ANIMAL MODELS IN CARDIOVASCULAR RESEARCH

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PREFACE

Currently in the U.S.A., and in some European countries, the pressure to sharply curtail animal studies has intensified. The shrill rhetoric and horror stories of the antivivisectionists have been toned down by the animal's rights groups. A more sophisticated approach, which effectively focuses and sustains public attention on the problem, has emerged. Animal welfare groups now appear in political forums, such as congressional hearings, armed with facts and figures, sometimes regretably true, or only slightly distorted. They are well versed in scientific terminology and capable of debating with scientists in a very calm and orderly manner. This approach has had a noticeable effect on politicians.

Regardless of pressure from animal welfare groups we, as scientists, must continue our work, but with constant appreciation and regard for the moral and ethical issues raised by animal research. The concept of "reverance for life" does not, a priori, preclude the use of animals to further scientific knowledge and understanding. It does demand an awareness of the importance and responsibility involved in using and/or taking the life of any creature. It is important to recognize that as much care must be taken in the choice and handling of animal models as in any other facet of research methodology.

It is possible, through institutional lab animal committees and the critical peer review process, to police ourselves against colleagues who violate social and professional standards for humane treatment of animals. We are now being "aided" in this task by legislated restrictions which will, certainly, become more and more stringent, unless our own policing efforts become more effective.

To become more sophisticated, more scientific and, therefore, less vulnerable to criticism in our use of animal models is a difficult task. Our information retrival systems and/or patience to keep searching are often lacking and we are

then doomed, through ignorance, to repeat work that has been done or, worse, to repeat work that has been done better. Through ignorance of comparative physiology, a less than appropriate species may be chosen for a particular study. Through ignorance of comparative anesthesiology inappropriate chemical restraint or anesthesia may invalidate results. It is to these problems that this text is addressed.

In a statement submitted to the House Subcommittee on Science, Research and Technology, Professor Earl H. Wood, while President of FASEB, made the following observations. "...Scientists do not, by definition, walk on water. They are human and there will be those who, either from defect of instinct or indifference to social and professional values, betray the spirit of their profession and the public trust. The scientist who finds himself in this category should be prepared to live with the censure of his peers and whatever penalties society sees fit to inflict. But he or she should be dealt with as an individual aberration..." (FASEB Newsletter, Vol. 14, No. 16, Nov., 1981). There is increasing political pressure being applied to find alternatives for animal studies. Again quoting from Professor Wood: "...The so-called alternative methods -- computer simulation, mathematical models, use of cell and tissue culture, etc. -- are, in fact, aids, adjuncts, supplements or shortcuts which help an investigator decide whether an experiment on an animal is likely to produce a useful result..."

CHAPTER 1

GENERAL PRINCIPLES OF ANIMAL SELECTION, PREOPERATIVE CARE, PREANESTHESIA, CHEMICAL RESTRAINT AND ANALGESIA

It seems obvious that physiological results cannot be obtained from pathological specimens. Despite this universally accepted axiom, the number of so-called physiological experiments conducted on animals incubating disease or obviously ill can be appalling. The importance of this point cannot be overemphasized. Animals used for physiological experiments must be held long enough in approved facilities to insure that they are not incubating infectious diseases. They should be vaccinated against the diseases likely to cause a problem in that species and they should be verified free of internal and external parasites prior to use. These precautions are expensive but miniscule compared to the overall cost of conducting experiments where the results are suspect because of the condition of the subject.

Physical examination:

Prior to use, the animal should be given a thorough physical examination. One of the most neglected aspects of this examination is some history, easily supplied by observant animal care personnel, of the appetite displayed by the animal and the character of the urine and feces. It is a rare animal that eats normally when it is ill. The physical examination should include an assessment of the rectal temperature, feasible in most species commonly used. A list of normal rectal temperatures is provided as Table 1.1.

The physical examination should also include an evaluation of the mucous membranes, with particular attention to abnormal discharges from the eyes and nose. Animals with inflamed

mucous membranes should not be used. Judicious use of the stethoscope can rule out the possibility of using an animal for cardiovascular studies with a congenital or acquired heart murmur, unless that model is of particular interest. Animals which originate from areas where heartworms or parasitic blood diseases are a problem must be shown to be free of these afflictions. It is probably a good idea to take an electrocardiogram, especially if one of the giant breeds, or giant breed-crosses is being used. Although congenital and acquired arrhythmias are relatively rare in dogs they do occur (see Chapter 11).

It may not be necessary to do a complete hematological evaluation on every animal, but in studies where extensive surgical preparation is necessary and when considerable time and money are to be invested in the model, such an evaluation could be essential. Tables 1.2-1.8 provide normal hematological data for most of the species now in common use. It should be pointed out that these normal values may vary with geographical location (i.e. sea level versus high altitude) and with prevalent breeds in the particular region as well as with gender and age. The values provided are from the Texas Veterinary Diagnostic Laboratory, the Clinical Pathology Laboratory of the Texas A&M Veterinary Teaching Hospital, our Laboratory of Physiology and Applied Physics and the Texas A&M Laboratory Animal Resources Facility.

The state of hydration is an important consideration in cardiovascular studies. All animals should be well hydrated before they are used and if the procedure or preparation of the model requires extensive surgery, or is of long duration, the state of hydration must be maintained during the course of the experimental procedure. Again, these considerations seem obvious and redundant but, unfortunately, they are often ignored or forgotten.

Most procedures in most species do not require that the animal be without water prior to the anesthesia. Free choice of water to a healthy subject should insure proper hydration. As a matter of convenience, and to prevent inspiration of ingesta, it is advisable to withhold food overnight or for 12 hours prior to general anesthesia.

Recent knowledge and understanding of blood coagulation and coagulopathies have created interest in hematological parameters related to this system. Platelet counts have been done on most species and found to range from about 2-5 X 10^5/ml. Cats, sheep and calves range slightly higher, from

about 3-8 X 10^5/ml. Other commonly conducted tests include activated prothrombin time (A-PTT), prothrombin time (PT), thrombin time and Fibrinogen levels. The use of the heat precipitation method for the latter results in falsely low values. The capillary tube clotting time is insensitive and limited to detection of severe clotting defects. Results of measurements of the other parameters will vary with; the volume of sample used, the incubation time, the commercial reagent used (including freshness), the anticoagulant used for sample collection and the concentration of CA^{++} present. There also appear to be optimal conditions for the assays which vary for each species. Most knowledgable researchers in this field seem to agree that, if these data are required, it is best to compare subjects with normal controls at the time and in the same lab where the evaluation will be made.

Table 1.1. Range of normal rectal temperatures for some commonly used animal species (references; 1, 2)

	^{o}C	^{o}F
Dogs	37.9-39.9	100.1-102.8
Cats	38.1-39.2	100.5-102.5
Cattle	36.7-39.1	98.0-102.4
Sheep	38.3-39.9	100.9-103.8
Goats	38.5-39.7	101.3-103.5
Horses and Ponies	37.2-38.2	99.0-100.8
Swine	38.7-39.8	101.6-103.6
Rabbits	38.6-40.1	101.5-104.2
Monkey, Rhesus	38.4	101.1
Guinea pigs	38.6	101.5
Hamster	36.38	98-101
Rats	37.5	99.5
Mice	37.4	99.3

Table 1.2. Averages (single values) or ranges of normal blood count data.

	WBC ($x10^3$)	Neutrophiles (%)	Lymphocytes (%)	Monocytes (%)	Eosinophiles (%)
Dogs	6-17	60-77	12-30	3-10	2-10
Cats	5.5-19.5	35-75	20-55	1-4	2-12
Rats	9.76	25.6	73.9	0.29	0.48
Mice	13.5	17.5	72.3	2.19	2.25
Rabbits	8.45	44.7	40.4	8.5	2.0
Hamsters	8.10	25.6	70.7	2.45	0.8
Guinea pigs	11.2	36.5	56.2	3.05	3.75
Pigs	14.8	34.0	55.5	4.3	0.24
Sheep	4-12	31.8	59.4	3.25	5.2
Goats	4-13	30-48	50-70	0-4	1.8
Horses	5.4-14.3	22-72	17-68	0-14	0-10
Calves	4-12	15-45	45-75	2-7	2-20
Primates (old world)	10.8	38.6	58.8	1.10	1.85
Primates (new world)	4.3-28.5	46-82	13-54	0-3	0-4

Table 1.3. Averages (single values) or ranges of normal red blood cell parameters.

	Hemato-crit (%)	Hemo-globin (g/100ml)	RBC ($X10^6$/mcl)	Mean Corpuscular volume (micron)^3	Mean Corpuscular Hemoglobin concen. (g%)
Dogs	35-55	12-18	5.5-8.5	60-77	32-36
Cats	24-45	3-15	5-10	39-55	30-36
Rats	45.8	14.2	8.47	56.0	31.1
Mice	41.8	11.1	9.2	49.25	26.5
Rabbits	40.6	13.4	6.51	62.8	32.8
Hamsters	50.7	16.4	7.35	70.0	32.3
Guinea pigs	43.7	14.3	5.18	84	32.8
Pigs	41.0	12.4	6.99	58.5	30.2
Sheep	27-45	9-15	9-15	28-40	31-34
Goats	23-38	8-12	8.18	15-30	35-42
Horses	32-53	11-19	6.8-12.9	37-58.5	31-38.6
Calves	24-46	9.5-12.5	5.2-6.8	49.7-64.3	30.2-35.8
Primates (old world)	27-44	10-13	5.17	82.0	31.9
Primates (new world)	17-48	5-12.5	1.97-4.22	54.8-15.4	15.4-38.5