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## **FURTHER STUDIES ON CANINE ERYTHROCYTE FRAGILITY IN THE TROPICAL SUBREGION: THE EFFECTS OF SODIUM PENTOBARBITONE ANAESTHESIA IN NIGERIAN DOGS**

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The osmotic fragilities of the red blood cells of 5 local dogs were determined before and during sodium pentobarbitone anaesthesia using different concentrations of sodium chloride solution. It was observed that before the anaesthesia, the osmotic fragility decreased with increasing concentration of sodium chloride solution, exhibiting a sharp drop from a high (43%) at 0.5% to a low (19%) at 0.6%. Between 0.6 - 0.9% saline concentrations, the decrease in osmotic fragility was rather more gradual. Under anaesthesia, the osmotic Fragility curve was shifted to the right of the pre-anaesthesia level. The pattern of response was however basically the same- decreasing value with increasing concentration of saline solution. The increases in osmotic fragility values recorded during anaesthesia over those of the pre-anaesthesia levels were significant ( $P < 0.05$ ) for saline concentrations 0.5% - 0.8%. The values of some other hematological parameters such as the PCV, Hb, RBC and WBC only showed insignificant decreases. It was concluded that veterinary practitioners employing sodium pentobarbitone as anaesthetic agent should exercise more caution than hitherto.

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### **INTRODUCTION**

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In a previous article (Olaifa *et al*, 1998), the rationale for embarking on this series of studies was established. Many dogs of different breeds are being imported into Nigeria by many people and for many reasons including security. These dogs are very expensive and this calls for greater care by veterinarians in handling them. There is also the need to establish a firm scientific basis for the use of many of the drugs found on the shelves of most veterinary pharmacists and surgeries across the country. Sodium pentobarbitone (Nembutal, Bayer<sup>R</sup>) is one of such drugs. This barbituric acid derivative is very popular with Veterinarians in Nigeria as an intravenous anaesthetic agent for many small animal interference even though it has become less fashionable in countries that have facilities for inhalation anaesthesia (Hall and Clarke, 1983). It is marketed in this country as a sterile 6.5% (W/V) solution containing propylene glycol and as a powder in gelatine capsules. For

euthanasia it is sold as 20% (W/V) solution.

Although it is often presented as a good anaesthetic agent, especially for small animal practice, sodium pentobarbitone is still subject to the major disadvantage of all intravenous agents - the fact that the practitioner loses control of the drug once it has been administered. The drug must then run its full course in the patient.

From this perspective therefore, there is the need to study its effects in the animals in the rather hot and humid environment of this tropical sub-region. This study has been designed to provide information on how it affects the osmotic fragility of the red blood cells of the dog.

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### **MATERIALS AND METHODS**

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The methods adopted for this study had been described in the previous paper (Olaifa *et al*, 1998). Briefly, 5 local dogs

were procured from a local dog market. They were acclimatized to the experimental conditions in the Kennels of the small animal clinic of the veterinary teaching hospital, University of Ibadan. At the start of sampling, four weeks post-procurement, they were certified to be clinically fit and free of any worm burden. The dogs were then weighed and 5ml blood was taken from the cephalic vein of each dog and put in a bijou bottle containing EDTA as anticoagulant. This process was done twice for each dog before and during anaesthesia. The dogs were then given sodium pentobarbitone solution (30 mg/Kg body weight), again via the cephalic vein. Half the total dose for each dog was administered rapidly while the remaining half was given gradually depending on the rate of induction of anaesthesia. This, on the average took less than five minutes for each dog. There was no pre-medication and struggling occurred in all the dogs. The characteristic signs or stages of induction of sodium pentobarbitone anaesthesia viz. followed struggling: excitement, salivation, urination, defaecation, quietness and recumbency. A complete relaxation of the jaw muscles and the total absence of corneal and pedal reflexes determined surgical anaesthesia. Twenty minutes into surgical anaesthesia the second blood sample (%ml) was taken from each dog.

### Blood Sample Analyses

The blood samples were analysed to determine the osmotic fragility and other hematological indices as previously described (Olaifa et al, 1998). Determination of the hematological parameters such as red blood cell (RBC) and white blood cell (WBC) counts, Packed cell volume (PCV) and hemoglobin concentration (Hb.) was done using standard methods (Jain, 1986).

Statistical analysis of mean values was by the method of variance (ANOVA) in which both animal numbers and saline concentrations were used as determinants of the degree of freedom.

## RESULTS

The means of the osmotic fragilities for the five dogs in the different saline concentrations are presented in Table 1.

The cumulative osmotic fragiligram before and during anaesthesia are presented graphically in Fig. 1. Table 2 shows the test of significance while Table 3 shows the mean values of other hematological parameters before and during sodium pentobarbitone anaesthesia. The mean osmotic fragility of the dogs before the pentobarbitone anaesthesia (Table 1) decreased as the concentration of the sodium chloride solution increased. There was actually a sharp drop in value from a high  $43.35 \pm 19.9\%$  at saline concentration 0.5% to a low  $19.67 \pm 6.20\%$  recorded at saline concentration 0.6%. Between saline concentration 0.6% - 0.9% the fall in value was more gradual and indeed

**Table 1.**  
**Mean osmotic Fragility as % haemolysis of local Nigerian dogs in different saline concentrations before and during Sodium Pentobarbitone anaesthesia.**

SALINE CONC. (%)	OSMOTIC FRAGILITY % Haemolysis	
	BEFORE ANESTHESIA	DURING ANESTHESIA
0.1	100.00 + 0.00	100.0+0.00
0.2	100.00 + 0.00	100.0 + 0.0
0.3	79.40 + 0.00	100.0 + 0.0
0.4	75.80 + 6.16	75.53+ 13.2
0.5	43.35 + 19.90	72.50+14.5
0.7	10.88 + 2.06	68.17+ 18.7
0.8	8.40 + 0.75	52.78+24.9
0.9	8.09 + 3.34	7.50 + 2.27

virtually absent between saline concentrations 0.8% - 0.9%.

Similar trend of fall in osmotic fragility value with increasing concentration of the saline solution was observed with the samples collected when the dogs were in the state of surgical anaesthesia. But the mean values in this case were higher at each saline concentration than the corresponding pre-

anaesthesia value (fig. 1). This was the case for all concentrations of sodium chloride solution except at the concentration of 0.4% where the two values were coincident (see Fig. 1). Variance analyses (Table 2) revealed that these differences between the pre-and during anaesthesia mean value of osmotic fragility were significant ( $P>0.05$ ) especially between saline concentration 0.6-0.8%.

ANOVA also revealed that differences in mean osmotic fragilities between individual animals were not significant whether pre-or during anaesthesia.

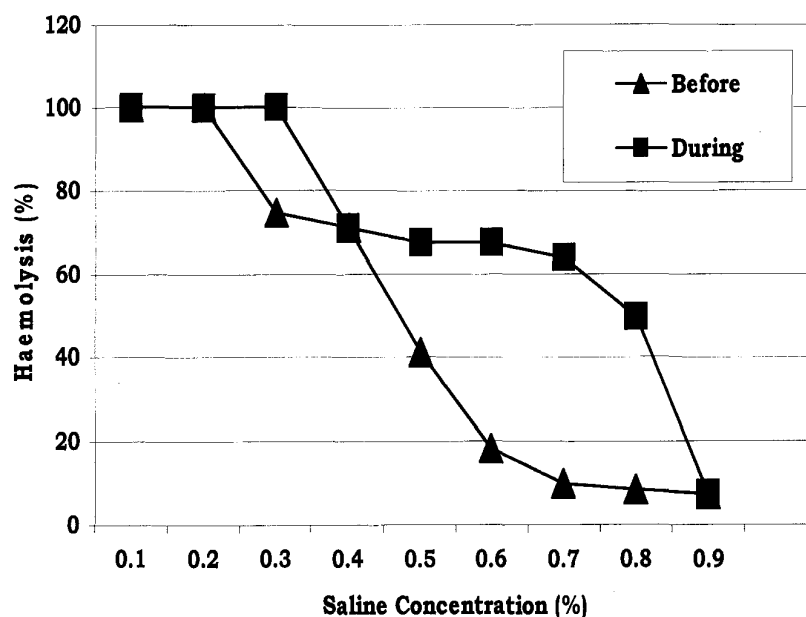
As Table 3 shows the other hematological parameters investigated showed marginal decreases in value during sodium pentobarbitone anaesthesia when compared to the pre-anaesthesia levels. None of these changes were however significant.

**Table 2.**

**Statistical comparison of canine erythrocyte osmotic fragility before (B.A) and during (D.A) Sodium Pentobarbitone anaesthesia.**

		DF	F value	P.F.
B.A	NaCl	8	48.2	0.0001 <sup>a</sup>
	Animal	4	2.29	0.08 <sup>b</sup>
D.A.	Saline conc.	8	11.55	0.0001 <sup>a</sup>
	Animal	4	2.33	0.07 <sup>b</sup>

a = significant b = not significant



**Fig. 1.**

**Osmotic Fragiligram of Canine Erythrocytes Before and During Sodium Pentobarbitone Anaesthesia**

**TABLE 3.**

**Mean value of some hematological parameters in local Nigerian dogs (n=5) before and during sodium pentobarbitone anaesthesia.**

	PCV (%)	Hb. (gm/dl)	RBC ( $\times 10^6$ /ml)	WBC ( $\times 10^3$ /ml)
<b>Before anaesthesia</b>	34.20 $\pm$ 7.12*	11.36 $\pm$ 2.39	5.90 $\pm$ 0.82	11.14 $\pm$ 3.28
<b>During anaesthesia</b>	31.00 $\pm$ 7.38	10.40 $\pm$ 2.51	5.39 $\pm$ 1.41	8.82 $\pm$ 0.17

Mean  $\pm$  SEM

## DISCUSSION

The results showed that pentobarbitone sodium increased the lysis of canine erythrocytes when used as anaesthetic agent in that species. This could be attributed to several factors one of which is the action of the drug on the cardiovascular system of the dog. According to Olmsted and Page (1966), administration of pentobarbitone to a dog causes an immediate large increase in heart rate, decrease in stroke volume and a transient to moderate fall in both cardiac output and arterial blood pressure but with no change in peripheral resistance. Similarly, Brander *et al* (1991) stated that pentobarbitone had a spasmolytic effect on the blood vessels and that it could cause decrease in myocardial contractility. If that is the case, it could result in venous stasis and pooling of blood in body organs that may predispose to increased cell lysis.

Another possible cause of the increase in the osmotic fragility of the erythrocytes could be the effect of respiratory distress. Hall and Clarke (1983) associated pentobarbitone anaesthesia in the dog with the risk of hypoxia. This could lead to cellular damage especially in the liver and kidney where the drug is metabolised and excreted respectively. Damaged cells might release toxic substances, which could enhance the rate of homeless.

Furthermore it has been shown (Matsuzawa and Ikarashi, 1979) that changes in the hydrogen ion concentration (pH) of solutions can effect the osmotic

fragility of red blood cells. Barbituric acid, the parent acid of sodium pentobarbitone is weakly acidic in nature (pKa 7.4 - 8.0). Its sodium salt therefore is likely to have a strong tendency to alkalinity (pH 9-11). Such an influence will likely lead to increased cell lysis in hypotonic solutions.

It would seem from this study that for dogs the critical saline concentration to watch when administering any form of anaesthesia is 0.6%. The rise in the rate of osmotic homeless at concentrations hypotonic to this strength was very remarkable. This was potentiated by the administration of the pentobarbitone which would therefore appear to be contraindicated in conditions such as aldosterone hormone insufficiency because low plasma salt concentration may lead to increased hemolysis which could weaken the patient. So it will amount to a good practice to always check the plasma electrolyte of the dog before pumping sodium pentobarbitone into it.

The phenomenon observed at saline concentration of 0.4% where the osmotic fragility values for both pre- and during anaesthesia were similar is difficult to explain. A comparable situation was not observed in xylazine sedated dogs (Olaifa *et al* 1998). Plasma electrolyte concentration of 0.4% seems therefore to be some kind of threshold in the dog for anaesthetic purposes. This however calls for more investigation.

The marginal decreases in the values of the other hematological parameters of dogs under pentobarbitone anaesthesia are not to be unexpected and have indeed

been previously reported (Gilmore, 1958). A depressive effect on the cardiovascular system if it leads to sequestration and pooling of blood in certain organs such as the spleen and hemodilution would necessarily cause decreases in blood cellular values. All these effects call for

the exercise of greater caution by veterinary practitioners in the use of pentobarbitone sodium as an intravenous anaesthetic in the dog, especially in the tropical subregion where parasitism is endemic.

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