A microchip implant system as a method to determine body temperature of terminally ill rats and mice

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Summary

In a series of experiments, *Klebsiella pneumoniae* was inoculated intratracheally into rats and mice, and the temperature of the animals was recorded twice daily using microchip transponders. Transponders are interrogated by radio frequencies and were implanted either subcutaneously or intraperitoneally. The microchip temperatures were compared with rectal temperatures taken at the same time. The purpose of the experiments was (a) to investigate the practicability and reliability of the ELAMS^{TM} for temperature recording; (b) to compare values given by subcutaneously or intraperitoneally implanted transponders with rectal temperatures; and (c) to determine a 'temperature-cut-off point' as an alternative for 'death of the animal' as an end point for the experiment. The results showed that the ELAMS^{TM} was easy to operate and no important drawbacks in the use of the system were observed. The temperatures generated by the transponders implanted subcutaneously and intraperitoneally did not differ significantly from rectal temperatures. In two out of three experiments on rats, it was shown that when the temperatures reached values below 36°C, the median survival time of the animals was 24 h. In the one experiment on mice the same median survival time was observed at 36°C. In one experiment using rats, however, the disease was so acute that death occurred before any temperature drop was seen. The results show that when a 36°C cut-off point is used instead of the time of death in this particular animal model, the statistical analysis was not altered, but that it would spare animals further suffering for approximately 24 h. The argument that measuring body temperature is a laborious job and stressful to the animals is overcome when the ELAMS^{TM} system is used.

Keywords Animal; body temperature; humane end point; refinement alternative; microchip transponder

Refinement is the 'R' in the Three Rs of Russell and Burch's (1959) that has received relatively little attention (the other Rs are Replacement—of animals by other means, and Reduction—in the number of animals used). Refinement means that animal pain and suffering is reduced. To illustrate the lack of research into Refinement, in the journal *ATLA* (*Alternatives to Laboratory Animals*) which covers all three Rs, only three out of more than 100 articles were dedicated to this subject between 1991–1995. One article was on animal housing (Sharmann 1991), and the other two were more

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like overviews (Morton 1995; Rowan 1995). However, in the past 10 years Refinement has been introduced into animal experiments through 'Guidelines', 'Recommendations' and 'Codes of Practice'. For example, guidelines were introduced into cancer research (Workman *et al*. 1988), on the welfare of animals in rodent protection tests (Acred *et al*. 1994), and on pain, distress and discomfort in general (Morton & Griffith 1985). In The Netherlands, Codes of Practice were introduced for raising polyclonal and for monoclonal antibodies. Furthermore, progress has been made by introducing telemetric systems (Kramer *et al*. 1993), non-invasive techniques like NMR (Fransen *et al*. 1996), the use of analgesics in painful experiments (Flecknell 1994), and the use of improved biostatistical methods in data analysis e.g. quantitative parameters replacing dichotomies (like life or death). Another important aspect of Refinement is the use of 'humane end points' i.e. programmed euthanasia instead of death of the animal (Olfert 1995). It is our opinion that death as an end point is not acceptable when other parameters with the same statistical power can be used. Acceptability, however, of such a parameter is dependent on the practicability of the alternative procedure. Therefore, in the present study we focused on experiments in which mice and rats were challenged intratracheally with bacterial suspensions of *K. pneumoniae* and in which classically bacterial virulence or efficacy of drug therapy are measured by the number of animals dying at a certain timepoint after inoculation.

It is well known that the progression of illness is accompanied by a decrease in body weight, hypothermia and clinical symptoms like piloerection, a badly groomed coat and decreased locomotion. In earlier studies in which *K. pneumoniae* was used to induce bacteraemia, body temperature and body weight were sometimes also determined (Roosendaal 1988) but no effort was made to study the possibility that either of these parameters could be used as a criterion for euthanasia of the animals. In particular body temperature seemed to be most discriminating and predicting oncoming death (Soothill *et al*. 1992), but because measuring rectal body temperature is rather time consuming and stressful, the method was considered to be not practical. Using an implanted temperature transponder system could overcome these drawbacks and increase acceptability. We therefore undertook a study in which we tried to answer the following questions:

(1) How practical is an implanted temperature transponder system?
(2) How accurate is such a system, and what is the preferred site of implantation, subcutaneously or intraperitoneally?
(3) What is the temperature-cut-off point, i.e. what is the body temperature after which death of the animal can be predicted accurately?

Materials and methods

In this experiment the rats and mice were part of an ongoing study in which new therapeutic regimens were tested using the *K. pneumoniae* model.

Animals and husbandry

Thirty SPF female RP rats and 10 male NMRI mice were obtained from Harlan and Iffa-Credo, (Harlan Nederland BV, Austerlitz, The Netherlands; Iffa-Credo Belgium). The animals were used for experiments after an acclimatization period of at least a week after arrival at the Institute.

Until the start of the experiment rats and mice were maintained under standard conventional conditions: acidified tap water (pH 3.0) and food (Hope Farms AM II, Woerden, The Netherlands) *ad libitum*; a 12:12 light:dark cycle (lights on 07:00 h), temperature of $22 \pm 1^{\circ}$C and a relative humidity of 40–60%; a minimum of 15 air changes per hour; housed in polycarbonate cages (dimensions, $40 \times 25 \times 15$ cm, $1 \times b \times h$) in groups of four rats or five mice. After inducing experimental pneumonia the animals were singly kept in filtertop cages (polycarbonate; dimensions, $30 \times 15 \times 13$ cm, $1 \times b \times h$) in an animal room especially designed for infection studies and in which the entrance was restricted to persons wearing gloves, masks,

surgical caps and clean coats. Cages, animal bedding, food and water were sterilized.

The experimental protocols adhered to the rules laid down in 'The Dutch Animal Experimentation Act' (1977) and the published 'Guidelines on the Protection of Experimental Animals' by the Council of the EC (1986). The present protocols were approved by the Institutional Animal Care and Use Committee of the Erasmus University, Rotterdam, The Netherlands.

Experimental design

Three experiments each with one group of rats ($n = 10$) and one experiment with a group of 10 mice were performed in the study. Half of the animals received the transponder subcutaneously, the other half intraperitoneally. At the start of each experiment (day 0) the animals were weighed and rectal temperature recorded, and animals were then anaesthetized and the transponders implanted. The rats were inoculated at the same time as implantation, but the mice were given a second anaesthetic and inoculated the next day. In the experiments using rats the temperatures were recorded at T = 0, 24, 48, 72, 78, 96 and 102 h. In mice, temperatures were measured exactly every 12 h, i.e., 0, 12, 24, 36 etc. The remaining animals were killed at the end of day 7 (180 h).

Infection model of Klebsiella pneumoniae *pneumonia in rats*

Experimental pneumonia was induced under anaesthesia with Hypnorm® (0.1 ml/rat, i.m.; Janssen Pharmaceutical Ltd, Oxford, UK) together with Nembutal® (0.3 ml/rat of a 4× diluted solution in saline, i.p.; Sanofi Sante BV, Maassluis, The Netherlands). The left main bronchus was intubated and the left lung inoculated with 0.02 ml of a saline suspension of *K. pneumoniae* (ATCC 43816, capsular serotype 2). The number of viable bacteria varied between experiments from 5.9×10^6 to 8.0×10^6. As confirmed in previous studies (Bakker-Woudenberg *et al.* 1982) infection develops within 24 h and generally leads to spontaneous death within 5 days. No antibiotics were given in this experiment.

Infection model of Klebsiella pneumoniae *pneumonia in mice*

Klebsiella pneumoniae infection was induced using a Hamilton syringe under anaesthesia (Enflurane: 3%, $O_2 : N_2O = 1:2$). The mice received either 4×10^3, or 4×10^5 or 4×10^7 cfu/ml bacteria in 50 μl, endotracheally (the present study was part of a dose-response study). The three doses were 'equally' distributed between the 10 mice ($4 + 3 + 3$, respectively). The transponders were implanted one day before inoculation of the bacteria using the same anaesthesia.

Measurement of the body temperature

All measurements of body temperature were carried out twice a day. Measurement of the rectal temperature was done by means of a digital recording with a thermistor system (Ellab type Du-3, Ellab Instruments, Copenhagen, Denmark; calibration by internal calibration). Recordings were carried out without anaesthesia. When necessary the rats were restrained by a towel. In the rats the probe was put 6 cm into the rectum. Recordings were taken when the temperature seemed to be stable, which was after approximately 15 s. Between measurements the probe was cleaned with sterile saline and lubricated with vaseline. After measurements the probe was cleaned with 70% alcohol. For the mice the procedure was the same, although it was not necessary to restrain the animals. By grasping the mice by the tail and lifting a few centimeters off the table, the thermistor could easily be introduced into the rectum (approximately 1.5 cm).

The first recordings of the transponder temperature were carried out with the animal still under anaesthesia, shortly after the transponder was inserted and the identification code was programmed. The recordings at T = 0 were not further used in the evaluation of the results as the temperatures were clearly lower than normal because of the anaesthesia induced hypothermia.

All other recordings (after T = 0) were carried out while the animals were freely roaming around their cage with the lid removed. To identify animals and to measure

the temperature the scanner of the system had to be directed toward the animals within 5 cm of the transponder, it was not necessary to touch the animals. The transponder temperature was measured in duplicate: in rats within 15 s between the measurements; in mice the transponder temperature was measured just before and just after the rectal temperature was carried out.

BioMedic Data Systems' transponder system

The system we used, ELAMS™ (Electronic Laboratory Animal Monitoring System by BioMedic Data Systems, Inc. Seaford DE, USA) consisted of a notebook (DAS-5002), which is a portable data acquisition system connected to a detachable scanner wand. The system is powered with an internal rechargeable battery. All data, i.e. the unique code of the animal, the temperature and the clinical observation data are stored in the memory and can be transmitted to other data systems. The implantable programmable temperature transponders (IPTT-100) are encapsulated in biocompatible glass capsules and covered with a polypropylene cap for anti-migration. They measure 2.2×14 mm and weigh 120 mg. The identifier that can be programmed on the microchip is a maximum of 16 characters. According to the manufacturer, the temperature can be read with a $\pm 0.5^{\circ}$C accuracy, and a resolution of 0.1°C in the calibrated range of 32–43°C. To read the transponder the scanner has to be held at a distance of around 50 mm. To programme the transponder after it has been injected into the animal the scanner has to be held at a distance of around 25 mm. To programme the transponder before injection a needle programmer must be used. Each transponder is packed in sterile (sterilized with EtO) needles (o.d 2.2 mm) and in each box of 100 transponders a specially designed 'insertor' is added. These insertors were used for rats and mice, and for both subcutaneous implantation and intraperitoneal implantations. For the subcutaneous site, the neck region of the animals was chosen.

Although the manufacturer meant the transponders to be used just once, we have reused them. After death the transponders were removed, gently cleaned with tap water, then 70% alochol, and dried. The transponders were placed in their covering needles and resterilized with Cidex® (22.5% glutaraldehyde solution; Johnson & Johnson Medical BV, Amersfoort, The Netherlands) for at least one hour and then rinsed with sterile tap water and dried.

Statistics

Repeatability of temperature measurements by transponders was evaluated according to ISO/DIS 5725 (1994). The repeatability value is defined as the critical limit for the absolute difference between two single independent measurements obtained under repeatability conditions (same instrument, same observer, within a short time), which will not be exceeded in 95% of cases. This value may be interpreted as the 'limit of detection' of a significant difference between single independent measurements under repeatability conditions.

The repeatability value r is derived from (if $n > 30$): $r = 1.96 \times \sqrt{2} \times \mathrm{SD}_{\mathrm{repeat}}$.

The SD for repeatability can be computed from duplicate measurements on n cases by using the formula

$$\mathrm{SD}_{\mathrm{repeat}} = \sqrt{\frac{\sum(d^2)}{2n}}$$

with n degrees of freedom, where $\Sigma(d^2) =$ sum of squared duplicate differences.

Whether or not $\mathrm{SD}_{\mathrm{repeat}}$ depends on the level of measurements is checked by analysing scatter diagrams relating duplicate differences to duplicate means. In addition any systematic shifts between first and second measurements are tested for significance using Student's paired t-test. Similar procedures are followed in analysing paired temperature measurements by transponder or rectal thermisters. Mean differences were considered statistically significant if $P_{(2\text{-sided})} \leq 0.01$.

Results

Body weight change

The rats entered the study at an age of 21–24 weeks and at the beginning of the experiments weighed 196–230 g. Mean body weight of the animals 2 days after inoculation of the bacterial suspension was 90% of the day-0 values. In the mice model, the 10 mice weighed 26–30 g at the start of the experiment and most animals did not lose weight (actually most of them gained weight). One animal, surviving 7 days, lost 17% of its original body weight.

Body temperature change

Convenience and ease-in-use is, of course, rather subjective however, the ease by which the ELAMS^{TM} from BioMedic Data Systems generates the temperature as well as the unique animal code cannot be compared with any other way of detecting animal body temperature. Programming the transponders proved to be easy, without any data errors occurring, and took a matter of seconds. Collecting the data was also simple when the scanner was placed at the proper distance from the animal. The data could be collected within seconds without hindering the animal in its movements.

Although of minor importance some disadvantages in the practical application of the ELAMS^{TM} system were assessed: the beep that comes with data collecting sometimes seemed to disturb the animals (in more recent models it can be disabled). Furthermore, the reflection of light at the display of the notebook sometimes made it hard to read the data. The weight of the apparatus is approximately 2 kg, which is considerable for an apparatus that is designed to be held in the hand. For this reason our technicians collected the data mostly with the notebook placed on the table, which decreased the reflection.

Measuring body temperature rectally in rats and mice was a stressful procedure and sometimes difficult to carry out without restraining the animals. For this reason we wrapped the rats in a towel during the rectal temperature readings. For mice this was not necessary but distress could certainly not be excluded. To put a thermister into a constant depth into the rectum is difficult, and although we marked the thermister at a certain length we could not guarantee that the procedure was standardized. Moreover, it takes some time for the thermister to adapt to body temperature, which means that the time between inserting and reading has to be a minimum standard as well.

Although the injector, which was a 2.2 mm diameter needle, appeared large for a 20 g mouse, in particular for introducing intraperitoneally, no apparent complications (i.e. bleeding, paralysis etc.) were seen. All 40 transponders were inserted properly, i.e. none came out spontaneously and all gave accurate data recordings during the course of the experiment. The only complication seen, was that two of the inserted transponders in rats and one in mice, which were meant to be implanted intraperitoneally, were actually placed subcutaneously. This was because we were very careful not to harm the internal organs and implanted the transponders under an angle of less than 45° With experience this complication was not repeated.

Collecting data was easier when the transponders were implanted subcutaneously compared with intraperitoneally as the exact location of the transponder is easier to reach. There were no differences in the procedure of collecting data for rats and mice.

At autopsy, (a maximum of one week) all the transponders were easily found and there was no visible tissue reaction. The implanted transponders produced no detectable clinical effects in the animals. As mentioned earlier they were cleaned and used for three consecutive experiments without any problem.

Repeatability of the ELAMS^{TM} system with regard to temperature recordings was established on the basis of *all duplicate values*, i.e. values of normal temperatures as well as of temperatures of animals during the infection. The results (Tables 1 and 2) showed mean differences ($\pm$SEM) of -0.12 ± 0.04 ($n = 34$) for rats subcutaneously and $+0.01 \pm 0.04$ ($n = 44$) for rats intraperitoneally. For mice the values were -0.23 ± 0.06 ($n = 43$) and -0.22 ± 0.07 ($n = 55$), for subcutaneous and intraperitoneal

Table 1 Mean body temperatures of rats during the course of experiment, either recorded by transponder or rectally

	Transponder ($n=34$)	Transponder ($n=44$)
Rats	Subcutaneous	Intraperitoneal
Mean T1	36.6 ± 0.22	37.1 ± 0.20
Mean T2	36.7 ± 0.23	37.0 ± 0.20
Mean T3	36.7 ± 0.24	36.8 ± 0.20
Shift T1–T2	-0.12 ± 0.04	$+0.01 \pm 0.04$
Shift T1–T3	-0.07 ± 0.05	$+0.25 \pm 0.06$
SD_{repeat} T1–T2	0.15	0.16
SD_{repeat} T1–T3	0.21	0.28

T1 = first recording of the body temperature by transponder
T2 = second recording of the body temperature by transponder
T3 = body temperature established rectally
Shift T1–T2 = mean difference between T1 and T2
Shift T1–T3 = mean difference between T1 and T3
SD_{repeat}: see M&M Statistics

Table 2 Mean body temperatures of mice during the course of experiment, either recorded by transponder or rectally

	Transponder ($n=43$)	Transponder ($n=55$)
Rats	Subcutaneous	Intraperitoneal
Mean T1	37.2 ± 0.42	36.7 ± 0.19
Mean T2	37.4 ± 0.43	36.9 ± 0.18
Mean T3	36.9 ± 0.46	37.2 ± 0.21
Shift T1–T2	-0.23 ± 0.06	$+0.22 \pm 0.07$
Shift T1–T3	$+0.23 \pm 0.11$	$+0.55 \pm 0.13$
SD_{repeat} T1–T2	0.29	0.36
SD_{repeat} T1–T3	0.49	0.70

See Table 1 for abbreviations

sites, respectively. The second recordings of the body temperature in mice were slightly higher than those of the first. This may partly be due to the fact that the second reading was taken after the (stressful) rectal temperature measurement. In rats, where the duplicate values were taken within 15 s of each other and both before the measurement of the rectal temperature, differences were smaller and not consistent in trend.

Differences between the body temperatures recorded either by ELAMS^{TM} transponders or by rectal measurements were small (Tables 1 and 2) and well within the bounds of the specifications given by the factory (accuracy = $\pm 0.5^{\circ}$C). It seems that on the basis of the SD for repeatability the subcutaneous values approach more closely the rectal values than the intraperitoneal values. From these data it also seems that repeatability in rats is better than in mice. Finally, the difference in values between transponder temperature and rectal temperature was higher in mice than in rats. Paired *t*-tests proved that differences between the pairs (i.e. first and second measurements; transponder and rectal) could be considered extremely significant with 2-tailed *P* values of < 0.001.

In Tables 3–5 the results of two out of three infection studies in rats and one in mice are given. In the one experiment with rats not given here, the infection was so acute that all the animals died within 4 days without any detectable temperature decrease. In the tables, 36°C, 35°C and 34°C are taken as cut-off points, and correlated with the number of

Table 3 Number of rats with a ΔT of either < 6, 12, 24, 36 and 48 h at a certain point of hypothermia: experiment 1

Bt	ΔT < 6	ΔT = 12	ΔT = 24	ΔT = 36	ΔT = 48
< 36°C	0	4	2	2	2
< 35°C	1	7	2	0	0
< 34°C	2	6	2	0	0

Median 36°C = 24 h; 35°C = 12 h; 34°C = 12 h
ΔT = time difference (h) between the time of measured body temperature at a certain °C and the time of observed death
Bt = body temperature

Table 4 Number of rats with a ΔT of either < 6, 6, 12, 18 and 24 h at a certain point of hypothermia: experiment 2

Bt	ΔT < 6	ΔT = 6	ΔT = 12	ΔT = 18	ΔT = 24
< 36°C	0	1	3	2	4
< 35°C	6	1	2	1	0
< 34°C	7	1	2	0	0

Median 36°C = 24 h; 35°C = 12 h; 34°C = 12 h
ΔT = time difference (h) between the time of measured body temperature at a certain °C and the time of observed death
Bt = body temperature

Table 5 Number of mice* with a ΔT of either < 6, 6, 12, 18 and 24 h at a certain point of hypothermia

Bt	ΔT < 6	ΔT = 6	ΔT = 12	ΔT = 18	ΔT = 24
< 36°C	1	2	2	2	1
< 35°C	1	3	3	0	1
< 34°C	3	3	2	0	0

Median 36°C = 24 h; 35°C = 12–24 h; 34°C = 12 h. *2/10 animals survived
ΔT = time difference (h) between the time of measured body temperature at a certain °C and the time of observed death
Bt = body temperature

animals dying during that time period. What is striking is that the progression of the infection is very rapid, most rats dying within 48 h and mice within 24 h after the first decrease in temperature. After reaching a body temperature of 34°C the median survival time of the rats is 12 h, whereas at 36°C the median survival time is 24 h.

As all rats eventually died when the temperatures reached values below 36°C a sensitivity (discrimination between survivors and non-survivors) of either one of these cut-off points could not be given. In mice, however, 2 out of 10 ('80%' sensitivity!) animals recovered from having had temperatures below 36°C. The lowest temperature these animals reached before recovery of the body temperature was 35.4°C. This means that with a cut-off point of 35°C a 100% sensitivity can be reached. These two mice had received the lowest dose of *K. pneumoniae* (4×10^3 inoculum).

Discussion

Death as an end point in animal experiments is criticized by many who take the welfare of animals seriously. In many studies in which death was the parameter of choice, for instance in experiments on transplant survival or cancer, death has been successfully replaced by other parameters (i.e. for kidney transplants in dogs: serum creatinine >1000 μMol/l (Bijnen *et al.* 1979), for cancer a specific weight pattern (Redgate *et al.* 1991) with the same or even better statistical

power. The benefit was to both science and the animals. However, infection studies in which animals are challenged with bacteria and therapeutic regimens tested, death is commonly used as the end point, the arguments for this is that no other parameter exists, or are not validated, or not accepted, or are expensive, or are time consuming, or interfere with the results, etc.

It is a fact that disease in many of such experiments progresses very rapidly, with a short time between clinical signs of illness and death. Nevertheless death as an end point should be avoided as stated in 'Guidelines for the welfare of animals in rodent protection tests' (Acred *et al*. 1993) where it is recommended to kill the animals with: (a) hind limb paralysis; (b) hypothermia (not further defined!); (c) nasal discharge; (d) signs of respiratory distress; and (e) loss of righting reflex. However, to use clinical signs as a parameter in protection tests may not easily be accepted because of their relative subjectiveness and possible lack of reproducibility. Another possible parameter is to take blood samples and quantitate the bacteraemia. This procedure would also have drawbacks, not least of all because the investigator is primarily interested in the clinical signs and how to prevent and postpone these, and not the bacteraemia as such.

Changes in body weight and temperature are widely accepted as general clinical signs of the condition of an animal, and for this reason these parameters are sometimes taken into account as well. However, body weight is not seen as discriminatory enough to decide whether an animal should be euthanized. Arguments to support this are that even with substantial weight loss, animals are able to recover and, on the other hand, animals can die without any weight loss at all. We think that for body temperature these arguments are less valid and it should be possible to determine a cut-off point below which the animals are terminally ill. Other authors came to the same conclusion. In the article by Soothill *et al*. (1992) in which mice were challenged with different bacterial agents, the authors found that when 34°C is taken as a cut-off point not only an objective end point is chosen, but that this temperature is reached before clinical signs of illness can be judged. The authors did not give data on the number of hours or days that one would have been able to prevent the animals suffering when using this end point.

If one accepts the value of body temperature as a parameter of illness and as an indicator for euthanasia, recording body temperature per rectum several times a day would not be acceptable. Moreover, the procedure of measuring temperature in this way is certainly stressing to the animals but by using temperature transponders this problem is overcome. The present results and those of others (Ball *et al*. 1991) proved that such a system can be very accurate in displaying the body temperature, and in particular temperature changes.

Ideally it would be useful to measure the temperature as often as possible, even continuously. Not only do the commercially available data systems not provide for such a possibility, but at night when the temperature might reach a terminal value, there is no technician to euthanize the animals (we do not have a 'technician for the night' as was suggested at the 2nd World Congress on Alternatives in Utrecht, The Netherlands, October 1996). Therefore, in the present study we measured the temperature with the same frequency as the animals' condition was routinely observed in these experiments, which is twice a day, although we consider this is rather a low level of observation for such experiments.

One may wonder if investigators can be convinced of the value of using temperature as an end point just for the prevention of a few hours of animal suffering. In other infection models, however, this period may be longer with more to gain. Furthermore, when animals become comatose some investigators consider the animals are beyond suffering. However, suffering is likely to have been severe before that state. True or not, it seems logical that when the first signs of suffering can be assessed one is obliged to relieve the suffering of the animal especially if the scientific end point has been achieved. In the present experiment using *K. pneumoniae*, the condition of the animals deteriorated rapidly after temperatures below 36°C,

and the time between the cut-off point and coma is very short indeed—a matter of hours rather than days. When the cut-off point is chosen on the safe side as proposed by Soothill *et al*. (1991) at 34°C, 24 h was gained, but more suffering could have been avoided had the cut-off temperature been taken higher (Morton DB, personal observation).

The present results show that temperatures taken either subcutaneously or intraperitoneally do not differ significantly, despite the fact that intraperitoneal, rather than subcutaneous would better reflect the core temperature of the animal. Nor did we find evidence that transponders placed subcutaneously were influenced by differences in room temperature. We did find, however, an important influence of anaesthesia on body temperature and this is to be expected, but because the anaesthetic was given only at the start of the experiment, it did not interfere with the interpretation of the infection-induced hypothermia.

The subcutaneously implanted chips were read more easily by the data scanner, so we consider subcutaneous site as the first choice. However, if for some reason the intraperitoneal site is preferred, this can be done with almost the same ease.

From the results of this study we conclude that the ELAMS™ data system as used by us was practical and accurate in measuring body temperatures and can be of great help in designing humane end points in infection experiments, although with *K. pneumoniae* the progression of the disease was so fast that not much could be done in terms of time to reduce the animal suffering. We have to realize, however, that in other experiments in which death is reached more slowly, more will be gained in terms of prevention of suffering.

A shortcoming in the design of the present experiment, in which no therapy was given, is the fact that most animals died (rats 100%). Only when therapy is being studied and some animals are expected to survive will we be able to establish the temperature below which animals are certain to die. One may wonder if in these experiments the same power of arguments to assess the quality of a certain therapeutic regimen could be reached, when instead of life/death, body temperature is taken as the end point. Perhaps, the number of animals per group could be reduced as instead of a dichotomic variable (life/death), a continuous variable (temperature) is taken. Statistical power may even be increased if, for instance, body weight is taken into account as well.

The temperature transponders are still relatively expensive (approximately $10, each), although when the transponders are reused (as was done by us successfully) the costs can be reduced, particularly when related to the benefits in accuracy of the experiment and the wellbeing of the animal. Certainly, we hope the manufacturer will be willing to deliver separate polypropylene caps for the transponders as these will wear off with time. Even so the price of this system is not a valid argument not to measure body temperature in terminally ill animals.

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