

A questionnaire-based inventory of the orbital puncture method in the Netherlands

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Introduction

Orbital puncture is frequently used to collect blood from the retro-ocular blood vessels. In the Netherlands, and also in other European countries, there is considerable controversy and concern as to this technique. Debatable questions relate to extent and severity of lesions induced and the impact of orbital puncture on welfare of the animals. In attempt to gain information on these points a number of experiments has been carried out (*Beynen et al.* 1988, *Beynen, Van Tintelen & Baumans* 1988, *Van Herck et al.* 1991, *Van Herck et al.* 1992). We also collected information with the use of an inquiry among so-called laboratory animal welfare officers in the Netherlands. The Dutch animal welfare officer acts as an adviser for the institution where he/she is employed or of which he/she has the supervision, and also acts as a local inspector and as the contact between the institution and the governmental and regional inspectorate (*Van der Gul-*

den 1989). In the Netherlands, each institute being a licence holder for experiments on animals, has to appoint an animal welfare officer. The results of the inquiry are listed here. A preliminary report in Dutch has been published earlier (*Baumans et al.* 1988).

Inquiry

A questionnaire was sent to all thirty-one Dutch animal welfare officers; thirty officers responded. Eleven of the respondents had passed on the questionnaire of the individual laboratories falling under their supervision and returned the completed questionnaires as such. Twenty-nine returned one completed questionnaire covering the various laboratories falling under their supervision. We received 40 completed questionnaires. Unless indicated otherwise, the numbers given below refer to the number of completed questionnaires. The questions (translated from Dutch) are in italics.

- | | | |
|-----|--|----|
| 1a) | <i>Is orbital puncture (O. P.) being performed in your institute?</i> | |
| | Yes | 28 |
| | No | 12 |
| 1b) | <i>If not performed, what is the reason?</i> | |
| | No reason mentioned | 7 |
| | No need + emotional/ethical objections | 3 |
| | No need + scientific/technical objections | 1 |
| | No need | 1 |
| 1c) | <i>If performed, how often per year?</i> | |
| | No number mentioned | 7 |
| | Number mentioned | 21 |
| | The range of O. P.'s/year was 10–15000. | |
| | The total number of O. P.'s from the 21 questionnaires was 45000/year. | |

1d)	<i>In which animal species is O. P. being performed?</i>		
	Mouse		7
	Rat		2
	Mouse + rat		11
	Mouse + rat + hamster		3
	Mouse + rat + guinea pig		3
	Mouse + hamster		1
	Rat + guinea pig		1
2a)	<i>Is O. P. being performed in the same animal only once or more often?</i>		
	Only once		4
	More than once		12
	Once or more often		12
2b)	<i>If performed more than once, what is the interval between O. P.'s?</i>		
	< hour		2
	2 days		1
	4 days		3
	1-2 weeks		12
	3 weeks		4
	4-6 weeks		3
	> 2 months		3
2c)	<i>If performed more than once, is one eye used or both?</i>		
	One eye		15
	Both eyes		13
	In 10 questionnaires, alternating puncture of the two eyes was indicated.		
3)	<i>How much blood is being collected per puncture?</i>		
	<u>Species</u>	<u>Volume (ml)*</u>	
	Mouse	0.1	1
		0.2	4
		0.3	3
		0.4	2
		0.5	2
		1.0	2
		Rat	1.0
		1.5	3
		2.0	1
		3.0	1
	Guinea pig	1,5	1
		4.0	1
	No species mentioned	0.2	1
		0.3	1
		0.5	3
		0.6	1
		1.0	2
		2.0	1
	No volume mentioned		1
	* Maximum volumes are indicated.		

In several questionnaires more than one animal species was indicated so that the total number of answers exceeds that of 28.

- 4a) *Are the animals being anaesthetized prior to O. P.?*
- | | |
|-----------------------------|----|
| Always anaesthesia | 24 |
| Occasionally no anaesthesia | 3 |
| Never anaesthesia | 1 |
- 4b) *If no anaesthesia is being applied, what is the reason?*
- | | |
|---|---|
| Anaesthesia interferes with experiment | 3 |
| High mortality in mice when ether is used | 1 |
- O. P. in non-anaesthetized animals because anaesthesia would interfere with the objective of the experiment concerned rat experiments.
- 4c) *What is being used?*
- | <u>Species</u> | <u>Anaesthetic</u> | |
|----------------------|--------------------|----|
| Mouse | Ether | 8 |
| Rat | Ether | 1 |
| | Ketamine/xylazine | 1 |
| Guinea pig | Ketamine/xylazine | 1 |
| | Halothane | 1 |
| No species mentioned | Ether | 17 |

In several questionnaires more than one animal species was indicated so that the total number of answers exceeds that of 28.

In one questionnaire, the anaesthetic used for rats was mentioned to depend on the type of experiment, but no details were given.

- 5a) *Does O. P. cause complications?*
- | | |
|-----|----|
| Yes | 19 |
| No | 9 |
- 5b) *What kind of complications do you see and how often do they occur?*
- | <u>Species</u> | <u>Complication</u> | <u>Frequency</u> | |
|-----------------------|--------------------------------|------------------|---|
| Mouse | Blindness | 1-5 % | 3 |
| | Blindness/necrosis | < 5 % | 1 |
| | Death due to ether anaesthesia | < 5 % | 1 |
| Rat | Eye abnormalities | < 5 % | 1 |
| Mouse + rat | Inflammation | 1 % | 1 |
| | Blindness | < 5 % | 2 |
| | Blindness | 5-10 % | 1 |
| | Inflammation of lacrimal gland | < 5 % | 1 |
| | Haematoma | < 5 % | 1 |
| | Lesions of optical nerve | Not specified | 1 |
| | Corneal lesions | < 5 % | 1 |
| Mouse + rat + hamster | Blindness | 5-10 % | 1 |

Mouse + rat + guinea pig	Inflammation	< 5 %	1
	Blindness, haematoma	5-10 %	1
	Blindness, bleeding nose	< 5 %	1
Rat + guinea pig	Blindness	< 5 %	1

In one questionnaire, mortality in rats due to anaesthesia was mentioned, but the frequency was not given. Likewise, in one questionnaire perforation of the nasal septum in rats and mice was indicated.

- 6a) *Do employees of your institute(s) have objections against O. P.?*
- | | |
|-----|----|
| Yes | 16 |
| No | 12 |
- 6b) *What is the nature of objections?*
- | | |
|---------------------------------|----|
| Emotional/ethical objections | 13 |
| Scientific/technical objections | 2 |

In one questionnaire, the two types of objections were claimed to occur simultaneously.

Discussion

On the basis of the questionnaire, we assess that orbital puncture is being performed in the Netherlands more than 45000 times a year. In the Netherlands, about 1.1 million vertebrate animals are used per year for scientific purposes. Orbital puncture is used only in the smaller rodents such as mouse, rat, hamster and guinea pig. Often, an animal is punctured more than once, the period between successive punctures generally being one to two weeks. In about half of the cases, puncture is invariably being performed in the same orbit. Ether appeared to be the anaesthetic of choice, especially in mice. Ether anaesthesia produces a dramatic endocrine stress response in rats, which fully masks that caused by puncture (*Van Herck et al. 1991*). Three questionnaires indicated that, if necessary for the experiment, orbital puncture in rats is being performed without anaesthesia.

Orbital puncture appears to cause a wide array of complications. Most frequently blindness is observed. This can be seen in up to 5 % of the animals punctured. It is likely that skill of the executor and method of puncturing determine the frequency of complications. Histological changes in the orbital region of rats after puncture depend

on the puncturing technique used (*Van Herck et al. 1992*). We have reported that orbital puncture in rats may induce enophthalmia (*Beynen et al. 1988*).

In more than half of the institutes where orbital puncture is being performed, there are objections to this technique. Scientific and/or technical objections were not common. One technical objection was that blood collected by orbital puncture is unsuitable for coagulation research. The majority of the objections were of an emotional/ethical nature.

The results of this inquiry indicate that orbital puncture may cause pain and distress. The abundance of emotional and ethical objections to orbital puncture indicates that this technique also causes distress in the people performing and/or observing it. Thus, it appears important to investigate experimentally the impact of orbital puncture on the animal subjected to it and to improve the technique or seek for alternative blood sampling techniques.

Summary

To contribute to the assessment of the degree of discomfort in rodents as caused by orbital puncture, we made an inventory in the Netherlands on the basis of an inquiry. Orbital puncture is being performed in laboratory rodents more than 45000 times a year. Usually, ether is used as anaesthetic.

In about two-third of the institutes where orbital puncture is being performed, complications are noted. In 1 to 5 % of the animals punctured blindness can occur. In more than half of the institutes where orbital puncture is being performed there are objections to this technique. Which are essentially of an emotional/ethical nature.

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