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Haematology in mice after weekly blood sampling for 7 weeks

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Introduction

Blood sampling in rodents from the orbital vein plexus is a controversial procedure due to potential animal welfare implications. As a consequence the procedure is regulated differently in European countries. It is also controversial whether the animals should be anaesthetised while the sampling takes place. Recommendations for removal of blood from laboratory animals have recently been given in a report by a BVA/FRAME/RSPCA/UFAW (1993) working group. This working group dealt specifically with retro-orbital blood sampling and stated that "there are many potential adverse effects to this technique and these should be given full consideration before use".

The Danish Animal Experiments Inspectorate has recently conducted an in depth investigation of blood sampling by puncture of orbital vein plexus in guinea pigs, rats and mice. Several members of the Inspectorate have had the opportunity at inspections to supervise this sampling procedure in numerous animals and seen animals being sampled by this procedure weekly for seven weeks. The present study was followed closely by the Inspectorate. The procedure these members has seen has not been the course of remarks *(Kjærsgaard,* 1997). In a recent work by *Herck et al.* (1997) it is concluded that orbital bleeding in rats while under diethylether anaesthesia does not influence telemetrically determined heart rate, body temperature, locomotor and eating activity when compared with anaesthesia alone. The Danish Animal Experiments Inspectorate came up with the following conclusion: Anaesthesia of mice is not required at blood sampling from the periorbital plexus. Sampling in conscious mice is only acceptable if carried out by trained and experienced technicians. Anaesthesia is required at blood sampling from the periorbital plexus of rats and guinea pigs. Sampling from the periorbital plexus of rats without anaesthesia may be allowed by the Inspectorate in special cases in animals with a body weight below 120 g *(Kjærsgaard,* 1997).

In a series of immunisation studies in mice, sampling of 0.5 ml of blood took place at a weekly interval for seven weeks. The mice were supposed to develop a certain level of anaemia *(BVA/FRAME;RSPCA/UFAW working group,* 1993; *McGuill and Rowan,* 1989).

For this reason haematological parameters were determined after the final blood sample for immunology was taken.

*)Abbreviation: British Veterinary Association (BVA), Fund for the Replacement of Animals in Medical Experiments (FRAME), Royal Society for the Prevention of Cruelty to Animals (RSPCA) and Universities Federation for Animal Welfare (UFAW

Materials & Methods

Haematological parameters were determined in 24 BALB/c Bom female mice (7-8 weeks of age). The mice were divided into three groups, each of 8 animals. Group 1 was a blank control group and only one blood sample for haematology was taken simultaneously with the sample taken for haematology from the other two groups. From the mice in the other two groups, a blood sample of 0.5 ml for immunology was taken weekly for six-seven weeks before a blood sample for haematology was taken 1 day (group 2) and 9 days (group 3) respectively after the final blood sample for immunology. All blood samples were taken on the same date.

The immunological part of the study included immunisation on 4 occasions and subsequent blood sampling once weekly for seven weeks. At the blood sampling for haematology the body weight range was 20 - 27 g.

The mice were kept in macrolone type III cages with softwood sawdust bedding. A pelleted complete rodent diet ""Altromin 1314" (for growing animals) and domestic quality drinking water were available *ad libitum.* The room temperature was 21°C ± 3°C, relative humidity 55% ± 15% and air changes 10 times/hour. Light was on from 6 am to 6 pm.

Blood samples for immunology were taken by puncture of the retroorbital vein plexus on days 0, 28, 35, 42, 49, 56, 63 and 72 (only group 2).

For haematology a volume of 0.5 ml EDTA stabilised blood was taken simultaneously from the control animals and the day 73 animals (group 2 - one day after the 7th weekly blood sampling). On day 72 (9 days after the 6th weekly blood sampling) a similar blood sample was taken from group 3. The haematological parameters were determined using Cobas Minos (Roche, Montpellier, France).

Data were processed to give group mean values and standard deviations where appropriate. Possible outliers were identified, too. Thereafter each continuous variable was tested for homogeneity of variance with Bartlett's test. If the variance was homogeneous, analysis of variance was carried out for the variable. If any significant differences were detected, possible intergroup differences were assessed with least-squares means. If the variance was heterogenuous each variable was tested for normality by the Shapiro-Wilk method. In case of normal distribution, the possible intergroup difference was identified with Student's t-test. Otherwise the possible intergroup difference was carried out with Wilcoxon Rank-Sum test.

Results

One day after the seventh blood sampling all parameters apart from platelet count were lower than control level (Table 1). Haemoglobin concentration, red blood cell count and haematocrit were 15-18% lower whereas white blood cell count was 35% lower than control level.

Nine days after the seventh blood sampling the haemoglobin, red blood cell and haematocrit levels were 18-22% higher than control level. The white blood cell level was not different from control level whereas the platelet level was 13% lower than control level.

No macroscopic lesion to the eye was observed in any of the animals.

Discussion

It is rather unlikely that the results obtained should be influenced by the immunological part of the study since this only included administration of an antigen. Only relatively slight anaemia was seen one day after the final blood sampling when compared to the control group. However, the levels obtained one day after final sampling were 29-35% lower than the levels found nine days after final sampling. At each blood sampling a volume of 0.5 ml was taken. This corresponds to about 30% of the total blood volume. Based on the results from the present study it is concluded that mice are capable to compensate the blood loss induced by weekly sampling of 0.5 ml per week for seven weeks.

Removal of a volume of this magnitude should according to the literature *(BVA/FRAME; RSPCA/UFAW working group,* 1993; *McGuill and Rowan,* 1989) have more severe consequences than seen in the present study.

Table 1. Haematology in mice 1 and 9 days after weekly blood sampling (0.5 ml/sample) for 6 - 7 weeks

Group	Haemoglobin (mmol/l)		Red blood cell count (10^{12}/l)		Haematocrit (ml/100 ml)		White blood cell count (10^9/l)		Platelet count (10^9/l)	
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
1 - Control (n=8)	9.5^b	0.5	9.30^b	0.53	44.0^b	2.1	7.4^b	1.4	758^{ab}	65
2 - Day 1 (n=8)	7.9^a	1.0	7.64^a	0.95	37.4^a	4.7	4.8^a	0.8	791^b	110
3 - Day 9 (n=8)	11.2^c	0.9	11.01^c	1.13	53.5^c	5.0	7.4^b	1.5	660^a	113

Mean = mean value of eight animals
Mean values with different letters are statistically significantly different ($p<0.05$)
S.D. = standard deviation

References:

BVA/FRAME/RSPCA/UFAW working group: Removal of blood from laboratory mammals and birds. Laboratory Animals, *27*, 1-22, 1993.

Kjærsgaard, C.J.: Personal communication. Chairman Danish Animal Experiments Inspectorate, 1997.

McGuill, M.W, & Rowan, A.N.: Biological effects of blood loss: Implications for sampling volumes and techniques. ILAR News, *31*, 5-18, 1989.

Van Herck, H., De Boer, S.F., Hesp, A.P.M., Van Lith, H.A., Baumans, V. & Beynen, A.C.: Orbital bleeding in rats while under diethylether anaesthesia does not influence telemetrically determined heart rate, body temperature, locomotor and eating activity when compared with anaesthesia alone. Laboratory Animals, *31*, 271-278, 1997.