10[a] (0.11)	0.26[b] (0.16)	0.00	0.01 (0.03)	30	30
9 – 10	12.29 (2.67)	12.52 (2.49)	8.18[a] (1.45)	9.25[b] (2.25)	2.17 (0.66)	1.92 (1.04)	1.24 (0.47)	1.13 (0.48)	0.21[a] (0.11)	0.28[b] (0.08)	0.00	0.01 (0.03)	45	45
12 – 13	14.00 (2.14)	12.70 (3.28)	9.41 (2.28)	9.40 (2.36)	2.76[a] (0.83)	2.03[b] (0.62)	2.14[a] (0.81)	1.08[b] (0.44)	0.25 (0.18)	0.24 (0.15)	0.03 (0.06)	0.02 (0.04)	30	36
15 – 16	10.11 (2.99)	10.08 (3.51)	7.48 (2.25)	7.84 (1.76)	1.86[a] (0.84)	1.37[b] (0.22)	1.09[a] (0.10)	0.50[b] (0.39)	0.36[a] (0.18)	0.22[b] (0.13)	0.04 (0.06)	0.03 (0.04)	36	30
18 – 22	10.90 (2.86)	10.03 (2.27)	7.26 (1.75)	7.81 (1.63)	2.21[a] (0.36)	1.09[b] (0.56)	1.15[a] (0.41)	0.77[b] (0.48)	0.47 (0.36)	0.37 (0.15)	0.00	0.06 (0.03)	30	45
24 – 26	12.78[a] (2.40)	8.12[b] (1.84)	7.11 (2.09)	6.32 (1.32)	3.41[a] (0.91)	0.88[b] (0.76)	1.82[a] (0.93)	0.62[b] (0.39)	0.58[a] (0.23)	0.30[b] (0.02)	0.08 (0.07)	0.03 (0.03)	30	30
60 – 72	14.81[a] (2.25)	16.47[c] (4.04)	9.30[a] (2.36)	12.30[b] (3.71)	3.51[a] (1.26)	2.44[b] (0.39)	1.39 (0.21)	1.39 (0.52)	0.59[a] (0.45)	0.34[b] (0.27)	0.03 (0.07)	0.00	36	30

** Results are presented as means X 10^3 cells per microlitre of blood with standard deviation in brackets; M = Males, F = Females. .[a b c] Different superscripts in an age set row indicate significant differences between the mean values of the parameters between males and females: ab = p < 0.01; ac = p < 0.05*

The pattern of variations in absolute basophil counts was not definite for both sexes and different age sets, and in some cases basophils were not found in blood for certain age sets. The inconsistency in absolute basophil numbers and sometimes their total absence in blood of rats is a normal occurrence (Schalm *et al*., 1975; Coles, 1986). A comparison of the differential leukocyte counts obtained in this study with that reported by Schalm *et al*., (1975) showed that there were significant differences in the absolute numbers of lymphocytes, neutrophils, monocytes, eosinophils and basophils even when the TLC results of the two studies did not differ significantly; the current study being reported found comparatively lower absolute lymphocyte counts in males mainly from 3-4 weeks of age up till 18 - 22 weeks of age, higher neutrophil counts from 3 - 4 weeks of age up to 12 - 13 weeks of age in both sexes, higher absolute monocyte and eosinophil counts all through the age sets studied and inconsistent variations in absolute basophil counts for both sexes.

Results of this study have shown significant differences in the normal reference values of some haematological indices of the SD albino rat in both sexes and certain age sets when compared with standard reference values generated in temperate regions. The determination and establishment in this study of the erythrocytic and leukocytic profile of the SD albino rat in Nsukka Nigeria is of great significance because of the indispensable relevance of haematological studies in laboratory animal experimentation/research in the University of Nigeria's biomedical research departments and independent life science research centres in the Nsukka and Enugu town and other universities and research centres in Eastern Nigeria. The significance of the study is further buttressed by the fact that the SD albino rat is the most commonly used model animal for experiments and biomedical research worldwide (NIH, 1999b) including in Nsukka, Nigeria.

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