Orbital sinus blood sampling in rats as performed by different animal technicians: the influence of technique and expertise

H. van Herck[1], V. Baumans[2], C. J. W. M. Brandt[1], A. P. M. Hesp[1], J. H. Sturkenboom[1], H. A. van Lith[2], G. van Tintelen[3] & A. C. Beynen[2]

[1]Central Laboratory Animal Institute (GDL), Utrecht University, [2]Department of Laboratory Animal Science, Faculty of Veterinary Medicine, Utrecht University and [3]Laboratory Animals Centre, Agricultural University, Wageningen, The Netherlands

Summary

In this study the influence of orbital sinus blood sampling on clinical signs was studied within the framework of various nutritional experiments. In order to assess the clinical signs in a random design, the rats were punctured in either the left or the right orbit. Thus, the effect of puncture within rats could be determined by comparing the left and right eye. Four animal technicians punctured a total of 303 rats, using different techniques. Orbital sinus blood sampling caused clinically visible alterations. The type, frequency and prognosis of the alterations differed with the person performing the puncture. Two experienced animal technicians were able to perform the technique without causing a statistically significant increase in alterations in punctured orbits. One less experienced animal technician caused severe abnormalities. The use of either a Pasteur pipette or a haematocrit capillary did not necessarily produce different results. Neither did puncturing the lateral *vs* the medial canthus of the orbit. By not applying chloramphenicol eye ointment in the conjunctival sac after puncture, the number of abnormalities in 'ocular discharge' and 'corneal alterations' in the punctured orbits was significantly decreased. Four punctures in the same orbit with 14-day intervals by a skilled animal technician did not cause a significant increase in abnormalities.

Keywords Blood collection; orbital sinus blood sampling; rodent; rat; clinical lesions

Orbital sinus blood sampling is a technique used frequently in rats, but it is controversial, particularly due to arguments of an emotional nature (Van Herck *et al.* 1992a). In The Netherlands, diethyl ether is often chosen to anaesthetize rodents before collecting blood from the orbital venous plexus (Van Herck *et al.* 1992a). The behaviour of rats after orbital sinus blood sampling under light diethyl ether anaesthesia, as performed by a skilled animal technician in the medial canthus of the orbit, was studied previously in an open field (Beynen *et al.* 1988), and telemetrically as diurnal locomotor activity and eating pattern (Van Herck *et al.* 1997). In those studies, the behaviour of punctured rats did not differ from that of those treated with only diethyl ether. The clinical condition of rats after a singular orbital puncture in the medial canthus of the orbit by an experienced animal technician showed no alterations, apart from a possibly higher incidence of enophthalmia in the punctured eyes (Beynen *et al.* 1987).

Correspondence to: H. van Herck, PO Box 80.190, NL 3508 TD Utrecht, The Netherlands. Fax: +31 30 2 510964, E-mail: H.VanHerck@GDL.UU.NL

Accepted 18 May 1998

It is debatable whether puncture should be performed at the medial or lateral canthus of the orbit, or at the dorsal aspect of the eye (Morton *et al.* 1993, Timm 1979). Also, the skill of persons and the admissibility of multiple punctures in the same orbit are thought to be important issues when collecting blood in this way (Morton *et al.* 1993).

This study presents the clinically visible alterations caused by orbital sinus blood sampling in outbred Wistar rats. All rats described here were used in current nutritional experiments, implying that no animals and no blood samples were taken for this study alone. All punctures were performed while the rats were under diethyl ether anaesthesia. This paper consists of four parts. In the first part we looked at the influence of a singular orbital puncture as performed by four animal technicians, who differed with respect to the technique used and/or experience. In the second part we studied the effect of orbital puncture performed in the lateral versus the medial canthus of the orbit. In the third part the influence of applying chloramphenicol eye ointment was determined. In the fourth part we studied the clinical signs after multiple puncturing.

Materials and methods

The appropriate animal ethics committees had approved the experimental protocols.

Animals and housing

HsdCpb:WU rats in current nutritional experiments were used either at the Utrecht University or at the Wageningen Agricultural University. After acclimatization, animals were housed individually under conventional conditions. The cages were placed in a room with controlled temperature (20–24°C), relative humidity (40–60%), lighting (12 h light) and ventilation (15–20 air changes/h). Further details are given in Table 1 and the various papers describing the nutritional experiments for which the rats were used. Experimental groups fed magnesium-deficient diets (Bergstra *et al.* 1993) were excluded. At the beginning of the experiments (at the age of 5 weeks), the rats were punctured while they were under light diethyl ether anaesthesia. About 1 ml of blood was collected per puncture.

Part I: Comparison of clinical signs after single puncture as performed by four animal technicians

Experimental design Each technician had performed the punctures in his routine way. To prevent bias during the clinical observations, the animals were punctured in random order in either the left or the right orbit. This implied that the technicians had to puncture at a site that was not routine. Additional information is listed in Table 1. After puncture, animal technician D applied chloramphenicol eye ointment in the conjunctival sac of the punctured orbit. Animal technicians A, B and C did not. Upon recovery after puncture, animals were put back into their home cage.

All rats were clinically examined by two experienced veterinarians on days 2, 7 and 14 after puncture, the day of puncture being day 0. Animals were examined in random order and blinded with respect to the site of the puncture. Examination took place between 09:00 h and noon. Assessors independently examined the animals outside their home cage in the animal room. The following variables were scored as 'normal' or 'abnormal' by gross observation: eye position, ocular discharge, nasal discharge, corneal alterations and intra-ocular alterations. If abnormalities were found, the orbit or nose side (left or right) considered abnormal was identified. If the eye position was found to be abnormal (i.e. left and right eyeball not equally deep in the orbit), the type of abnormality (en- or exophthalmia) was scored.

Part II: Comparison of puncture in the lateral versus medial canthus of the orbit

Animals were punctured by animal technician D in either the lateral or the medial canthus of the orbit (Table 1). No chloramphenicol eye ointment was applied after puncture. Animals were examined on days 2

Table 1 Main characteristics of the punctured orbits studied as classified per animal technician

	Technician A		Technician B		Technician C		Technician D		Technician D			
Reference[1]	Beynen *et al.* 1996		Suprijana *et al.* 1997		Veenendaal *et al.* 1992		Bergstra *et al.* 1993		Zhang & Beynen 1993			
Location of animal house	Wageningen University		Utrecht University		Utrecht University		Utrecht University		Utrecht University			
Cage type	Stainless steel cages with grid floor[2]		Metabolism cage[3]		Metabolism cage[3]		Metabolism cage[3]		Metabolism cage[3]			
Type of diet	Mash		Powder		Powder		Powder		Powder			
Puncture needle	Haematocrit capillary[4]		Pasteur pipette[5]		Haematocrit capillary[6]		Micro haematocrit capillary[6]		Micro haematocrit capillary[6]			
Number of punctures/rat	4		1		1		1		1			
Gloveticol eye ointment[7]	No		No		No		Yes		No			
Punctured canthus of orbit	Medial		Medial		Lateral		Lateral		Lateral		Medial	
Punctured orbit[8]	Routine	Non-routine	Routine	Non-routine	Routine	Non-routine	Routine	Non-routine	Routine	Non-routine	Routine	Non-routine
Number of rats	38	34	25	25	25	25	15	17	24	25	25	25
Frequency[9]	Weekly	Incidental	Weekly	Incidental	Weekly	Never	Weekly	Incidental	Weekly	Incidental	Never	Never
Years of experience	> 10 years		> 10 years		> 10 years		> 10 years		> 10 years			

[1] Nutritional study in which the rats were used
[2] Stainless steel cage: type SKS4 (RUCO metaalindustrie, Valkenswaard, The Netherlands)
[3] Metabolism cage: type 3701MO-000 (Tecniplast Gazzada, Buguggiate, Italy)
[4] End-to-end minicaps 75 mm / 60 μl (Hirschmann Labogeräte, Germany)
[5] Pasteur pipette: QTE 250, length 150 mm (Bilbate, Daventry, England)
[6] Heparinized capillary tubes, type BS4316 (Hawksley & Sons Ltd, Lancing, England)
[7] Gloveticol eye ointment: 10 mg chloramphenicol in 5 g (Mycofarm, de Bilt, The Netherlands)
[8] For each technician either the left or the right eye was the site of routine orbital sinus blood sampling
[9] The common-practice frequency by which orbital sinus blood sampling was carried out for the routine or non-routine site

Table 2 Comparison of the results of four animal technicians after the rats had undergone a single orbital sinus blood sampling. Results are shown as relative frequencies (%) of scores per variable

		Technician A									Technician B								
		Non-punctured orbits (*n* = 72)			Punctured orbits[4] Routine site (*n* = 38)			Punctured orbits[4] Non-routine site (*n* = 34)			Non-punctured orbits (*n* = 50)			Punctured orbits Routine site (*n* = 25)			Punctured orbits Non-routine site (*n* = 25)		
Variable	Day	<0[1]	0[2]	>0[3]	<0	0	>0	<0	0	>0	<0	0	>0	<0	0	>0	<0	0	>0
Eye position	2		100		1	99			100			100			100		4	94	2
	7		100		5	95		6	94			100			100		2	98	
	14		100			100		1	99			100			100			100	
Ocular discharge	2		100			99	1		99	1		97	3		94	6		96	4
	7		100			97	3		100			100			100			100	
	14		100			100			97	3		100			100			100	
Nasal discharge	2		97	3		93	7		100			86	14		66	34		68	14
	7		83	17		71	29		93	7		97	3		90	10		94	6
	14		99	1		96	4		100			93	7		94	6		96	4
Corneal alterations	2		100			100			100			100			100			100	
	7		100			100			100			100			100			100	
	14		100			100			100			100			100			100	
Intra-ocular alterations	2		100			100			100			100			96	4		94	6
	7		100			100			100			100			100			100	
	14		100			100			100			100			100			100	

		Technician C									Technician D								
		Non-punctured orbits (*n* = 50)			Punctured orbits Routine site (*n* = 25)[5]			Punctured orbits Non-routine site (*n* = 25)[5]			Non-punctured orbits (*n* = 32)			Punctured orbits (eye ointment) Routine site (*n* = 17)			Punctured orbits (eye ointment) Non-routine site (*n* = 15)		
Variable	Day	<0	0	>0	<0	0	>0	<0	0	>0	<0	0	>0	<0	0	>0	<0	0	>0
Eye position	2		100		4	92	4		94	6		97	3	3	82	15	7	93	
	7		100			96			94	2		100		3	94	3	17	80	3
	14		100			96		4	92			100		6	94		10	83	7
Ocular discharge	2		100			82	18		84	16		98	2		79	21		83	17
	7		100			90	6		92	4		100			79	21		90	10
	14		100			88	8		92	4		100			100			83	17
Nasal discharge	2		91	9		80	20		76	24		89	11		44	56		93	7
	7		97	3		92	8		90	10		98	2		68	32		100	
	14		98	2		94	6		94	6		98	2		97	3		100	
Corneal alterations	2		100			92	8		92	8		98	2		85	15		77	23
	7		100			92	4		92	4		100			88	12		70	30
	14		100			90	6		92	4		100			94	6		67	33
Intra-ocular alterations	2		100			90	10		92	8		100			100			93	7
	7		100			90	6		94	2		100			94	6		86	14
	14		100			94	2		96			98	2		94	6		77	23

[1] <0 = enophthalmia (only for variable 'eye position'). [2] 0 = normal (all variables). [3] >0 = exophthalmia (for variable 'eye position') or abnormal (for variables 'ocular discharge', 'nasal discharge', 'corneal alterations', 'intra-ocular alterations'). [4] On day 7 only one veterinarian scored the animals. [5] Because of severe eye lesions, in both the routine site and the non-routine site groups one animal was euthanized on day 2 after scoring

and 7 after puncture, as described above in part I.

Part III: Comparison of puncture with versus without eye ointment

To assess the effect of applying the chloramphenicol eye ointment, the rats punctured by animal technician D in the lateral canthus of the orbit described in part II were compared with the rats punctured by the same animal technician, described in part I.

Part IV: One animal technician, four subsequent punctures per rat

The animals punctured by animal technician A were punctured four times in the same way, in the same orbit, at 14-day intervals. Subsequent punctures were performed after clinical observations 14 days after the previous puncture had taken place.

Statistical analyses

After assessment of the variables, the code (site of punctured orbit) was broken and the results were subjected to statistical analysis. Log-linear models were formulated for the analysis of categorical data. These models are useful for uncovering the potentially complex relationships among the variables in a multi-way cross tabulation. Therefore, for each part of this study, a Hierarchical Log Linear test (i.e. a log-linear model) was performed to detect possible associations between the examined variables and the various factors. Since log-linear analysis does not distinguish between independent and dependent variables, the number of cases in parts I, II, III and IV are in fact multiplied by 12 (two orbits/sides of the nose, two assessors, three time points), 8 (two orbits/sides of the nose, two assessors, two time points), 8 (two orbits/sides of the nose, two assessors, two time points), and 48 (two orbits/sides of the nose, two assessors, three time points, four punctures), respectively. In the statistical analyses the factors group of rats, time, nutritional experiment, location of animal house and puncture needle were all considered to be a part of the factor animal technician. To take into account the greater probability of a type I error due to the multiplications, the Chi-square was adjusted; i.e. divided by 12, 8, 8 and 48 respectively. For the sake of simplicity only two-way associations (i.e. between a variable and one factor) were examined. The level of statistical significance was pre-set at $P < 0.05$ throughout this study. All statistical analyses were carried out according to Steel and Torrie (1981) using a SPSS PC^{+} computer program (SPSS 1990).

Results

The scores of the assessors did not differ significantly, except for the variable 'nasal discharge' in part IV. For this reason the variables are presented as averages of both assessors (Tables 2, 3, 4, 5), except for 'nasal discharge' in part IV (Table 5).

Part I: Comparison of clinical signs after single puncture as performed by four animal technicians (Table 2)

As would be expected, more abnormalities were found in the punctured orbits than in the non-punctured orbits. Two of the animals punctured by technician C were euthanized on day 2 after clinical examination, because of severe eye lesions.

In the punctured orbits as to eye position animal technician A caused enophthalmia only. The other three animal technicians caused both en- and exophthalmia. All four animal technicians induced ocular discharge. Compared with the non-punctured orbits, this increase reached the level of statistical significance (DF 1, adjusted χ^2 5.489). With respect to nasal discharge none of the factors studied had a significant effect. Corneal lesions were not found in animals punctured by animal technicians A and B. Animal technicians C and D caused statistically significantly more corneal lesions than technicians A and B (DF 3, adjusted χ^2 10.103). Compared with the non-punctured orbits, the increase in corneal lesions after puncture reached statistical significance (DF 1, adjusted χ^2 5.897). Intra-ocular alterations were not found in the orbits punctured by animal technician A. Hardly any were present in the orbits punctured by animal technician B.

Table 3 Comparison of rats punctured in either the lateral or medial corner of the eye (animal technician D). Results are shown as relative frequencies (%) of scores per variable

					Punctured orbits No eye ointment											
		Non-punctured orbits (*n* = 99)			Routine site						Non-routine site					
					Lateral (*n* = 24)			Medial (*n* = 25)			Lateral (*n* = 25)			Medial (*n* = 25)		
Variable	Day	<0[1]	0[2]	>0[3]	<0	0	>0	<0	0	>0	<0	0	>0	<0	0	>0
Eye position	2		99	1		100			100			100			100	
	7		99	1		96	4		100			100			100	
Ocular discharge	2		100			100			100			98	2		100	
	7		100			96	4		100			100			96	4
Nasal discharge	2		96	4		94	6		98	2		94	6		96	4
	7		96	4		90	10		94	6		96	4		98	2
Corneal alterations	2		100			100			100			98	2		100	
	7		100			100			100			100			100	
Intra-ocular alterations	2		99	1		100			94	6		94	6		92	8
	7		100			98	2		100			100			100	

[1]<0 = enophthalmia (only for variable 'eye position'). [2]0 = normal (all variables). [3]>0 = exophthalmia (for variable 'eye position') or abnormal (for variables 'ocular discharge', 'nasal discharge', 'corneal lesions', 'intra-ocular alterations')

Table 4 Effects of applying eye ointment in the conjunctival sac directly after orbital bleeding. Animals were punctured by animal technician D in the lateral corner of the eye. Results are shown as relative frequencies (%) of scores per variable.

		Eye ointment									No eye ointment								
					Punctured orbits									Punctured orbits					
		Non-punctured orbits (*n* = 32)			Routine site, lateral (*n* = 17)			Non-routine site, lateral (*n* = 15)			Non-punctured orbits (*n* = 50)			Routine site, lateral (*n* = 25)			Non-routine site, lateral (*n* = 25)		
Variable	Day	<0[1]	0[2]	>0[3]	<0	0	>0	<0	0	>0	<0	0	>0	<0	0	>0	<0	0	>0
Eye position	2		97	3	3	82	15	7	93			99	1		100			100	
	7		100		3	94	3	17	80	3		99	1		96	4		100	
Ocular discharge	2		98	2		79	21		83	17		100			100			98	2
	7		100			79	21		90	10		100			96	4		100	
Nasal discharge	2		89	11		44	56		93	7		94	6		94	6		94	6
	7		98	2		68	32		100			93	7		90	10		96	4
Corneal alterations	2		98	2		85	15		77	23		100			100			98	2
	7		100			88	12		70	30		100			100			100	
Intra-ocular alterations	2		100			100			93	7		99	1		100			94	6
	7		100			94	6		86	14		100			98	2		100	

[1]<0 = enophthalmia (only for variable 'eye position'). [2] 0 = normal (all variables). [3] >0 = exophthalmia (for variable 'eye position') or abnormal (for variables 'ocular discharge', 'nasal discharge', 'corneal lesions', 'intra-ocular alterations')

Table 5 Effects of four subsequent punctures per rat (animal technician A). Results are shown as relative frequencies (%) of scores per variable

			Non-punctured orbits ($n=72$)			Punctured orbits: Routine site ($n=38$)			Punctured orbits: Non-routine site ($n=34$)		
Variable	P #	Day (Ass.)	< 0[1]	0[2]	> 0[3]	< 0	0	> 0	< 0	0	> 0
Eye position	1	2		100		1	99			100	
		7[4]		100		5	95		6	94	
		14		100			100		1	99	
	2	2[4]		100		5	95		3	97	
		7		100		1	99		1	99	
		14		100		1	99			100	
	3	2		100		3	97			100	
		7		100		4	95	1		100	
		14		100		5	93	3		100	
	4	2		100		5	95			100	
		7		100		3	97			100	
		14		100		4	95	1		100	
Ocular discharge	1	2		100			99	1		99	1
		7[4]		100			97	3		100	
		14		100			100			97	3
	2	2[4]		100			92	8		100	
		7		100			92	8		100	
		14		99	1		97	3		100	
	3	2		99	1		99	1		99	1
		7		99	1		97	3		100	
		14		100			100			100	
	4	2		100			97	3		100	
		7		100			100			97	3
		14		100			95	5		100	
Nasal discharge	1	2 (A)		94	6		92	8		100	
		2 (B)		100			95	5		100	
		7 (A)		73	17		71	29		97	3
		7[4] (B)		-			-			-	
		14 (A)		97	3		92	8		100	
		14 (B)		100			100			100	
	2	2[4] (A)		-			-			-	
		2 (B)		100			100			100	
		7 (A)		76	14		79	21		90	10
		7 (B)		100			100			100	
		14 (A)		77	13		74	16		75	15
		14 (B)		100			100			100	
	3	2 (A)		94	6		92	8		100	
		2 (B)		100			100			100	
		7 (A)		92	8		95	5		97	3
		7 (B)		100			100			100	
		14 (A)		93	7		72	18		100	
		14 (B)		100			100			100	
	4	2 (A)		75	15		74	16		72	18
		2 (B)		100			100			100	
		7 (A)		76	14		77	13		72	18
		7 (B)		100			100			100	
		14 (A)		75	15		79	11		91	9
		14 (B)		99	1		100			100	

Continued

Table 5 Continued

Variable	P #	Day (Ass.)	Non-punctured site (*n* = 72)			Punctured orbits: Routine site (*n* = 38)			Punctured orbits: Non-routine site (*n* = 34)		
			< 0[1]	0[2]	> 0[3]	< 0	0	> 0	< 0	0	> 0
Corneal alterations	1	2		100			100			100	
		7[4]		100			100			100	
		14		100			100			100	
	2	2[4]		100			92	8		100	
		7		100			95	5		100	
		14		100			95	5		100	
	3	2		100			95	5		100	
		7		100			95	5		100	
		14		100			97	3		100	
	4	2		100			95	5		100	
		7		99	1		96	4		100	
		14		100			95	5		100	
Intra-ocular alterations	1	2		100			100			100	
		7[4]		100			100			100	
		14		100			100			100	
	2	2[4]		100			95	5		100	
		7		100			95	5		100	
		14		100			95	5		100	
	3	2		100			95	5		100	
		7		100			96	4		100	
		14		100			99	1		100	
	4	2		100			99	1		100	
		7		100			97	3		100	
		14		100			100			100	

[1]<0 = enophthalmia (only for variable 'eye position'). [2]0 = normal (all variables). [3]>0 = exophthalmia (for variable 'eye position') or abnormal (for variables 'ocular discharge', 'nasal discharge', 'corneal lesions', 'intra-ocular alterations'). [4] On day 7 (puncture 1) and day 2 (puncture 2) animals were scored by only one of the two veterinarians

Their occurrence was higher in the orbits punctured by animal technicians C and D, but this technician difference did not reach the level of statistical significance.

Part II: Comparison of puncture in the lateral versus medial canthus of the orbit (Table 3)

No statistically significant associations were found between the variables scored and the factor site of puncture within the orbit.

Part III: Comparison of puncture with versus without eye ointment (Table 4)

In all variables scored, higher frequencies of alterations occurred in the orbits treated with chloramphenicol eye ointment after puncture. However, only the variable 'corneal alterations' reached statistical significance (DF 1, adjusted χ^2 3.744), whereas for the variable 'ocular discharge' the effect was of borderline statistical significance (DF 1, adjusted χ^2 3.685). Compared with the non-punctured orbits, the increase in ocular discharge reached borderline statistical significance only (DF 1, adjusted χ^2 3.699), but the increase in corneal lesions after puncture was significant (DF 1, adjusted χ^2 3.949).

Part IV: One animal technician, four subsequent punctures per rat (Table 5)

In the punctured orbits the frequency of abnormalities was higher than in the non-punctured orbits, but this difference did not reach the level of statistical significance.

There were no statistically significant associations between the eye position, ocular discharge, corneal alterations or intra-ocular alterations and the factor number of punctures per orbit. For nasal discharge there was a significant difference between assessors (DF 1, adjusted χ^2 4.384).

Discussion

Collecting blood inevitably damages the tissues punctured. The type of tissues that will be damaged by orbital sinus blood sampling and the degree of damage depends on the technique, including the type of puncture needle, and the location, direction and depth of puncture (Van Herck *et al.* 1992b). Also the skill of the person performing the puncture and the method of immobilization of the animal punctured are expected to be important. Abnormalities may occur not only in the tissues punctured, but also in adjacent tissues such as the corneal conjunctiva. Direct damage can be caused by the invasiveness of blood collection, but secondary damage can also occur as a result of the animal grooming injured tissues. In addition, conditions such as disturbed blood flow (arterial and/or venous) and inflammatory reactions may cause alterations in the orbital region after puncture. Occurring lesions may heal without remnants (Krinke *et al.* 1988, Van Herck *et al.* 1992b), but may also cause scar tissue or loss of function (Le Net *et al.* 1994).

The frequency of induced abnormalities differed between animal technicians. When all the variables were combined, technicians A and B had the best results and technicians C and D the worst. Animal technicians A (Tables 2 and 5) and B (Table 2) caused only a few abnormalities. Those abnormalities found were mainly related to the puncture channel ('eye position' and 'ocular discharge') and were clinically (almost) healed 14 days after puncture. This coincides with results from previous clinical (Beynen *et al.* 1987) and histological (Van Herck *et al.* 1992b) observations, in which technicians A and B participated. Technicians C and D had less favourable results (Table 2). As to the variable 'corneal alterations' the difference between technicians was statistically significant. The alterations seemed to persist for a longer period of time. Furthermore, two of the animals punctured by technician D were euthanized because of the severity of their eye lesions. All of this indicates that the lesions caused by animal technicians C and D were more severe than the alterations caused by animal technicians A and B. Corneal tissue is one of the most sensitive tissues of the body (FELASA 1992). The type and severity of the lesions indicates that a number of animals have probably experienced pain and/or discomfort after puncture by technicians C and D (Table 2).

Orbital sinus blood sampling can be performed in numerous different ways, a few aspects being listed in Table 1. The results obtained for technicians A and B (Table 2) give no reason for prefering a Pasteur's pipette to a haematocrit capillary or vice versa. Also, no differences were found between puncturing either the lateral or the medial canthus of the orbit (Table 3). Leaving out the chloramphenicol eye ointment improved the results of technician D dramatically (Table 4). No direct adverse effects of chloramphenicol eye ointment are known in animals (M. H. Boevé, Veterinary Ophthalmologist, Faculty of Veterinary Medicine, Utrecht University, personal communication). It is likely that the eye lesions found after applying the eye ointment were caused by extra grooming. This may be induced by the ointment itself, sticking of the powdered diet (Table 1) to the eye ointment, and/or by a direct effect of the powdered diet on the conjunctiva. Comparison of the results of animal technicians A (mash diet) and B (powdered diet), indicates that use of a powdered diet alone does not cause a higher number of alterations after orbital sinus blood sampling.

An overall comparison of the four animal technicians (including the experiment in which animal technician D did not apply chloramphenicol eye ointment) might indicate that the frequency with which persons perform orbital sinus blood sampling (Table 1) is an important factor in reducing the number and severity of alterations. Skilled animal technicians performed equally well for the site of orbit punctured routinely as for that punctured non-routinely. Four subsequent punctures by technician A in the same orbit at 14-day intervals did not cause an increase in alterations that reached the level of statistical significance. The alterations caused by the second puncture decreased in the following weeks and were (almost) healed at the end of the 8-week period of the experiment, even though the orbit was punctured another two times within that period.

Acknowledgment The authors are grateful to Dr Ir. J. A. J. Faber (Center for BioStatistics, Faculty of Veterinary Medicine, Utrecht University) for his highly appreciated advice on statistical analyses.

References

Bergstra AE, Lemmens AG, Beynen AC (1993) Dietary fructose *vs* glucose stimulates nephrocalcinogenesis in female rats. *Journal of Nutrition* **123**, 1320–7

Beynen AC, Baumans V, Haas JWM, Van Hellemond KK, Stafleu FR, Van Tintelen G (1987) Assessment of discomfort induced by orbital puncture in rats. In: *New Developments in Biosciences: their Implications for Laboratory Animal Science* (Beynen AC, Solleveld HA, eds). Dordrecht: Martinus Nijhoff Publishers, pp 431–6

Beynen AC, Van Tintelen G, Baumans V (1988) Orbital puncture may not influence open field behaviour in rats. *Zeitschrift für Versuchstierkunde* **31**, 121–3

Beynen AC, Weusten-Van der Wouw MPME, De Roos B, Katan MB (1996) Boiled coffee fails to raise serum cholesterol in hamsters and rats. *British Journal of Nutrition* **76**, 755–64

FELASA (1992) Report of the working group on pain and distress in laboratory rodents and lagomorphs. *Laboratory Animals* **28**, 97–112

Krinke A, Kobel W, Krinke G (1988) Does the repeated orbital sinus puncture alter the occurrence of changes with age in the retina, the lens, or the Harderian gland of laboratory rats? *Zeitschrift für Versuchstierkunde* **31**, 111–19

Le Net JL, Abbott DP, Mompon RP, Leblanc B (1994) Repeated orbital sinus puncture in rats induces damages to optic nerve and retina. *Veterinary Pathology* **31**, 621

Morton DB, Abbot D, Barclay R, *et al.* (1993) Removal of blood from laboratory mammals and birds. *Laboratory Animals* **27**, 1–22

SPSS (1990) SPSS/PC^{+TM} 4.0 Advanced Statistics Manual for the IBM/PC/XT/AT and PS/2V (Release 4.0). Chicago, IL: SPSS Inc

Steel RGD, Torrie JH (1981) *Principles and Procedures of Statistics. A Biometrical Approach*, 2nd edn. Singapore: McGraw-Hill

Suprijana O, Terpstra AHM, Van Lith HA, *et al.* (1997) Plasma lipids and apolipoproteins in rats fed diets with type of fat (fish oil versus corn oil) and fiber (pectin versus cellulose) as variables. *Nutrition Research* **17**, 1187–97

Timm KI (1979) Orbital venous anatomy of the rat. *Laboratory Animal Science* **29**, 636–8

Van Herck H, De Boer SF, Hesp APM, Van Lith HA, Baumans V, Beynen AC (1997) Orbital bleeding in rats while under diethyl ether anaesthesia does not influence telemetrically determined heart rat, body temperature, locomotor and eating activity when compared with anaesthesia alone. *Laboratory Animals* **31**, 271–8

Van Herck H, Baumans V, Stafleu FR, Beynen AC (1992a) A questionnaire-based inventory of the orbital puncture method in the Netherlands. *Scandinavian Journal of Laboratory Animal Science* **19**, 189–96

Van Herck H, Baumans V, Van der Craats NR, *et al.* (1992b) Histological changes in the orbital region of rats after orbital puncture. *Laboratory Animals* **26**, 53–8

Veenendaal M, Zhang X, Lemmens AG, Beynen AC (1992) Liver and plasma copper concentrations in rats fed diets containing various proteins. *Biological Trace Element Research* **34**, 213–18

Zhang X, Beynen AC (1993) Lowering effect of dietary milk-whey protein *vs* casein on plasma and liver cholesterol in rats. *British Journal of Nutrition* **70**, 139–46