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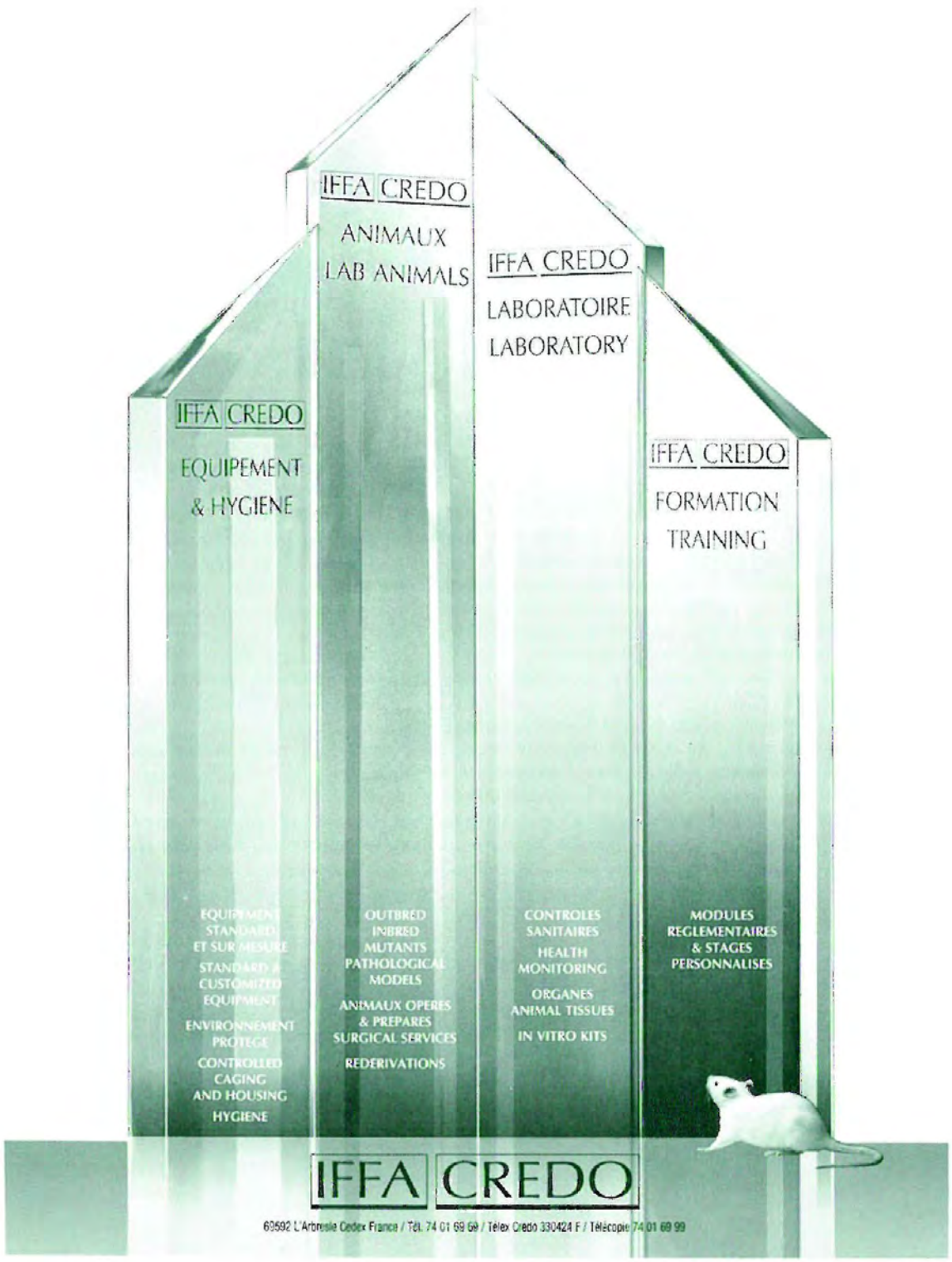

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New methods of anaesthesia and analgesia

Abstract

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The changes in the techniques used to anaesthetise laboratory animals which have occurred over the last five years have been influenced by several factors. The introduction of novel anaesthetic agents, such as propofol, have provided the opportunity to develop techniques of total intravenous anaesthesia in a range of species. Other agents, notably the alpha-2 adrenoreceptor agonists, such as medetomidine have enabled the introduction of reversible or partially reversible anaesthetic regimens.

A second major influence on choice of anaesthetic regimen has been the growing significance attached to the welfare of laboratory animals. Agents which do not provide a consistent level of surgical anaesthesia, or in which the margin of safety is so small that their use may be associated with increased anaesthetic mortality, have declined in popularity. Information concerning the undesirable side-effects of some agents, for example tissue reactions to intramuscular drug administration, or peritonitis after intraperitoneal dosing, have led to a re-evaluation of some methods of anaesthetic administration. Concern for animal well-being has also led to attempts to assess the stress or distress of induction of anaesthesia with volatile anaesthetic agents.

A further significant influence on anaesthetic methodology has been the increased availability of electronic monitoring apparatus at moderate cost. Irrespective of the anaesthetic agents used, effective monitoring of the animal during anaesthesia and in the post-operative period reduces both anaesthetic mortality and morbidity, and leads to an improvement in the quality of scientific data which is obtained.

In the area of pain relief, the introduction of new analgesics, notably potent non-steroidal anti-inflammatory agents such as carprofen and ketoprofen, have increased the scope for analgesics use in research animals. More significantly, more information concerning pain assessment is now becoming available. This information is essential if analgesic agents are to be used in a rational manner. Finally, novel approaches to analgesic administration have been explored, for example orally in food pellets or drinking water, or by intrathecal or epidural administration, and these techniques offer the possibility of providing prolonged periods of pain relief following surgical procedures.

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Evaluating pain and distress in laboratory animals

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Introduction

The FELASA Working Group on Pain and Distress has (*FELASA* 1994) published the results of its deliberations on pain and distress in laboratory rodents and lagomorphs. These animals account for the vast majority of vertebrates used in experimentation and there is a legal and moral requirement throughout the European Union to balance pain and distress suffered by animals against the intended scientific gain from experimentation. The FELASA document (op. cit.) is intended to be easily accessible but is backed by a substantial body of reference material.

Defining pain, distress and suffering

Defining the essentially human concepts of pain, distress and suffering is inherently difficult especially when dealing with such experiences in non-human animals. 'Pain' should be limited to physical or nociceptive pain in the sense used by the *International Association for the Study of Pain* (1979) namely that 'pain is an unpleasant sensory and emotional experience associated with actual or potential damage or described in terms of such damage'. 'Distress' should be defined as advocated by *UFAW* (1989) as 'a state where the animal has to devote substantial effort or resources to the adaptive response to challenges emanating from the environmental situation'. States such as 'anxiety', 'frustration', 'depression' and 'discomfort' can be regarded as being encompassed by distress. Interestingly, there is recent evidence that apparently minor husbandry problems (including switching lights on at variable times, exposing animals to unpredictable noise and tilting their caging) which render the environment unpredictable, impair the coping responses of rodents and induce a depression-like state in rats and mice. These procedures must essentially cause distress. 'Suffering' is a specific state of 'mind' which may result from pain or distress if they are of sufficient intensity or duration or both. Obviously suffering necessarily results in detrimental effects such as retarded growth, impaired breeding and inadequate body care and is something to be avoided wherever possible.

Pain mechanisms

There are a variety of peripheral and CNS mechanisms involved in processing nociceptive (pain-related) signals. The existence of these complex mechanisms confirm that pain is not a single sensation. All mammals have the capacities for perceiving and experiencing pain as well as remembering situations associated with this sensation. Although rodents and lagomorphs probably have a lesser capacity for making advanced interpretations of a pain situation than, for example, humans and apes, we should, in general, give the animal the benefit of the doubt whenever possible, in attempting to ameliorate effects of procedures and husbandry.

Measurement of analgesia and environmentally-induced analgesias

A number of techniques (*Brain* 1992) have been employed to assess the efficacy of analgesic compounds in rodents and lagomorphs. Only some (notably the radiant-heat tail flick and the hot plate test) are likely to prove ethically acceptable and useful in assessing the impact of husbandry and surgical techniques that might alter pain sensitivity. An enormous range of environmental stimuli have been shown to alter pain sensitivity in rodents (so-called environmentally-induced analgesias). It will for

this reason be extraordinarily difficult to rate the severity of procedures employing laboratory animals using additive numerical scales as advocated by *Morton & Griffiths* (1985).

Sensitivity of tissues and organs to pain

The sensitivity of particular tissues and organs depends on their innervation (cornea and dental pulp being the most sensitive and bones and brain tissue the least). There seems a prima facie case for a classification of sensitivity being useful for identifying severe or deleterious procedures. The classification is, however, unrealistic as sensitivities are greatly modified by pathologies *and* experimental procedures. It is more important to attempt to evaluate the overall severity of individual experimental procedures rather than dwelling on tissue sensitivity.

Effects of pain and distress

It is difficult to predict the detailed effects of pain and distress in individuals or in particular experimental procedures. There must be an examination of individual cases *and* a recognition that pain and distress increase experimental subjects. Consequently, amelioration of pain and distress will result in better experiments, perhaps requiring fewer subjects.

Sources of pain and distress

The *FELASA* (1994) review notes that many obvious and less obvious features of the operations of animal facilities can give rise to pain and distress. They include transport, physical factors associated with the macroenvironment (e. g. ambient temperature within the animal house and exposure to ultrasound from running taps), physical factors associated with the microenvironment (e. g. cage design and construction, the act of cleaning and social interactions with cagemates) and factors associated with experimental procedures (as attempted by *LASA* 1990). All of these have to be considered if one wishes to provide a complete audit of the conditions.

Signs of pain and distress

Much effort has been devoted to assessing the pain or distress in laboratory animals by looking at changes in individual behaviour. One has to be fully familiar with the normal behavioural characteristics of the particular strain and species with which one is dealing. There is ample evidence (e. g. *Brain* 1992) that different species show different behavioural responses to putative pain or distress-inducing stimuli, i. e. there is no "gold standard" for behaviourally assessing these phenomena. For example, some species run from such stimuli whereas others become immobile. Some workers have advocated measuring levels of "stress" hormones as an indicator of pain or distress but *Brain* (1990) has noted that many hormonal factors change in a complex way and sampling itself is likely to be stressful. Combining behavioural, physiological (including hormonal assays and heart rate) and immunological measurements as well as injury, growth and reproductive performance may provide a complete indication of "welfare". The results of pathological investigations to retrospectively assess the impact of housing conditions and procedures on distress may have some merit.

Grading of pain and distress

In authorising experimental programmes, many European regulatory bodies advocate balancing the scientific gains against the severity of the procedures (a so-called cost/benefit analysis). For example, the UK Animals (Scientific Procedures) Act of 1988 classifies invasive procedures as mild, moderate and substantial (*Home Office* 1990). There has been considerable debate on how one can best assess severity banding. The potential role of physical signs in rodents and the importance of good husbandry and housing in scientific refinement are strongly

emphasized. 'Enrichment' (a relatively neglected area for rodents) may provide further means of reducing 'distress' in laboratory animals.

An unresolved problem

Brain (1993) has pointed out that the whole area of dealing with pain and distress in laboratory animals is replete with examples of people making assumptions from too little scientific information. A real problem is that it is evident that, in some circles, there is a belief that once we have identified behavioural and physiological indicators of 'pain', 'distress' and 'suffering', all we need to do is eliminate these from the lives of our laboratory animals. This is overly simplistic because abolishing events or conditions that acutely change indicators of 'stress', environmentally impoverish animals *and* reduce their capacity for dealing with subsequent 'stressors'. Welfare involves achieving a fine balance between enrichment and stress, a process which should clearly take into account differences in strains, species and life experiences.

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Recent and promising alternatives to animal experiments

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Examples of recent and promising new developments in alternatives to animal experiments: validation; legislative, regulatory and related issues; refinement; reduction and prospects for the use and development of non-animal techniques are described. The three Rs (replacement, reduction and refinement) have dominated the search for alternatives to animal experiments, although developing appropriate, non-animal models is still a goal for most areas of toxicity. One problem is the need for validation (assessing relevance and reliability during test development and during interlaboratory, collaborative trials), which may be confounded by scientific shortcomings and politics. Only 3 tests have received regulatory acceptance as replacements: the Limulus Amoebocyte Lysate (LAL) test, listed in British and US Pharmacopoeias to replace rabbit pyrogenicity testing; CORROSITEX^{R}, a biomacromolecular system, and SKIN^{2TM} (cultured human skin tissue), approved by the US Department of Transportation (DOT), for labelling according to UN packing classification.

Finding alternatives for skin/eye irritation, and skin sensitisation, has been stimulated by EC Cosmetics Directive 76/768 (*Anon.* 1993), banning animal testing of cosmetics from 01 January, 1998, where validated alternatives exist. For example, an EC/UK Home Office Draize validation of 9 tests for eye irritation with 36 laboratories from 9 countries is in progress. Future methods, using cultured human corneal cells, modelling phenomena responsible for corneal opacity *in situ*, may provide more definitive information. More difficult endpoints for alternatives to model include sensitisation, carcinogenicity, reproductive toxicity, teratogenicity and absorption, distribution, metabolism and excretion (ADME). Also, *in vitro* methods for phototoxicity, due to uvA and uvB exposure, in conjunction with sunscreens, are being developed.

Two important events occurred in 1993: establishment of the European Centre for the Validation of Alternative Methods (ECVAM) in Italy, whose main goal is to promote the scientific and regulatory acceptance of alternatives (*Balls* 1994), and secondly the US National Institute of Health (NIH) Revitalisation Act, passed by Congress, decreeing that NIH should concentrate more on alternatives research. In 1994, two reports suggested reducing protocols for chronic rodent carcinogenecity testing (*Betton et al.* 1994, *Lai et al.* 1994), and *Apostolou & Helton* (1993) recommended a 5-fold reduction in maximum dietary doses of non-toxic chemicals.

Harmonisation of regulatory guidelines, to reduce numbers of animals, has been the subject of two reports: the International Workshop on Standardisation of Genetoxicity Test Procedures (Melbourne, Australia, Feb. 1993, see *Kirkland et al.* 1994), and the International Conference on Harmonisation (ICH) (*D'Arcy* 1994). A report in 1994 on regulatory animal testing from a subcommittee of the UK Animal Procedures Committee (APC), recommends better experimental design, harmonisation of guidelines to minimal criteria, increased mutual acceptance of data, with more funding and cooperation between scientists and regulators for development and acceptance of alternatives. Other attempts to reduce and refine animal experimentation include validating a Fixed Dose Procedure (FPD) to replace the LD_{50} test (*van den Heuval et al.* 1990), and de-

veloping transgenic organisms, which are considered promising for toxicity testing (*Sullivan et al.* 1993).

The use of cell cultures *in vitro* provides many advantages for studying toxicity (*Balls & Clothier* 1992), such as different cell types, including human cells (*Bardsley* 1994), and those from specific tissues for target organ specificity. A wide range of endpoints and a comprehensive selection of assays are available, with flexible protocols for detecting short- and long-term effects, and recovery outside the whole organism. Advances facilitating the use of cultured cells include the use of filter inserts providing 2 compartment systems, and immortalised cells, replacing primary cells, which often dedifferentiate and senesce in culture (*MacDonald et al.* 1994). Two developments have contributed especially to *in vitro* genotoxicity testing: mammals cells with cloned genes for cytochrome P-450 isozymes and other foreign compound metabolising enzymes (*Combes* 1992), and 'painting' chromosomes with fluorescent antibodies, improving cytogenetic analysis (*Savage & Simpson* 1994). Other types of non-animal assays include commercial kits, based on biomacromolecular systems, human skin equivalents, bioluminescence, and stress gene induction. Also, several computer methods exist for studying structure activity relationships (*Lewis* 1992).

In conclusion, there is a need to improve the way large amounts of data are handled and interpreted. Our ability to devise new tests exceeds our facility to improve the way in which we assess risk. Also, we require more human data, and more mechanistically-based assays, as well as tests for non-genotoxic carcinogens. Moreover, strategies are necessary for validating and using batteries of complementary tests, as are new approaches to animal husbandry (environmental enrichment) for refining animal experiments. Although due recognition should be given to advances being made in reduction and refinement, we should not be distracted from the principal goal of alternatives research, the eventual replacement of animal experimentation.

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COMPMED – The comparative medicine bulletin board

Abstract

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Many laboratory animal-related issues, including scientific breakthroughs, regulatory changes, and ethics are in constant transition. This makes it difficult for any one person to keep track of all advancements or efficiently manage the deluge of information at their disposal. Traditional means of communicating information include familiar standbys, such as: journals, newsletters, conferences, phones, and faxes. However, a different approach, through computer networking, is rapidly emerging to supplement or even replace conventional communication methods with far more efficiency and convenience. Computers and networks provide several solutions to the general phenomenon known as the "information explosion". Electronic mail distribution lists, computer bulletin board systems, commercial online services, and the now ubiquitous internet, are gaining popularity every day as problem-solving resources in all fields of endeavor. Many institutions well-known to laboratory animal professionals either have gone "online" or are planning to do so in the near future. Just a few of the organizations with computer networks in place today include: the American Association for Laboratory Animal Science (AALAS), the National Agricultural Library – Animal Welfare Information Center (AWIC), the National Library of Medicine (NLM), the American Veterinary Medical Association (AVMA), the Jackson Laboratory, the National Institutes of Health (NIH), and the National Science Foundation (NSF) This lecture will provide detailed information on the internet service called COMPMED (Comparative Medicine), as well as other examples of computer resources available to laboratory animal professionals, what they are about, and how to access them.

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Ketamine anesthesia in pig

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For induction of anaesthesia fixation is necessary in most cases. Fixation is, however, not only strenuous for man but induces, especially in pigs, fulminant stress reactions like an increase of heart rate, blood pressure and lactate levels (7). These stress reactions should be avoided prior to anesthesia. Therefore we looked for an intramuscular injectable general anesthetic in pigs. Numerous substances like phenothiazines, butyrophenones, phencyclidines, benzodiazepines, morphines, α-2-agonists and their combinations were investigated. The most effective substances were the phencyclidines with the two substances ketamine-HCL and tiletamine (5).

Ketamine is not only a good analgesic but produces a special dissociation of the function of the neocortex – thalamus and of the limbic – reticular parts of the brain, that account for the hypnotic-cataleptic effects of ketamine. The state designated as catalepsia is characterized by absence of response, unconsciousness and deafferentation.

The main advantage of this substance is the early onset of action after intramuscular administration. After injection of 15 mg ketamine-HCL/kg b. wt. IM ataxia of the pigs occurs within 1 minute. Lateral recumbency was achieved within 5 minutes. In some cases little excitation is induced within the initial 5 minutes. In some cases tonic-clonic spasms occured in newborn piglets and gnotobiotics. Relaxation could not be achieved by increasing the ketamine-dose.

Maximum of anaesthesia is reached about 10 to 11 minutes after injection. At this time all animals are immobilized. In adult pigs an analgesic effect is also detectable by skin prics. In piglets analgesia is achieved quickly. All reflexes can be stimulated at any time. Although only some pigs show a severe rigidity of the skeletal muscles during the initial period, mucle relaxation during the whole anesthesia is insufficient. Most of the pigs show excessive salivation.

Deepness and length of anesthesia depend on age and weight of the animals. About 20 minutes after injection, piglets begin with spontaneous agitation. Later they frequently show grunting, smacking, chewing and stereotypic contractions of the neck muscles. Young pigs stand up within 30 to 90 minutes but adults may not be able to rise before 3 hours. During the recovery period vomiting and, in adults, anxious behaviour and panic may occur.

Ketamine induces an increase of respiratory rate and rhythm of breathing becomes irregular. This results in an increase in blood pO_2 and a slight respiratory alcalosis. Due to the excitements a slight metabolic acidosis occurs during both the initial and the recovery period (4).

After IM administration maximal plasma concentrations are rapidly reached but peak concentrations show considerable variations. After IV administration ketamine kinetics follow a three-term exponential decrease with rapid distribution to highly vascular tissues (e.g. brain) followed by redistribution into less vascularized tissues, and elimination. The minimal plasma ketamine concentration for induction of immobilization is about 2 µg/ml. The major metabolite in plasma is norketamine. Elimination half-life of ketamine is about 2 hours after either IM or IV administration. Anesthesia is terminated by redistribution of the drug from the brain into other tissues, whereas metabolism and excretion are less important for duration of anesthesia. The differences in analgesia between adult and young pigs could not be related to pharmacokinetic differences (6).

Insufficient muscle relaxation, variation of clinical effects and side effects like excitations and anxiousness need supplementation with other substances. With the two combinations ketamine plus climazolam (a new short acting 1,4 benzodiazepine, very similar to midazolam) in a dose of 15 mg ketamine + 1 mg climazolam/kg b. wt. (= K + C) and ketamine plus xylazine (= K + X, 15 mg + 18,5 mg/kg) in adults a degree of anesthesia can be achieved that is sufficient for most clinical purposes. Especially climazolam prevents some side effects of ketamine like excitations, convulsions, floundering or vomiting. Onset of action after IM injection is early. Anxiousness and excitations may still occur after K + X.

Reactions of cardiopulmonary and metabolic parameters are similar after both the two combinations as after Ketamine alone. A side effect of xylazine is the cancellation of temperature regulation. After K + X the mean body temperature falls from 39,0 ± 0,4 C° prior to anesthesia, to 36,3 ± 1,4 C° two hours after administration.

Especially after K + C, muscle relaxation is excellent. Climazolam should not be used for obstetrics as climazolam cannot be metabolised by newborn piglets (2).

In adults, analgesia may be achieved with both combinations. In accordance to *Bloch et al* (1986) no differences can be observed between skin and deep analgesia. Analgesia is often insufficient for painful surgery. Only in adults, insufficient analgesia can be supplemented by additional IV injection of ketamine. In piglets trials to improve analgesia by supplementation with opiates administered IM were only successful in the combination ketamine/climazolan/levomethadone (15 mg + 1 mg + 0,5 mg/kg b. wt.). In other combinations opiates caused postanesthetic excitations with hyperthermia (5).

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Dissociative and adjunctive anesthetics in swine

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Dissociatives

Ketamine is one of over 200 congeners of phencyclidine, the original "Dissociative Anesthetic". Phencyclidine (Sernylan) was synthesized by Maddox[1]. Basic studies were conducted by *Chen & colleagues*[2]. The drug was evaluated as an anesthetic in people by *Greifensteins*[3], *Corssen*[1] and *Johnstone*[4] *et al.* Human responses to phencyclidine were often bizarre, being variously described as hallucinations, aberrations, delirium and other descriptive psychotic disturbances. The most useful derivative of these drugs is ketamine (2-(o-chlorophenyl)-2-(methylamino)cyclohexanone). Ketamine's S(+)-isomer possesses the major pharmacologic and anesthetic characteristic of the racemic mixture. Administration of the S(+)-isomer results in less spontaneous movement and fewer psychotic emergence reactions i.e. disorientation, excitement and delirium than either the racemate or the R,(–)-isomer. Ketamine anesthesia is characteristic of a cataleptoid state. Unlike that seen with the barbiturates, the patient's eyes remain open with a slow nystagmic gaze and an appearance of wakefullness.

Drugs disconnecting higher brain centers (thalamocortical) from lower centres (limbic systems) are referred to as "dissociative" anesthetics. The first dissociative available was phencyclidine hydrochloride. While its use in swine and bears gained some popularity, illicit use resulted in its removal from the veterinary market. Subsequently, tamer and, perhaps, less addictive derivatives were synthesized, namely, ketamine and tiletamine. These drugs have become popular for induction of anesthesia and short surgical procedures when combined with adjunctive drugs (eg., alpha-2 agonists, opioids and benzodiazepines).

In the early 70's, the first clinical paper was published on the use of ketamine in swine[3]. It has since become the most commonly used injectable anesthetic in all animal species. However, as a mono-anesthetic, ketamine is not an effective analgesic unless extremely high doses are given. Adjunctive drugs are required to enhance analgesia and ameliorate ketamine's unusual side effects in swine[5].

Tiletamine is similar to ketamine and is available only in a proprietary compound. This drug combination consists of a 1:1 mixture of tiletamine and zolazepam. The latter is a benzodiazepine that is similar to diazepam but more potent. Anesthesia induced with these drugs is characterized by minimal muscle relaxation often referred to as a cataleptoid state.

Zolazepam seems to increase muscle relaxation, prolongs the action of tiletamine but does not add measurably to analgesia. High doses may result in bladder relaxation and urine retention.

Studies proving this clinical observation have not been conducted. Analgesia induced by ketamine appears to be in response to action on N-methyl-D-aspartate (NMDA) and, perhaps, on opioid receptors subsets. However, attempts to antagonize ketamine's action with naloxone have not been decisive[6].

With dissociative anesthesia, the patients eyes remain open and the swallowing reflex usually remanins intact. This, however, does not prevent the patient from aspirating foreign material should vomition occur, nor laryngeal spasms, so common in swine. Vomition can be a real threat when pigs are not properly fasted. When intubating the trachea after ketamine induction, the swallowing reflex can be obtunded with a small

dose (2–4 mg/kg IV) of a barbiturate or local application of lidocaine. While a muscle relaxant would be useful, it could induce malignent hyperthermia (eg., succinylcholine). In humans, ketamine induced analgesia has been described as intense[7]. Clinical and laboratory observations do not confirm this in swine[5].

Because ketamine induces excessive salivation in swine, it seems logical to administer an anticholinergic (atropine 0.4 mg/kg IM). However, atropine should be avoided in patients with tachycardia or fever. When given IM at a dose of 10–12 mg/kg, ketamine will immobilize swine in approximately 5 minutes. Although xylazine does not provide good muscle relaxation or sedation when given alone, it will greatly enhance the anesthetic effect of ketamine. The xylazine dose-range is 2–3 mg/kg IM or 1–2 mg/kg IV. When xylazine is combined with ketamine, the result is improved analgesia and muscle relaxation[4]. When necessary anesthesia can be prolonged in healthy patient with 2–4 mg/kg of ketamine and 0.5–1 mg/kg of xylazine mixed in the same syringe and injected IV slowly. When an auricular vein can not be cannulated, the IM dose has to be increased at the expense of a prolonged recovery. A dose of 4–8 mg/kg IM may be required. Recovery will be quicker and an excitatory recovery is less likely to occur with the lower IV dose. Excitement during recovery is most often seen in mature sows and boars but is less likely to occur when xylazine is a part of the anesthetic regimen. when confronted with an excitatory situation, it is easily controlled with pentobarbital (4–8 mg/kg IV). If IV injection is impossible, the larger dose should be diluted with an equal parts of sterile saline or water and injected deeply IM. The injection is best made behind the ear. A 3–4 cm needle is required to insure injection into muscular tissue.

Because physical restraint is frequently difficult in mature swine, the IM route is often used to immobilize large patients. Drug combination that reportedly works for this situation is ketamine (4 mg/kg), oxymorphone (0.15 mg/kg) and xylazine (4 mg/kg). If an IV injection can be made, the dose of each drug is decreased by one-half[4, 8]. This drug combination, although expensive, offers relatively good analgesia and muscle relaxation. Recovery is generally smooth and can be hastened with naloxne and yohimbine. However, these antagonist will also antagonize postoperative analgesia. *Telazol®* is a proprietary drug combination. It is available in 5 ml vials contain 250 mg of tiletamine and 250 mg of zolazepam. Zolazepam is similar to diazepam but it is water soluble and more potent than diazepam. Zolazepam has a central muscle relaxant action that partially relieves the cataleptoid state induced by dissociatives. Excessive or repeat IM dosing of Telazol® can cause prolonged recovery, particularly in older swine. Although only approved for IM use in dogs and cats, Telazol® is widely used in swine. This drug combination does not induce intense analgesia but it is an effective anesthetic in swine when combined with opioid or an alpha-2 proper adjunct. Presently, xylazine is the popular drug for this purpose. However, studies in our laboratory strongly suggest that medetomidine may be a more effective alpha-2 agonist, once widely available. Drugs within this class have been shown to decrease the requirement for general anesthesia by as much as 90 % (eg. medetomidine). Never alpha-2 agonists with greater receptor selectivity are being tested. Early work with Telazol® 6.6 mg/kg IM combined with xylazine 2.2 mg/kg IM immobilized 20–30 kg pigs in one to two minutes. The anesthetic time extended up to approximately one hour. Tracheal intubation could easily be performed[9]. In this study, xylazine was given before Telazol®. Subsequently, we have found that the two drugs can be mixed in the same syringe for easier handling. Prolonged recoveries have been experienced in elderly sows and boars with the dosage reported for 20–30 kg pigs.

Extended recoveries are more likely to occur when this drug regimen is given by the IM route or after redosing to extend anesthesia. It appears that prolonged recovery is due in large part to zolazepam's effects. Consequently, smaller IV doses are recommended for older swine (Telazol® 1–2 mg/kg and xylazine 1 mg/kg). When using the IV route, anesthesia may be safely extended by giving one-half the original dose as required.

Injectabile drug combinations

Telazol®-Ketamine-Xylazine

Zolazepam constitutes 50 % of the Telazol mixture and appears to be responsible for the posterior weakness during recovery of mature swine when given IM in anesthetic doses. The dissociative concentration is increased by adding 2.5 ml of ketamine (100 mg/ml). Xylazine, 2.5 ml (100 mg/ml) is also added to increase the sedative and analgesic effect. This provides 100 mg of dissociative/ml (i.e., tiletamine plus ketamine) and 50 mg/ml each of xylazine and zolazepam. In this drug mixture, zolazepam constitutes only 25 % of the total drug. In commercial swine, the dose of this drug combination is 1 ml/75–100 kg IM, depending on the depth of anesthesia required. "Potbellied" pigs appear to require a smaller dose, approximately one-half, that given to commercial swine.

Anesthesia may be extended by injecting one-half the IM dose IV slowly to avoid apnea or by administering either halothane or isoflurane in oxygen by nose cone. Inhalation anesthetic requirement will be significantly decreased with this drug mixture[2].

Guaifenesin-Ketamine-Xylazine ("Triple Drip")

This drug combination is prepared by adding 2 mg of ketamine and 1 mg of xylazine to each ml of 5 % guaifenesin prepared in 5 % dextrose in water. The drug combination must be given by the IV route. This can be a major problem because of an absence of accessible auricular veins in some individuals. The induction dose ranges from 2/3 to 1 ml of the mixture per kg. The average anesthetic maintenance dose is 2.2 ml/kg/hr. Using a standard IV delivery set (15 drops = 1 ml) the maintenance dose is calculated as follows: (body wt. kg) X (2.2 ml/kg/hr) X 15 drops/ml divided by 60 = drops/min again divided by 60 = drops/sec. In a 150 kg sow, induction would require approximately 100–150 ml depending on the rate of injection. Maintenance would be calculated as follows: 150 X 2.2 X 15 = 4950, drops/hr divided by 60 = 83 drops/min, divided by 60 = approximately 1.4 drops/sec[4].

This dosage rate is sufficient for the average sow. Sows that have been in prolonged labor usually require a smaller dose. On the other hand, young vigorous sows, in labor for only a short time, may require an increased dose. Animal response will serve as a guide to dose requirement. As with injectable mixture, it should be given to effect as measured by monitoring vital signs.

Induction and recovery from this anesthetic mixture is rapid (recovery occurring in 30–45 min.). Recovery time can be decreased by IV injection of a specific xylazine antagonist (eg., yohimbine 0.12–0.20 mg/kg or tolazoline 2–4 mg/kg). When the alpha-2 antagonist is given, postoperative analgesia is diminished. Rapid arousal to the antagonist suggests that xylazine is most likely responsible for residual anesthetic effect during recovery following continous infusion of "Triple Drip".

When "Triple Drip" is used for caesarean section, it provides excellent relaxation and analgesia. Piglets are only minimally depressed. But clearly, neonatal depression is directly related to the total dose of "Triple Drip" prior to fetal delivery. Speed of surgery is of essence. It is likely that xylazine is responsible for neonatal respiratory depression. This speculation is based on clinical observations that piglets will quickly commence breathing after a small dose of a specific alpha-2 antagonist. A minimal dose (ie., 0.25–0.5 mg/kg) of doxapram will stimulate breathing[4].

Anesthetic induction and recovery

Drugs and techniques for induction of anesthesia are numerous. The thiobarbiturates are the standard to which other injectable anesthetics are compared. Two other major classes of anesthetics that are commonly used in swine are the dissociatives (eg., ketamine and tiletamine [Telazol]) and inhalants. Induction drug popularity "ebbs and flows" but in large part is governed by appropriate preanesthetic medication. So long as pigs are not overdosed, the chosen induction drug(s) is/are of minimal importance.

The thiobarbiturates are mono-anesthetics but poor analgesics. The anesthetic dose is very close to the apneic dose. Repeated injection will result in tissue saturation and prolonged recovery. There is no specific antagonist for barbiturates. Some drug combinations will provide better laryngeal relaxation than others. For example, a popular combination that insures laryngeal relaxation consists of guaifenesin, ketamine and xylazine ("Triple Drip"), mentioned earlier.

When anesthesia is being induced with inhalant in oxygen eg., isoflurane, there is only a minimal amount of time to complete the intubation technique once the nose cone is removed. Thus, the general procedure is to remove the nose cone, open the pigs mouth and quickly spray the laryngeal opening with lidocaine, replace the nose cone and continue with the induction procedure. Until regular breathing and signs of surgical anesthesia occur, the nose cone should not be removed. For intubation, the laryngeal opening is exposed and identified with a laryngoscope and the tube put in place by one of two methods. Either by direct insertion or by the use of a guide tube to direct the endotracheal tube through the larynx and into the trachea. After properly positioning of the tube, the cuff is inflated so that when a pressure of 18–20 cm of H_2O is applied to the rebreathing circuit, a slight escapement of air can be heard from the pigs mouth.

Apnea is simply dealt with by rhythmically squeezing the rebreathing bag four to eight times per minute or by the use of an "Ambu" resuscitator bag when the patient is not connected to an anesthetic rebreathing system. With the latter, either oxygen or ambient air can be used. Excessive ventilation will decrease blood carbon dioxide concentration and may prolong apnea.

Experience gained in intubating pigs will increase the anesthetist's confidence and proficiency. Further, an assistant who understands the anatomy of the pig's airway and appreciates the importance of keeping the head and neck properly positioned is essential for a clean, safe, tracheal intubation.

Alpha-2 adrenoceptor agonists

The alpha-2 adrenoreceptor agonists available to the veterinarian in North America include xylazine and detomidine. Only xylazine has been used to any great extent in swine. While xylazine is an extremely potent sedative in other animal species, it does not have the same effect in swine. After injection of xylazine or detomidine some sedation is apparent, pigs will usually lie down in 10–15 minutes but, when approached, they will rapidly arise and flee[10]. Thus, to take advantage of xylazine's sedative effect it is usually combined with another drug. Xylazine and ketamine have become a popular anesthetic drug combination in swine. The dose of xylazine ranges from 1–2 mg/kg IM or IV. The effects of xylazine, when combined with ketamine or Telazol, appears to be greater than additive. However, studies have not confirmed this clinical observation. Recent clinical experience with medetomidine (15–30 μg/kg) and ketamine suggests that this drug combination offers major advantages over a xylazine and ketamine combination as previously described.

Alpha-2 agonist epidural analgesia

Controlled studies in swine receiving either detomidine or xylazine in the epidural space revealed signs of sedation and hemodynamic

changes commensurate with those expected after systemic injection.

Xylazine has both alpha-1 and alpha-2 activity, while detomidine acts predominantly at alpha-2 adrenoceptors. It has been demonstrated that both alpha subtype receptors are located in the dorsal horns of the spinal cord[11, 12, 13]. Further, it has been shown that analgesia can be induced by both alpha-1 and alpha-2 adrenergic agonists when injected intrathecally[14]. This finding supports the speculation that xylazine-induced analgesia is mediated by alpha-1 and/or alpha-2 stimulation. The proportion of alpha-1 to alpha-2 adrenoceptors in the spinal cord of domestic swine has not been determined. It seems that stimulation of alpha-1 spinal cord adrenoceptors may be more important for analgesia than alpha-2 adrenoceptors in swine. This speculation is based on the fact that xylazine induces more profound analgesia than does detomidine. It is interesting to know that in pigs receiving either intrathecal xylazine or detomidine the response is different after a pure alpha-2 antagonist (i.e., atipamezole) is administered. In detomidine treated pigs, sedation, analgesia and immobilization are quickly abolished after IV injection of atipamezole. In xylazine treated pigs, however, sedation is abolished but loss of motor and sensory responses posterior to the site of xylazine injection remains. Seemingly, this indicates the presence of a xylazine induced spinal local analgesic effect that has also been reported in horses and cattle[15, 16, 17, 18].

Epidural injection of xylazine or detomidine in swine induces sedation, analgesia and immobilization. The intensity of analgesia is greater with xylazine than detomidine. Xylazine's superior analgesic action appears to be mediated by either it's alpha-1 adrenoceptor activity located in the dorsal horns of the spinal cord and/or a local analgesic effect independent of alpha-adrenoceptor stimulation[13].

Sows weighing from 150–225 kg scheduled for caesarean were given an epidural injection of 10 ml of 2% lidocaine containing xylazine, 0.5–1.0 mg/kg. The onset of analgesia and sedation were rapid. Complete immobilization of the rearquarters remained for approximately four hours. The sows would lie quietly for over an hour at which time some front limb movement occurred. Thus, even though there is some xylazine induced sedation, the forelimbs should be tethered. The piglets were lively when delivered and effectively antagonized with either yohimbine (0.15–0.2 mg/kg IV) or tolazoline (2–4 mg/kg IV). Atipamezole is an effective alpha adrenoceptor antagonist, it is presently unavailable in North America. The local anesthetic effect of xylazine is not antagonized by alpha adrenoceptor antagonist.

In summary, pigs come in all size. They range from piglets (0.5–3 kg), miniatures (10–30 kg) to large adults that can weighing in excess of 400 kg. While most research is done in pigs weighing 50 kg or less, piglets lend themselves well for studying human neonatal diseases. Large blood samples can easily be taken from the anterior vena cave with only a minimal amount of practice. For these and other reasons, pigs are rapidly becoming the research animal of choice.

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Pain after surgery – it is getting better!

Abstract

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It is perhaps strange that despite the many advances that have been made in both the preoperative and intraoperative care of patients, their well-being after they have left the operating theatre has been sadly neglected. Although the fault for this cannot be assigned purely to the anaesthetists, they must be held to be significantly at fault. However the last few years have seen a dramatic change with many hospitals now providing acute pain teams, lead by anaesthetists, and responsible for the care of all postoperative patients.

The importance of postoperative pain was brought to the fore in the United Kingdom by a working party of the Royal College of Surgeons and Anaesthetists, published in 1990 and entitled Pain After Surgery. In the summary of their conclusions they stated "the management of pain after surgery in the U.K. is unsatisfactory". It is interesting to note that in a survey published in the same year by the British Medical Journal 41 % of patients regarded the pain they experienced postoperatively as very painful, while 53 % recorded painful experiences. The reasons for trying to deal with this pain are several. Humanitarian is obvious, whilst respiratory changes lead to cough suppression and sputum retention, cardiovascular effects cause a tachycardia with an increased risk of myocardial infarction.

Pain may be divided into three types. Nociceptive, both somatic and visceral, will be the most common encountered postoperatively. However neuropathic pain, for example from excessive surgical retraction, may also be present. The psychological aspects of pain, and that it may be induced by anticipatory anxiety, must also be remembered.

There have been many theories of pain and the pathways that the nerve fibres follow to the cortex. It is true to say that these are still not fully understood but the action of natural endorphines and the development of therapeutic analogues has clarified some of the issues. The opioid receptors (endorphine binding sites) can be broadly divided into three types. There is a range of activity in binding ability at these sites between the various opioid drugs, hence to achieve adequate analgesia the appropriate route of administration, and rate, must be established. This leads to the concept of patient controlled analgesia, whereby using a mechanically driven infusion pump, the patient is able to regulate their own pain control by pressing a button which provides them with a dose of an analgesic.

The development of non-steroidal anti inflammatory drugs, originally intended for use in arthritic conditions, has allowed them in addition to be used postoperatively. Aspirin is of course the oldest of these and despite its unwelcome side effects, still compares very favourably with morphine. The various propionic acids have all been used postoperatively, via various routes of administration. Probably the most useful of these are ibuprofen, diclofenac and ketorolac. Of the oxicam range of drugs, piroxicam is of value due to its twenty-four hour or more action. Whatever of these drugs are used it must always be remembered that their renal and gastric side effects can have a significant morbidity or even mortality.

Continuous postoperative epidural analgesia is now being used in many centres. The commonly administered drugs will be a mixture of local analgesics together with an opioid in a low concentration. Again the

rate of administration may be patient controlled.

Local analgesics may also be used for surface analgesia, wound infiltration, which may be via an indwelling catheter, or as regional or intravenous blocks.

As postoperative pain deminishes with time after surgery, less potent oral analgesics are appropriate. Paracetamol alone remains the most useful, as its combination with other agents, for example dextropropoxyphene, seems to have little or no advantage.

Finally alternative postoperative pain therapies must be considered. Ketamine in low dosages is valuable while the more recent development of alpha-2 agonists will probably further broaden the useful range of analgesic agents. Electromechanical therapies, for example transcutaneous nerve stimulation, cryoanalgesia and acupuncture all have their advocates although their application would appear to the rather limited. In conclusion, although some new analgesic drugs have become available the primary management of postoperative pain still relies heavily on the use of opioids. What has changed dramatically is the control of the administration of these drugs by an active pain management team consisting of doctors and nurses who are regularly able to visit patients during their recovery from surgery. This close liaison allows a prober assessment to be made of the degree of discomfort the patient is suffering and so the choice of the most appropriate drug regimen. So it is true to say in institutions where this facility is available that . . . pain after surgery – it is getting better.

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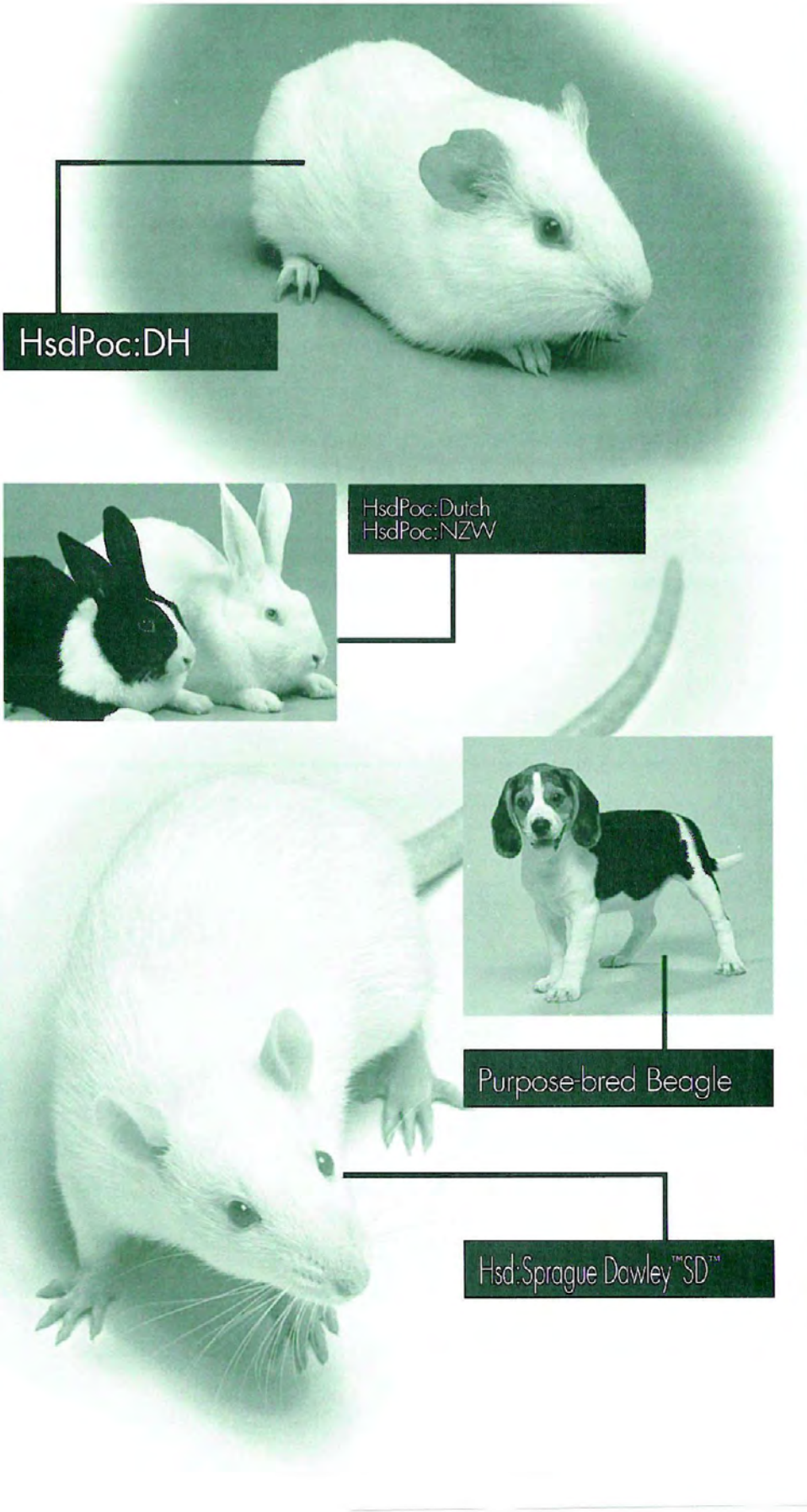
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Animals in hyperbaric medical research

Abstract

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Research to study the pathogenesis of diving disorders and physiology has been performed in all common research animal species, including dog, rat, rabbit, pig, and primates (*Butler & Hills* 1983, *Lethosalo et al.* 1983, *Tanoue et al.* 1987, *Hills & James* 1991, *Vik et al.* 1991, *Pearce et al.* 1989).

The animals are put in confined hyperbaric dry chambers either awake or anaesthetized. Anaesthetics per se often conbound physiological changes related to diving and one tends to avoid the use of anaesthesia. Adaption procedures have to be carried out, the animals often have limited space due to chamber sizes and operating costs.

Considerable noise is inflicted during compression and decompression procedures. Noise-reducing devices or ear protection may be considered. Barotrauma of the middle ear (ear squeezes) is avoided by performing paracentesis before compression starts. The chamber atmosphere is essential to the animals' survival and well being. A constant gas analysis (including contaminants) and temperature and humidity monitoring may be necessary. For long-term studies feeding and watering devices will have to be constructed as well as devices to remove excreta and urine through air-lock systems (*Pearce et al.* 1991).

The animal species must be considered when decompression procedures are done (*Berghage et al.* 1979). Often decompression tables and procedures are exceeded to produce symptoms of decompression illness (DCI) in the animals, these often include limb pain and/or neurological deficits. In studies including repeated dives special attention to the animal welfare between dives must be considered.

Generally there are considerable species differences between man and animals while at the same time there is a poor understanding of the pathogenesis of diving disorders. Further investigation seems warranted, including the use of animal models.

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Psychoneuroimmunology

Abstract

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During the last 30 years, a number of studies on the effects of stress on disease outcomes and on the immune system have contributed to the development of a new area of research; psycho-neuro-immunology. The topic of this lecture will be effects of stress on the immune system of laboratory animals, and to some extent humans. A review will also be given about the effect of stress on experimental disease in animals. An historical introduction will first be given. A summary of the knowledge today in regard to effects of stress on the function of the immune system will then be presented. The lecture will focus in particular on which stressors at what conditions have been documented to alter the function of the immune system or the resistance to disease. Some general conclusions in regard to stress in all experimental work with animals will be outlined. A brief summary of the effects of psychological stress on the function of the human immune system will subsequently be presented together with some experiments about the mechanisms of the effects of stress on the immune system. Finally, some concluding remarks about the overall importance of the effects of stress on the immune system in animals and man will be given.

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Building an animal P3 laboratory: Giving tail – biting advice for an agonizing experience

Abstract

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In view of the HIV/AIDS pandemic and the many scientific establishments doing active research on retroviruses, the need for a Category 3 animal laboratory facility is high on the agenda for most groups. The University of Bergen (UoB) has currently many research programmes on HIV/AIDS and excellent facilities for laboratory work with infectious retroviruses have been established at the National Virus Centre at Bergen High Technology Centre. So, did we need a corresponding animal facility? Many thought so.
When plans for building the new Vivarium at the UoB were set in motion a number of pertinent questions regarding the actual need and scope for the animal P3 facilities were put forward. Again, did we really need one? How large? What type of animal studies could be envisaged? What infectious agents should we take into consideration? Should the laboratory be constructed with options for upgrading to P4? What could be done within a very tight budget?
This talk will focuse on the discussions that finally metamorphosed into, we think, a mostly well designed Category 3 animal research laboratory that hopefully will serve us well for many years to come.

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Environmental enrichment, fact and fiction

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The intention to protect animals is based upon ethical considerations. But what animals need for their successful protection can only be deduced from a biological understanding of the specific animal species or strain in question. Any concept of animal protection is made up of conventions and assessments which are inevitably linked to those who have to make the decisions. The lines along which limits and minimum requirements are drawn depends on the "spirit of the times", on ethical considerations and, above all, on economical and political reasoning. Thus, animal protection must always be seen as a relative concept; the reasonableness of a restriction either for the animal or for the researcher and the animal technician may change according to both place and time.

What is reasonable?

The current housing standards for laboratory animals were worked out on the basis of practical experience as well as on scientific knowledge gained in the fields of hygiene, veterinary medicine, animal psysiology and work physiology. No doubt the great standardisation efforts which started some 30 years ago, resulted in better control over many aspects of the experimental animal and its environment (e.g. genetics, nutrition, microbiological state, microclimate); biomedical research was improved (e.g. by development of more sophisticated animal models) and the numbers of animals used for pharmacological screening and toxicity testing could be drastically reduced. But these standardisation efforts also led to a more and more impoverished environment. The barren cage environment may have negative consequences for the animals such as morphological damage, chronic stress and behavioural disorders despite good health and high reproductive success. The awareness of such problems, which are definitely related to reduced welfare, has been increasing recently due to public pressure as well as to feelings of uneasiness in scientists and animal technicians.

Which restrictions are reasonable for both the animals and the humans working with them can only be laid down bindingly by a compromise amongst the different parties involved. In working out new or revised housing standards which are more appropriate to the animals' needs, it is of the greatest importance that the animals' behavioural needs should also be taken into serious consideration. The aim of the *British Code of Practice for the Care of Animals used in Scientific Procedures* (1986) is "to maintain animals in good health and physical condition, behaving in a manner normal for the species and strain with a reasonably full expression of their behaviour repertoire . . .". But what does "a reasonably full expression of behaviour" mean? And what is "normal"? Behaviour is always the expression of a complex cause-and-effect network between an organism and its environment. Any behaviour actually observed relies on both, on the individual's actual spatial and social environment and on its ontogeny. The range of capacity to adapt behaviour to various and changing environments is species-specific (strain-specific, respectively) and genetically fixed. With respect to animal welfare, normal behaviour can be defined as follows: All behavioural expressions which lead to successful growth, to successful maintenance of bodily functions and (potentially) to successful reproduction and which contribute to successful avoidance of harm are said to be 'normal'. Thus, experimental animals should be kept "in such a way as not to interfere with their bodily functions or their

behaviour, or overtax their capacity to adapt" (*Swiss Ordinance on Animal Protection* 1981).

Solution 1: Intuitive-empirical enrichment of the environment

Attempts to solve animal welfare problems have so far been made mainly by methods which may be described as "Intuitive-empirical": Larger cage dimensions and an enrichment of the environment with occupational objects (e.g. towels, grain) and structures for withdrawal (e.g. huts, tubes) provide evidence that mice and rats are indeed able to make good use of such items. But problems rapidly provoke severe criticism. The consequences of "environmental enrichment" for the animals' well-being are rarely investigated at the level of behaviour elements selected on ethological grounds and hypotheses. Success or failure is determined empirically or measured by recording simple parameters (e.g. activity versus inactivity, locomotor activity, duration of stay). If the animals use enrichment elements without morphological damage or signs of stress, the changes are maintained and propagated on animal welfare grounds. But if problems arise e.g. higher levels of aggression against their own kind or against humans), such intuitive-empirical investigations are likely to be generalised and any enrichment may then be rejected with reference to an assumed and misinterpreted genetic adaption through selective breeding.

"Environmental enrichment" is mainly carried out on the personal initiative of animal technicians and staff members at laboratory animal facilities. In view of the growing awareness of animal welfare problems such actions are highly appreciated. But from a zoological point of view the long-term value of intuitive-empirical investigations is questionable, as experimental design and data sampling methods are primarily based on personal experience, on specialisation (e.g. veterinarian, zoologist, laboratory animal scientist or technician) and on affiliation (profit or non-profit making institution). Representatives of laboratory animal breeding facilities and of biomedical and toxicological research units, in particular, are more likely to agree to new and tighter recommendations for larger animals (e.g. primates, dogs) than for smaller (e.g. rabbits and rodents) where much higher costs are involved. It is odd that welfare specialists are frequently urged to prove that changes are beneficial to small laboratory animals by the same individuals who accept empirical enrichment for carnivores and primates. An empirical decision for species-specific minimum floor areas and stocking densities (e.g. the linear weight-space correlation laid down in *Appendix A of the EC Directive* 1986) can neither be discussed objectively between scientists nor made justifiable with respect to general animal welfare requirements (i.e. younger animals need more space for exercise and play than older subjects). Therefore, a linear increase in the minimum cage dimensions in order to provide a practical compromise in percentage terms has no scientific basis. The animal's behavioural and physiological needs are not only species-specific and strain-specific, but may also relate to the individual's position and experience within a given social community. Most of the animal species used for experimental purposes are highly social. Isolated housing has to be avoided except for incompatible animals (e.g. territorial adult males in rabbits and mice) and specific experimental demands. A normal social ontogeny can only be achieved in an adequately structured spatial and social environment.

But the expression of normal social behaviour and of species-specific dominance hierarchies (with dominant and subdominant animals per group) might contradict the strong interests in standardised animals. This fact may lead to basic conflicts between representatives of different interests (e.g. ethologists, animal experimentators, laboratory animal scientists), which have to be mediated in advance of any discussion and

before attempts to compromise on specific levels (minimum cage and pen dimensions, group size, group composition, density).

Solution 2: Scientifically-based development of enriched housing standards

As an alternative to the intuitive-empirical investigations in "environmental enrichment" we have developed an ethological research approach to the development of housing concepts appropriate to basic animal welfare requirements. This approach allows us to work out scientifically the behavioural (and physiological) needs of laboratory animals (species, strains, sex and age classes). It has proved to be a valuable basis for the revision of legal requirements (detailed references see *Stauffacher* 1993, 1994).

The most essential part of this ethological approach is the knowledge of adaptive behaviour patterns. Of particular interest are the mechanisms controlling the performance of normal behaviour (causation) and the immediate and long-term goals of normal behaviour (function). Such knowledge can only be achieved in a richly structured spatial and social environment. Quite apart from practical interests, every development of enriched housing systems starts with the qualitative and quantitative registration of behaviour and of morphological and physiological parameters in a rich (e.g. near-to-nature) environment. Also investigated are the stimuli and characteristics of the environment which release behaviour patterns and allow these to be carried out functionally. The following step involves reducing the environmental features to what is really essential for successful maintenance of normal bodily functions and normal behaviour. In order that the crucial stimuli and concrete characteristics required for the shaping of normal behaviour may be reduced to the essentials and may be brought into the right relationship with each other, ethological housing concepts have to be worked out. A clear division of the space available into functional compartments and the management of social behaviour, e.g. by the provision of blinds, should allow the animals to live together even within a very limited floor area and volume. The inherent nature of the reduction and substitution stages can be tested as to whether the substitute is actually recognised by the animals when it is part of an extensive environment.

Working out the animals' minimum requirements on zoological grounds it is most decisive that the following points are taken into account: 1. The animals must be provided with a choice of different stimuli (e.g. light gradation) and object qualities (e.g. solid and fissured floor) as the animals requirements depend on their actual activity. 2. Appetitive behaviour must lead to consummatory acts (e.g. construction of a nest should be allowed by own activity). 3. The ratio of environmental control and unpredictable events should be adapted to the animals' species-specific abilities (e.g. rats may respond to changes with more flexibility than quails). Routine management (e.g. automatic feeding and cleaning) should be regular and constant. The animals must be allowed to hide or escape when frightened. 4. Alertness and exploratory behaviour must be increased by provision of unpredictable elements, such as social partners. A social partner provides necessary diversion, occupation and probably also feelings of "safety" and "security". In contrast to occupational objects, a partnership creates a repetition of new and unpredictable situations to which the animal must react and on which it must take up a position.

A housing system should be easily manageable by the animal technicians and also should be in accordance with general standardisation requirements (e.g. hygiene). Therefore the housing concept has to be transformed into cage or pen prototypes which then are examined in terms of animal welfare and management. This last and very decisive stage can be very difficult indeed. Appropriate methods must be worked out in close co-operation with specialists working

in the various fields of laboratory animal science and with animal technicians.

Conclusions

Since empirical decisions could well create a situation which would effectively prevent scientifically based improvement from being realised at a later date, specific research needs should be worked out and scientific research in animal welfare should be coordinated at the international level. Moreover the parties involved (governments, industries, universities) not only should agree to principal research intentions but also should discuss the provision of infrastructure (logistic, financial, personnel) for efficient research following a politically justifiable schedule. In addition, serious education in laboratory animal ethology may lead to a better understanding of the animals' capacities and needs and may bring the animal welfare debate on a less emotional and more animal related biological level.

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Experimental models for rheumatic disease

Focusing on Sjögren's syndrome

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Summary
To identify potentially important immune responses leading to inflammatory reactions, studies of a model resembling the human disease is desirable. Early events in human inflammatory rheumatic diseases are difficult to study due to the relative late appearance of clinical signs of disease. Repeated sampling of tissues is hard to justify in human studies and thereby prospective analyses of mechanisms underlying progression of disease is limited. The ideal animal model of e.g. Sjögren's syndrome (SS) should have the clinical characteristics of dry eyes and dry mouth. The target organs should show inflammatory cell infiltration with destruction of normal tissue elements. Characteristic serologic abnormalities such as hypergammaglobulinemia, antinuclear antibodies, antibodies to extractable nuclear antigens SS-A (Ro) and SS-B (La), or rheumatoid factor (RF) should ideally be present. An appropriate animal model of autoimmune and rheumatic disease could greatly advance our possibilities to identify the target antigens, define the immune mechanisms, and characterize the evolution of tissue pathology. Such models have also been developed and characterised. Among the most useful have been the spontaneous models, but valuable information has also been obtained from induced models. More recently, gene-targeted mice have provided important contribution to our knowledge of human diseases and will hopefully permit animal models to be established for the development of gene therapy strategies. Thus, the decade of the mouse is here. With transgenic and knockout mice we suddenly have the ability to create tailor-made mammalian models of human disease which offers the opportunity to study complex physiological phenomena. Rheumatic inflammatory diseases are common and affect virtually every population or racial group studied. Most of these chronic inflammatory conditions affect predominantly females.
Although the etiology of connective tissue disease (CTD) is unknown, there is substantial evidence that immunologically mediated inflammation may be the most important factor leading to the chronic inflammatory lesions. It has been suggested that the genetic background and T-cell mediated autoimmune responses in the target organs are of central importance in the pathogenesis.
To identify potentially important immune reactions leading to inflammatory disease, studies of a model resembling the human disease is desirable. Early events in human CTD are difficult to study due to the relative late appearance of clinical signs of disease. For prospective analysis of mechanisms underlying progression of disease, we are also limited in human studies due to the fact that repeated sampling of tissues is hard to justify. In this context murine models are more easily justified. Our purpose with this short commentary is to illustrate some essential cellular and molecular aspects related to murine models of rheumatic disease. We have chosen to illustrate one of the more common CTDs, namely Sjögren's syndrome (SS).

Features of SS which should be fulfilled

The basic features of SS which should be satisfied by an experimental model are summarised below:

(a) clinical
In the ideal animal model dry eyes and dry mouth should be present. Reduced tear film can be objectively assessed by the Schirmer test and damage to corneal surfaces by Rose Bengal staining. Reduced salivary flow would be more difficult to demonstrate in small animals.

(b) histological
Both lacrimal and salivary glands should show infiltration by mononuclear cells, with progressive destruction of normal acinar and ductal epithelium and eventual epimyoepithelial island formation.

(c) serological
Sera from animals with SS should be hypergammaglobulinaemic and contain rheumatoid factors and autoantibodies to extractable nuclear antigens, specifically Ro/SS-A and La/SS-B.

(d) chronicity
Lesions in salivary glands should be progressive and long-lasting to mirror the human disease.

(e) immunohistochemistry
Glandular infiltrates should show the characteristic predominance of $CD4^+$ (helper) over $CD8^+$ (cytoxic/suppressor) T cells.

Additionally, the ductal epithelium should show aberrant expression of HLA-DR.

Examples of spontaneous models of SS
For a more comprehensive overview of available models the interested reader should consult an appropriate reference book (*Bona et al.* 1993). There are several inbred strains of mice which spontaneously develop autoimmune disease and which have been reported as models of SS. Among these are the NZB and NZB/NZW F1 hybrid mice, the MRL/Mp mice of which the substrain, *lpr/lpr* (homozygous for the *lpr* lymphoproliferation gene), the congenic substrain, +/+ (lacking the *lpr* gene) and NOD mice which spontaneously develop insulin-dependent diabetes mellitus. All these strains of mice have been reported to have glandular inflammation, which is a cardinal feature of human SS.
In all these strains $CD4^+$ T cells infiltrate the salivary gland, and expression of MHC class II products on the salivary gland ductal epithelium in the proximity of lymphoid infiltrates is thought to perpetuate activation of CD^+ T cells.

Cells involved in sialadenitis
In situ studies have shown that T-cells with different TCR Vβ-usage are oligoclonally expanded in the infiltrate of salivary glands in MRL/*lpr* mice (Skarstein et al. 1994). A relative decrease of T cells of certain Vβ families in the lymph nodes and the corresponding increase in the inflamed salivary glands, indicate that these cells have been specifically attracted to the salivary glands and possibly also expanded *in situ*.
Sialadenitis can be transferred *in vivo* to NOD neonates of both sexes by splenic T cells from diabetic males and females (*Goillot et al.* 1991). A recent report describes attempts to examine the cells involved in sialadenitis by studying the adoptive transfer of salivary gland inflammation in MRL-*lpr/lpr* mice (*Skarstein et al.* 1993). Cells from lymph nodes, spleen and infiltrating mononuclear cells enzymatically eluted from salivary glands were transferred *in vivo* to MRL-*lpr* neonates. Low grade sialadenitis was achieved by the transfer of total splenic cells but only occasionally by lymph node cells. In contrast, accelerating sialadenitis was achieved by the transfer of salivary gland infiltrating cells in some animals. These data indicate that sialadenitis in MRL-*lpr* mice is mediated by cellular mechanisms and suggest that the infiltrating salivary gland cells have the ability to accelerate autoimmune disease.

Role of autoantibodies
There is a single report on detection of anti-La antibodies by ELISA in MRL-*lpr/lpr* mice, although the pattern of recognition of

recombinant human La differed between mice and human patients (*St. Clair et al.* 1991). Approximately 30 % of older male MRL-*lpr* mice produce antibodies to the human La antigen, most of which reacted also with bovine and murine la. Spontaneously occurring anti-La antibodies in MRL-*lpr* mice show different epitope reactivity from those induced in MRL+/+ mice by immunisation with recombinant La protein. We have recently obtained results which show that anti-Ro is produced also in MRL-*lpr* mice (*Wahren et al.* 1994); 30 % of mice aged four months produced antibodies to human Ro 52 kD. Antibodies to Ro 60 kD and La were found in a low percentage. Immunohistological staining demonstrated anti-Ro 52 kD producing cells in spleen, lymph nodes and salivary glands of seropositive animals.

Cytokines involved in sialadenitis

The features of the T cells require further analysis with regard to the capacity to produce cytokines of potentially pathogenic significance. Initially, salivary gland inflammatory lesions of MRL/*lpr* mice were analyzed for local secretion of IFN-γ (*Jonsson & Holmdal* 1990). Additionally, we investigated the spontaneous and Con-A stimulated cytokine (Il-2, IL-3, IL-6, TNF-α and IFN-γ) production in purified thymus, lymph node, spleen and salivary gland mononuclear cell preparations. High levels of IL-6 was spontaneously produced by salivary gland infiltrating cells at all 3 ages investigated. Spontaneous production was also recorded for IL-3 and IFN-γ in salivary glands. This pattern of cytokine production certainly has a spectrum of biological consequences coordinating the immune response and/or autoimmune sialadenitis.

Apoptosis genes and Fas in MRL/lpr mice

Fas is a cell surface molecule that can mediate induction of apoptosis, and has recently been found to be mutated in MRL/*lpr* mice (*Watanabe-Fukunaga et al.* 1992). The defective *Fas* receptor on *lpr* lymphocytes and its resulting incompetence of primed cells to undergo *Fas*-mediated apoptosis, may lead to a prolonged survival and even expansion in peripheral tissues of activated cells. Perhaps peripheral T cells encountering autoantigen not previously seen in the thymus, fail to undergo deletion due to lack of functional *Fas* gene product. Recently, it was reported that culture with anti-CD3 antibody induced marked functional anergy and apoptis in normal murine T cells, but not in T cells from *lpr* mice (*Bossu et al.* 1993). This finding indicated that autoreactive T cells could resist growth-regulating stimuli and obtain a significantly extended lifespan.

Transgenic and knock-out mice

Transgenic mice containing the HTLV-I *tax* gene under the control of the viral long terminal repeat have been shown to develop an exocrinopathy involving the salivary and lacrimal glands (*Green et al.* 1989). Over the course of several weeks ductal proliferation dramatically increases and the structure becomes distorted accompanied by lymphocytic infiltration surrounding the nest of proliferating epithelial cells. Mice surviving 6–8 months exhibit extensive epithelial island enlargement with lymphocytic infiltration leading to destruction of acinar architecture. This model suggests that HTLV-I is tropic for ductal epithelium of salivary and lacrimal glands and may represent a primary event in the development of exocrinopathy by virally induced proliferation and perturbation of the function of ductal epithelium followed by a lymphocytic response. This sequence of events may differ slightly from SS pathogenesis where lymphocytic infiltration, although not clearly demonstrated, is thought to precede ductal cell proliferation. Transforming growth factor-β1 (TGF-β1) is a multifunctional growth factor that has profound regulatory effects on many developmental and physiological processes. Animals homozygous for a mutated TGF-β1 allele will at about 20 days suffer from a

wasting syndrome accompanied by a multifocal, mixed inflammatory cell response and tissue necrosis leading to organ failure and death (*Shull et al.* 1992). This includes also exocrine glands with periductal, primarily lymphocytic inflammation in about 50 % of the animals. It is hypothesised that absence of TGH-β1 could lead to lack of control of an autoimmune mediated inflammation.

Conclusion

The earlier reports on attempts to induce SS in animals by injection with salivary gland extracts with or without adjuvants and/or other supplements largely produced a transient inflammation which was self limiting and did not mirror the human disease in either the temporal course of events or in the serological profile. Better models are the mice with spontaneous autoimmune disease with longlasting and progressive exocrinopathy. Thus, it appears that there are currently some interesting experimental models which fulfil many of the here listed criteria of SS. However, elucidating the molecular aspects of human autoimmune exocrinopathy would certainly benefit largely from exploring relevant transgenic and knock-out mice.

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Extrapolation of animal data to man

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Between animal species, including man, there are physiological differences and therefore extrapolation of experimental data from one species to another has to be done with great caution. Thus the use of models instead of the target species itself has considerable limitations. It could be asked why use models or why use animals in biomedical research. Animals are used for the following reasons (1). a. Greater control over variables. b. Reduction of inter-individual variability because a group of laboratory animals of one species tends to react more homogeneously to treatment than does a group of humans. c. The specific sensitivity to treatment of certain animal species or certain strains of one species allows for greater experimental flexibility. d. Studies with animals are generally less expensive than those with man. e. Use of animals allow more demanding and invasive studies to be carried out. f. Because of the short lifespan of most laboratory animals, endpoint studies are feasible.

Only the last two reasons concerning invasive and endpoint studies are indisputable for using animals from the point of view of the extrapolation problem. In humans such studies simply cannot be performed. However, use of animal data entails the risk that they do not apply to humans. The other arguments are more of a practical nature but they may be important in speeding up the process of discovery. Rapid progress and reproducible data of animal research may generate well-substantiated ideas and hypotheses that can be tested in humans.

Extrapolation can take two forms; it may be qualitative or quantitative. Qualitative extrapolation deals with an animal's (pathophysiological) processes and its reactions to stimuli extrapolated to other animals or man. Quantitative extrapolation involves assessing, on the basis of animal tests, the dosage of a certain compound which would be beneficial or harmful to man or the target animal. Qualitative differences between species together with possible quantitative differences in physiological processes have a role to play here.

It could be suggested that for qualitative studies, it may be advantageous for models to have exaggerated or dissimilar metabolic characteristics to those of the target species. However, for quantitative studies, the metabolic or anatomic features of models should be as similar to those of the target species as possible. In order to reduce the risk of false extrapolation, different models may be used concurrently.

The criteria for the choice of a particular animal models are the following (2). a. Scientific value as a model. b. Availability. c. Economic considerations. d. Husbandry considerations. Although it would be difficult and perhaps impossible to prove, the laboratory rodents are used so frequently in research because these animals are readily available, relatively inexpensive and tractable.

The extrapolatability of results may also be affected by the degree of discomfort felt by the test animal. Table 1 gives a general indication of the extrapolatability of results from animal experiments, taking into account the degree of similarity between animal model and target animal, and also the degree of discomfort inflicted by experimental procedures during the experiments. When extrapolating results within the same species, one of the main differences recorded will be discomfort due to experimental procedures. Extrapolation may also be hampered by differences in genotype, sex, age and physiolo-

Table 1. Extrapolatability of results from animal experiments (3).

Animal model and target species	Discomfort during experiment	Type of extrapolation	
		Qualitative	Quantitative
Animal models matches target species	slight	+++	+++
	severe	+++	++
Animal model does not match target species	slight	++	+
	severe	++	+

+ = low degree of extrapolatability; +++ = high degree of extrapolatability.

gical status. The effect of differences in genotypes will be even more pronounced when the data is to be extrapolated to another species or to man. This is due to the differences in morphological and biochemical characteristics, to the response to substances and other stimuli, or to differences in the pathophysiological reaction patterns which exist between species.

Extrapolation from animal to man should always be carried out with reservation. Test results obtained from animals will ultimatively have to be verified in studies with humans. This means that it generally remains a matter of hindsight as to what extent extrapolation from animal to man was justified. It is often not possible to verify animal data in humans. It can only be suggested that, given this situation, animal tests can reduce risks imposed on man. Animal experiments for example set up to assess the safety of drugs and synthetic substances employed in agriculture, industry and food processing, reduce the risks involved for humans, even though toxicity data from test animals can never guarantee complete safety for human beings. The risk of false extrapolation can be minimized by using several species of animals in the experiments. This is the case in toxicological screening, where the authorities usually require the use of two species, one of which is to be a non-rodent. When carrying out research into the etiology and therapy of diseases, extrapolatability from an animal model is enhanced when the disease under study have a common origin in both man and the experimental animal. Animal tests can speed up the progress of research undertaken to combat sickness in man. Whilst observation of phenomena in animals provides ideas for directional research in humans, it also makes such research safer.

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Preliminary recommendations for health monitoring of mouse, rat, hamster, guinea pig, gerbil and rabbit experimental units

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Preamble

The health of an animal is always at risk from a variety of infections. Such infections may be inapparent or at least not made apparent by gross or obvious lesions. Clinical disease may thus not be observed until the animal is stressed, for example by an experimental procedure.

There is overwhelming evidence that infections in laboratory animals can often influence the outcome of experiments. Depending upon the specific infection, a variety of biological parameters may be affected, including behaviour, growth rate, relative organ weights, immune response and tumour developments. Subtle or overt infections can also lead to contamination of biological materials, tissue cultures, cell-lines, transplantable tumours and biological products. All infections, apparent or inapparent, are likely to increase biological variability.

Some laboratory animal diseases are zoonotic.

For all these reasons, a laboratory animal health monitoring programme is of vital importance, decreasing the risk of zoonotic infection and adding to the reliability and reproducibility of research data.

This report proposes a scheme for health monitoring of laboratory animal experimental units with the intention of harmonising procedures within the Scandinavian countries.

Report of the Scandinavian Federation for Laboratory Animal Science (Scand-LAS) Working Group on Animal Health.

General considerations

These recommendations constitute a common approach for all experimental colonies of mice, rats, hamsters, guineapigs, gerbils and rabbits. It is a prerequisite for the health monitoring of experimental animals units that all animals are obtained from breeding units that are subject to routine health monitoring according to the FELASA[1] or Scand-LAS[2] recommendations.

It must be emphasized that these recommendations contain only minimal requirements for health monitoring, and constitute a common baseline for researchers and technical personnel. Actual practice may exceed these recommendations in various ways, depending on local circumstances. Additional investigations may be deemed necessary; should these indicate the presence of an agent which, although not listed in these recommendations, is suspected of being important, that agent should be mentioned in successive reports and treated as are listed agents. In some experimental units it may not be necessary to use a complete health monitoring programme. In these cases the reasons must be stated and justified. Any modified programme should not, however,

[1] Recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit breeding colonies. Report of the Federation of European Laboratory Animal Science Associations (FELASA) Working Group on Animal Health, V. Kraft & al., Laboratory Animals 1994, *28*, 1–12.

[2] Recommendations for the health monitoring of gerbil breeding colonies. Report of the Scandinavian Federation for Laboratory Animal Science (Scand-LAS) Working Group on Animal Health, A. Hem & al., to be published.

be reported as a Scand-LAS approved health monitoring.
The term "unit" is here understood to describe a self-contained unit, which could be considered a microbiological entity. This unit may contain more than one room.
The existence of detailed written procedures – **S**tandard **O**perating **P**rocedures (SOP's) within monitoring laboratories is expected. Such SOP's must be available on request.
Monitoring laboratories should follow the principles of **G**ood **L**aboratory **P**ractice (GLP) where applicable.
Monitoring laboratories should participate in a Quality Assurance Programme.
It should be emphasized that negative results mean only that the presence of the micro-organisms monitored has not been demonstrated in the animals screened by the test(s) used. The results are not necessarily a reflection of the status of all the animals in the experimental unit.
An agent must be declared as present if it is identified or antibodies to it are detected in one or more of the animals screened. The results must continue to be reported as positive at subsequent screens until the agent has been eradicated by means of e.g. rederivation, restocking etc. However, agents known to be present need not be monitored at subsequent screens provided that they are declared in the health report.
The presence of antibodies in a colony is only an indicator of infection. Their significance can be elucidated using methods other than serological methods.
If a unit contains more than one animal species, each species must be screened separately, according to the test schedules.
It is important that all aspects of an experimental unit are monitored. This must include the animals, water and bedding. Also the environment, equipment eg cagewasher, air quality, temperature, humidity, noise and lighting levels.

Monitoring procedures

Animals

The animals to be monitored can be the same animals (principal population) as used in a study, or sentinel animals.

Sentinel animals

Sentinels are animals that are introduced into a laboratory animal unit for health monitoring purposes.
Sentinel animals must come from a colony in which health monitoring is performed according to FELASA or Scand-LAS recommendations for breeding units. The colonies must not have tested positive for any agent to be monitored in the experimental unit.
Sentinels are put into open cages which are placed either systematically or randomly throughout the unit or in places where exposure to infectious agents is thought or known to be at its maximum. The sentinel animals must be placed in clean cages with some dirty bedding used by the principal population. This will encourage the transmission of infectious agents from the principal population to the sentinel animals. During a study dirty bedding placed in the sentinel cages should be taken from different areas of the room.
Sentinels can be of the same strain or stock as the principal population. They may be of another strain suitable for monitoring or in special circumstances be strains/stock known to be susceptible to the infectious agents to be monitored.
It is emphasized that negative results in sentinel animals does not necessarily imply that the specific infection is not present in the experimental animals. There is a possibility that an infection has not spread to the sentinel animals.

Frequency of monitoring

Monitoring should be at intervals of not greater than 3 months. Sampling may be carried out more frequently if desired. After

the detection of an infection the frequency of monitoring for that particular agent should be increased. Animals to be monitored must be exposed in the room for at least a period of 4 weeks.

Sample size
Within a microbiological entity, at each sample point, all rooms must be monitored. It is sufficient to use 4 animals per room irrespective of whether the animals are part of the study or sentinels. The sex and the strain must be considered in relation to the facility being monitored.

Age of test animals
When studies are being monitored by the removal of the principal stock the age of the animals is obviously determined by the length of the study.
The age of sentinel animals when first placed in a facility should be 6–8 weeks for rodent species and 9–11 weeks for rabbits.
Note: It is inadvisable to expose animals to be used for monitoring for more than 3 month in any room. Thus, in long term studies it is advisable to use sentinel animals rather than old study animals.

Organisms to be monitored
The testing procedures for the organisms should follow the FELASA or Scand-LAS recommendations for breeding units for the species in question.

Non animal factors
The outcome of experiments may be influenced by many factors other than the animals themselves. Food, water and bedding may introduce microbes, chemicals or toxins. Noise and lighting levels as well as room temperature and humidity may affect animal behaviour and many other parameters. Equipment, such as cagewashers or the pans on the weighing machine can spread infections if not properly maintained and cleaned.
Non animal factors are important aspects of the defined animal and further elaboration is required.

Feed
Each batch of diet must be accompanied by an analysis of the feed, including the content of nutrients, microbiological status and a test report for possible residues or toxins. If considered desirable diet must be sent to specialised laboratories for duplicate analysis.

Water
The results of the analysis of the public water supply should be obtained from the appropriate authorities. In addition samples may be sent to specialised laboratories for analysis.

Bedding
Bedding must be accompanied by a certificate of analysis with special reference to chemicals and toxins. If considered necessary specialised laboratories can be sent samples for analysis.

Air supply
If the air is filtered the efficiency of the filters must be checked continuously by pressure gauges which indicate the flow.

Report
While Scand-LAS cannot accept responsibility for tests or their implication, breeders or users of laboratory animals who are reporting on health monitoring of their animal colonies may use words "in accordance with Scand-LAS recommendations" only where that is in fact the case.
A Scand-LAS approved health report for experimental units should follow the guidelines laid out in the FELASA recommendations appendix II. In addition information about the above non animal factors should be given.