This material may be protected by copyright law. (Title 17 U.S. Code)
Euthanasia by Decapitation: Evidence That This Technique Produces Prompt, Painless Unconsciousness in Laboratory Rodents

R. ROBERT HOLSON
National Center for Toxicological Research, Jefferson, AR 72079

Received 6 January 1992

HOLSON, R. R. Euthanasia by decapitation: evidence that this technique produces prompt, painless unconsciousness in laboratory rodents. NEUROTOXICOLOGICAL TERATOLOGY 14(4) 253–257, 1992. — Rapid euthanasia of laboratory rodents without the use of anesthesia is a necessary research technique whenever there is the likelihood of anesthesia or stress interfering with the chemistry of the tissues under investigation. Decapitation has long been the procedure of choice under such circumstances. Recently, however, the American Veterinary Medical Association (AVMA) panel on euthanasia recommended that decapitation be avoided on the grounds that the decapitated head may be conscious and suffering for as much as 15 seconds. The panel further recommended that if decapitation was scientifically necessary, the decapitated head be immediately immersed in liquid nitrogen. These AVMA guidelines now enjoy regulatory status; the recommendation that decapitation be avoided has thus caused considerable difficulty for all research requiring rapid, anesthesia-free collection of tissues. The scientific validity of these recommendations is consequently a matter of great practical as well as theoretical importance. The decision to discourage decapitation appears to have been based on a single literature report claiming that the EEG of the decapitated head revealed conscious suffering for more than 10 seconds (Mikeska and Klemm 1976). This review carefully examines the scientific literature on this subject. It is concluded that the report by Mikeska and Klemm of EEG activation in the decapitated head is correct, but that this phenomenon is also seen when the decapitated head is under deep anesthesia, and in normal brains under either anesthesia or during REM sleep. Hence these findings do not demonstrate either consciousness or the perception of pain. Furthermore, a substantial body of research indicates that unconsciousness due to hypoxia must occur in the decapitated head in at most 3 and more probably less than 3 seconds. Moreover, even if the decapitated head is conscious for some seconds, high cervical section immediately severs sensory input from the head and neck region to the brain. Thus, the severed head is anatomically incapable of experiencing substantial pain in the region of major trauma. Finally, no evidence was found to support the recommendation that the severed head be immersed in liquid nitrogen. It is, accordingly, urged that the AVMA panel recognize that decapitation produces rapid, pain-free unconsciousness in rodents, and that they alter their recommendations to allow rodent decapitation without qualification. Due to the rapidity of unconsciousness, it is further urged that the disposition of the severed head be left up to the investigator, without requiring immersion in liquid nitrogen or any other mandatory technique.

Euthanasia Decapitation Pain Hypoxia Consciousness Animal care and use

IN 1963, the American Veterinary Medical Association (AVMA) first published guidelines for animal euthanasia (2). These guidelines, since updated several times (3, 4, 5) were originally targeted primarily for veterinary practice, and were a laudable attempt to assure that animal euthanasia techniques minimized animal pain and suffering. However, the 1986 revision of these guidelines (5) greatly expanded the attention paid to animal research techniques. These revised guidelines now enjoy Federal regulatory status (25) and are an appropriate subject for scientific scrutiny. As this review will show, some aspects of the 1986 guidelines are not supported by such scrutiny.

The primary deficiency in the current guidelines concerns the recommendations for euthanasia techniques for laboratory rodents, especially euthanasia by decapitation. In their 1978 guidelines (4), the AVMA panel had approved decapitation as a method for rodent euthanasia, noting that “Although this procedure may be aesthetically offensive, it is rapid, inexpensive, and when properly done, produces instantaneous death” (4, p. 71). A year later a letter was published in the AVMA journal, citing an article by Mikeska and Klemm as evidence that the decapitated rat head was both conscious and suffering (33). The 1986 AVMA panel, perhaps alerted to the issue by this letter, cited the Mikeska and Klemm article as support for the assertion that “... data suggest that animals may not lose consciousness for an average of 13 to 14 sec following decapitation” (5, p. 265). This review will show that this assertion is incorrect. Data available by 1986, with the single exception of the Mikeska and Klemm article, did not make any such suggestion.

To complicate matters, the panel went further beyond the available evidence in recommending that if conscious ro-
dents are decapitated, the head must be "immediately frozen in liquid nitrogen subsequent to severing" (5, p. 265). As this article will show, neither recommendation had any foundation in the scientific literature available at the time, nor has subsequent research provided any support for these recommendations. Further, the recommendations for rodent euthanasia contain logical inconsistencies. All of the above problems will be detailed in this review.

There are four fundamental issues governing policy decisions on the use of decapitation in scientific research using rodents. These are:

1. Does decapitation produce prompt unconsciousness in severed head? If so, the technique is clearly humane.
2. If the severed head does retain full consciousness for more than a few seconds, does it experience pain or suffering?
3. If the head is conscious and experiencing pain, does immersion in liquid nitrogen in any way alleviate this suffering?
4. Are there valid scientific reasons for decapitation of conscious rodents?

There is considerable scientific evidence on each of these points; this evidence will be presented in the aforementioned order.

As to the question of consciousness in the severed head, it could be argued that any strong blow to the nape of the neck will stun an animal or render it unconscious. If such stunning does occur, however, it is probably because a sudden, sharp blow to the dorsal neck region snaps the unsupported head back, causing shock to the brain and stunning the subject. This effect is unlikely to occur at decapitation, because the head is typically immobilized at that time. Further, I am unaware of any solid evidence that severe trauma to any region of the body can in and of itself cause loss of consciousness. Hence, it seems unlikely that the severity of the trauma to this region would directly result in loss of consciousness in the decapitated head.

If unconsciousness does not necessarily result from the immediate trauma of decapitation, it is certain that, as we will show, unconsciousness will result promptly from the attendant loss of blood supply and hypoxia. It is a common human experience that the orthostatic hypotension which can result from suddenly standing upright after a long time in a prone position can produce an immediate loss of consciousness due to lowered blood flow to the brain. A drop of 25 mg in blood pressure may be sufficient to produce this effect (23). Clearly, unconsciousness will result even more quickly from the total loss of blood flow and blood pressure caused by decapitation, and there is no evidence that animals differ markedly from humans in this respect.

What light does the research literature throw on the question of the rapidity of unconsciousness in the severed head? By 1986, several laboratories had published reports on the EEG of the severed rodent head (14,18,19,20,24,28,34; see also 17,29, and 30). These reports are highly consistent (Table I). All agree that decapitation triggers an immediate slow direct current (DC) potential of 2 to 4 s duration, accompanied by a low-voltage, fast EEG trace. This EEG activation is gone (in the case of the electrocorticogram) by 10–13 s, although occasional episodes of activation can be seen for another 14 s or so, until the EEG is completely flat. It is also clear that decapitation of anesthetized rats produces the same EEG picture, with the exception that the activated low voltage fast EEG is of longer duration under anesthesia (14,17,18, 19,30,34). The well-replicated finding that anesthesia actually prolongs this EEG activation incontrovertibly establishes that the presence of such an activated EEG can not be interpreted as evidence for consciousness in the severed head.

Of the eight published reports of the EEG of the decapitated rodent brain available by 1986, only one (24) attempted to draw any conclusions regarding the suitability of decapitation as a method of euthanasia. This single report appeared in 1975 in the journal Laboratory Animal Science. Mikeska and Klemm replicated earlier findings (which they did not cite) of a DC shift and low-voltage fast activity (LVFA) in the electrocorticogram of the decapitated head. They then concluded that "... the EEG activation clearly indicates a conscious awareness of pain and distress" (p. 178). This conclusion was based primarily on the LVFA caused in the EEG trace by decapitation. The authors could not clearly interpret the DC shift, but thought it, too, might indicate pain or stress, perhaps from a galvanic skin response in the scalp.

This conclusion went far beyond their data, other published reports, and the state of scientific knowledge of the rodent EEG. Thus, as others have noted (1,7,16), it has been known since the 1930s that EEG activation of this kind is seen not just in aroused, conscious animals but can also be produced by inhalation anesthetics and REM sleep (6,26,27). Furthermore, by the time of publication of the Mikeska and Klemm article in 1975 there was already one published report of identical LVFA in the decapitated heads of anesthetized rodents (14).

In addition to EEG data, there was also information available on the rapidity of ischemia at the cortical surface of the decapitated rat brain, and comparative data for changes induced by 1 min exposure to inhalation of pure N2, a treatment which reliably produces unconsciousness (see citations in Table 2). These findings strongly suggest that consciousness, if any, in the decapitated rat head could not persist beyond a maximum of 3 to 6 s due to the extreme rapidity of hypoxia.

To summarize, when the AVMA panel revised its guidelines in 1986, the scientific literature contained the following findings:

1. Decapitation triggers a DC shift of 2–4 s duration accompanied by a 12–13 s LVFA EEG trace in the cortex.
2. Neither of the above EEG events can be interpreted as consciousness, much less the conscious perception of pain by the decapitated head.
3. Consciousness, if any, following decapitation could not persist beyond a maximum of 6 s, due to the almost immediate onset and rapid rise-time of cerebral ischemia.

As just noted, the literature also contained a single unsubstantiated claim to the contrary by Mikeska and Klemm (24). Because the AVMA panel cited only this last publication in support of their supposition that the severed head could be conscious for 13 to 14 s, it must be concluded that they did not adequately examine this issue in the light of the then-available scientific literature.

Since 1986, we are aware of only three publications which shed further light on this issue. Vanderwolf and colleagues have reported that the EEG activation of the severed rat head pharmacologically resembles that of an anesthetized animal rather than an animal consciously perceiving pain (30). They did so by demonstrating that two independent neurotransmitter systems could produce a LVFA "activated" EEG in neocortex, accompanied by rhythmic slow activity in the hippocampus. The first of these systems is an acetylcholine projection from basal forebrain. The EEG activation produced by this system is abolished by atropine, and is seen in both conscious
and anesthetized rats. Hence, it is unlikely that this form of activation is pain-related. The second of these systems is the serotonin projection from the raphe. The activation produced by this system is elicited by pain in conscious animals, is atropine-resistant and is not seen in anesthetized animals. The authors demonstrated that the EEG activation seen in the decapitated rat head was of the first type (present under anesthesia and atropine-sensitive), and hence quite unlikely to represent conscious pain. This study thus utilized a more sophisticated EEG analysis than any previous study, with results which again strongly suggest that there is, at best, extremely brief consciousness and no perception of pain in the severed head.

Similarly, EEG recording of lambs whose throats were cut indicated that unconsciousness occurred in under 7 s, and perhaps in less than 3 s (29). In regard to the probable duration of consciousness in the hypoxic decapitated head, Derr (8) calculated that, at known brain blood content and oxygen utilization rates, the decapitated rat brain could not maintain consciousness for more than 2.7 s.

Despite very substantial evidence to the contrary, however, let us assume that consciousness does persist in the severed head for some 14 s. Is there any evidence that such consciousness would be accompanied by substantial pain and suffering? There are two issues here. The first is anatomical. Section of the spinal cord at any level produces total and immediate loss of sensation, including all pain sensation, below the plane of section (11). Depending on the actual course of nerve fibers and the plane of section, spinal section can also effectively deprive areas anterior to the section of all feeling. This is

<table>
<thead>
<tr>
<th>Report</th>
<th>Anesthesia Used?</th>
<th>Species</th>
<th>LVFA Seen?</th>
<th>Duration (in seconds)</th>
<th>Seen</th>
</tr>
</thead>
<tbody>
<tr>
<td>King et al. (14)</td>
<td>Yes (barbiturates)</td>
<td>Mouse</td>
<td>Yes</td>
<td>10</td>
<td>Yes</td>
</tr>
<tr>
<td>Swaab and Boer (28)</td>
<td>No</td>
<td>Rat</td>
<td>Yes</td>
<td>9.6</td>
<td>Yes</td>
</tr>
<tr>
<td>Mikesa and Klemm (24)</td>
<td>No</td>
<td>Rat</td>
<td>Yes</td>
<td>13.6</td>
<td>Yes</td>
</tr>
<tr>
<td>Mayevsky and Chance (18)</td>
<td>No</td>
<td>Rat</td>
<td>Yes</td>
<td>12.5</td>
<td>Yes</td>
</tr>
<tr>
<td>Mayevsky (17)</td>
<td>Yes (nembutal or urethane)</td>
<td>Rat</td>
<td>Yes</td>
<td>16</td>
<td>Yes</td>
</tr>
<tr>
<td>Mayevsky and Zarchin (19)</td>
<td>Yes (chloroform)</td>
<td>Gerbil</td>
<td>Yes</td>
<td>39.9</td>
<td>Yes</td>
</tr>
<tr>
<td>Zarchin and Mayevsky (34)</td>
<td>No</td>
<td>Gerbil</td>
<td>Yes</td>
<td>23.9</td>
<td>Yes</td>
</tr>
<tr>
<td>(data for 400 gm adult male rats)</td>
<td>Yes (chloroform)</td>
<td>Rat</td>
<td>Yes</td>
<td>21*</td>
<td>Yes</td>
</tr>
<tr>
<td>McIntyre (20)</td>
<td>No</td>
<td>Rat</td>
<td>Yes</td>
<td>37*</td>
<td>Yes</td>
</tr>
<tr>
<td>(recording from Amygdala not cortex)</td>
<td>No</td>
<td>Rat</td>
<td>Yes</td>
<td>26</td>
<td>Yes</td>
</tr>
<tr>
<td>Tidwell et al. (29)</td>
<td>No</td>
<td>Lamb</td>
<td>Yes</td>
<td>8</td>
<td>Yes</td>
</tr>
<tr>
<td>Vanderwolf et al. (30)</td>
<td>No</td>
<td>Rat</td>
<td>Yes</td>
<td>17.2</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Yes (ether)</td>
<td>Rat</td>
<td>Yes</td>
<td>37.5</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Authors altered their definition of this variable using the more common measure of duration of EEG activation, figures suggest a value closer to 12 s. HC = hippocampus.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Time (s) to 50% of peak NADH Fluorescence (T 1/2)</th>
<th>Fluorescence at T 1/2 (% of baseline)</th>
<th>Fluorescence after 1 min N2 exposure (% of baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayevsky 1978</td>
<td>3.1</td>
<td>37%</td>
<td>44%</td>
</tr>
<tr>
<td>Zarchin &amp; Mayevsky 1981</td>
<td>3.3</td>
<td>35%</td>
<td>52%</td>
</tr>
<tr>
<td>200 gm immature male</td>
<td>6.1</td>
<td>34%</td>
<td>38%</td>
</tr>
<tr>
<td>400 gm, 1 yr male</td>
<td>6.1</td>
<td>34%</td>
<td>38%</td>
</tr>
</tbody>
</table>
especially true of the high cervical region. Region C1 of the spinal cord is reported to be devoid of sensory fibers. Sensory innervation of the neck muscles, skin, and posterior scalp enter the cord at C2 and C3 (10,12,13,31). Hence, decapitation above vertebra C2 (and section at decapitation typically occurs here or even higher, at the atlanto-occipital junction or C1) will effectively deprive the animal of sensory, including pain, input from the region of major trauma. The neuroanatomy of the rat thus makes it impossible for severe pain to result from a well-conducted decapitation.

Moreover, even if section is below this level, it is known that rapid, massive trauma of the sort produced by decapitation is seldom accompanied, in humans, by the immediate perception of pain (32). This fact has been cited as support for "gating" theories of pain perception. These theories posit that the massive somatosensory overload produced by extensive tissue damage serves to inhibit or "gate" accompanying pain messages (21,22). Thus, there is good reason to doubt that, even if there is a brief period of post-decapitation consciousness and the plane of section is below C3, there would be immediate perception of intense pain.

Turning to the question of immersion of the decapitated head in liquid nitrogen, this procedure is of course unnecessary if the decapitated head is either unconscious or conscious but not perceiving severe pain. Even in the extreme unlikely event that there is both prolonged consciousness and the perception of severe pain in the decapitated head, it is unclear whether immersion in liquid nitrogen would obliterate or exaggerate this putative pain and suffering. The only justification offered by the AVMA panel for this recommendation was the statement that "Decapitation is most often used to euthanize rodents... it also provides neurobiologists with a means to obtain anatomically undamaged brain tissue for study. In the latter case, the head is immediately placed in liquid nitrogen to halt metabolic processes" (5, p. 265).

Judging from this last statement, the panel may have mistakenly believed that freezing in liquid nitrogen is the standard scientific method for treating the decapitated head. There is no support for that belief. By way of example, a review of volumes 498 through 500 of the 1989 Brain Research showed that of 60 studies involving rodent sacrifice, 23 decapitated conscious animals without any subsequent freezing of the tissue, and only 5 involved any form of rapid freezing. Moreover, the panel seemed to feel that this procedure could somehow reduce the suffering of the decapitated head. There is also no evidence for this belief. Tissue freezes surprisingly slowly in liquid nitrogen due to a cavitation effect. It has been shown that as little as 0.5 grams of fresh liver still has core temperature of 30°C (9) 15 s after immersion in liquid nitrogen. Freezing of brain inside an intact skull would be still slower. Thus if for the sake of argument we assume that the severed head is conscious, it would be expected to experience more, not less suffering with this procedure, including pain from areas of the mouth and teeth where input to the brain is not severed by high cervical decapitation.

Finally, the panel's recommendations for cervical dislocation contradict those for decapitation. High cervical dislocation crushes the spinal cord, paralyzing breathing and producing eventual death from anoxia. In this regard, it is quite similar to decapitation but without the very rapid onset of unconsciousness due to loss of blood flow and blood pressure. There is certainly no evidence to support the panel's statement that "...cervical dislocation is a technique that may induce immediate unconsciousness" (p. 265), especially given their belief that decapitation may not do so. Indeed, recent papers report that cervical dislocation produces the same EEG changes as decapitation in rats (30), and that consciousness persists in lambs for several minutes after section of the spinal cord at the atlanto-occipital junction (29). Furthermore, even Klemm concedes that "...cervical dislocation probably causes more persistent pain than decapitation because blood supply of the brain is not completely disrupted" (15, p. 151). Thus, logic requires that the decapitated head and the cervically dislocated head be treated identically. However, the AVMA panel does not and should not recommend dipping the heads of cervically-dislocated rodents into liquid nitrogen.

I conclude that the 1986 AVMA panel's recommendations for use of decapitation are factually incorrect and logically inconsistent. The weight of scientific evidence indicates that unconsciousness due to loss of blood pressure and consequent hypoxia is almost immediate, and that the severed head is anatomically and physiologically incapable of perceiving intense pain even if conscious. There is also no basis in scientific fact, scientific practice, or in logic for the recommended immersion of the severed head in liquid nitrogen.

Despite these considerations, it is undeniable (as pointed out by the AVMA panel) that decapitation is an ugly, messy procedure. One can thus oppose this form of euthanasia on esthetic grounds, even granting that the technique produces almost immediate unconsciousness and that the severed head is anatomically incapable of feeling pain in the neck region. Ultimately, then, continued use of this technique will depend in large part on the scientific necessity of using decapitation to sacrifice conscious rodents. That such procedures are required for much current research is unquestionable: Despite the difficulties caused for researchers by the AVMA recommendations, published reports show that decapitation continues to be an important technique in biological research. This is because anesthesia interferes with a wide range of biological measures, from liver enzymes through hormones and blood chemistry to neurotransmitters. Decapitation is also much faster even than cervical dislocation, a major advantage in minimizing post-mortem changes in sensitive tissues. As evidence for the importance of this technique, we need only cite the considerable outcry from the scientific community provoked by publication of the 1986 recommendations (1,7,16), and the fact that Institutional Animal Care and Use Committees at many major research facilities, including the NIH, have found it necessary to allow continued use of decapitation in the interest of sound scientific research.

At the same time, the importance of the AVMA report on euthanasia must not be underestimated. These recommendations on decapitation policy have already caused great inconvenience, controversy, loss of valuable time, and in some instances, inability to utilize decapitation in cases where it was otherwise the technique of choice. These recommendations will continue to set policy for many institutional animal care and use committees, and of course will provide the animal rights groups with further ammunition to attack vital scientific research.

This year (1992) the AVMA panel is due to update their recommendations on animal euthanasia. This review has been written in the hopes that it will encourage the panel to reconsider their earlier position. It is my strong belief that decapitation is among the most humane of all euthanasic techniques, that the published literature on this subject amply bears out this contention, and that euthanasia of conscious animals will continue to be essential in many areas of research. Furthermore, requiring that rodents be stunned or anesthetized prior
to decapitation will probably increase, not decrease suffering. It is my observation that rodents find IP injections or inhalation anesthetics more stressful than the handling involved in being placed in a rodent guillotine. Based on these considerations, the AVMA panel is urged to approve decapitation of rodents, fully and without reservation or qualification. It is further urged that disposition of the head, once decapitated, be left up to the experimenter. The ritual of dropping the head into liquid nitrogen to make the procedure more "humane" is clearly not justified on any grounds, and could actually increase suffering in the unlikely event that the severed head is fully conscious for even a few seconds.

REFERENCES

COMPARISON OF EFFECTS OF DECAPITATION AND ANESTHESIA ON METABOLIC AND HORMONAL PARAMETERS IN SPRAGUE-DANLEY RATS

Sam J. Bhatena

Carbohydrate Nutrition Laboratory, Beltsville Human Nutrition Research Center, United States Department of Agriculture
Beltsville, MD 20705

(Received in final form March 18, 1992)

Summary

The modes of euthanasia by either anesthesia or by decapitation were compared by assessing several metabolic and hormonal parameters from plasma and hormone receptors from liver plasma membranes. Two different anesthetics were used. Compared to decapitation, euthanasia by anesthesia significantly increased plasma glucose and triglyceride levels but not plasma cholesterol. Plasma insulin was also significantly increased by anesthetics. No significant differences were observed in plasma glucagon levels or insulin and glucagon receptors from liver plasma membranes between rats euthanized by decapitation and anesthesia. Glucagon receptors were however, affected by dietary carbohydrates. It is concluded that in studies involving measurements of metabolic and hormonal parameters, the use of anesthesia is to be avoided for euthanasia and that decapitation should be the method of choice.

In most studies involving experimental animals, euthanasia is normally carried out by either decapitation or using anesthesia. Recently, serious objections to euthanasia by decapitation have been raised. Several laboratories have studied acute as well as chronic effects of different anesthetics on metabolic and hormonal parameters in rats (1-9). However, the comparison of euthanasia by anesthesia with decapitation is lacking. Dukiewicz and Chelstowski (10) compared the effects of ketamine and thiopental anesthesia on rat heart mitochondrial function and observed that decapitation caused some changes in the functional integrity of the mitochondria. Anesthetics affect brain as well as CNS system thereby altering the production and release of neuropeptides. Neuropeptides have been shown to be involved in carbohydrate and lipid metabolism. Hence it is anticipated that euthanasia by using anesthetics would alter the levels of metabolites in plasma and other tissues. Anesthetics also affect the pancreas (8) by altering the concentrations of pancreatic hormones such as insulin, glucagon and somatostatin which are also involved in carbohydrate and lipid metabolism. Therefore it is important to compare the effects of anesthesia on numerous metabolites and hormones. In the present study we compared the effects of amytal, pentobarbital and decapitation on plasma glucose, triglyceride, cholesterol, insulin and glucagon in rats fed different diets.

In order to see whether similar effects of anesthesia and decapitation are observed under different metabolic conditions the rats were fed either one of two synthetic diets differing in the source of carbohydrates or laboratory
chow. Since brain exclusively utilizes glucose as a source of energy, anesthesia may also alter glucose metabolism in the brain. Anesthetics affect brain membrane receptors. They may also affect receptors from other tissues. Chronic treatment of rats with phenobarbital has been reported to decrease receptors for epidermal growth factor and insulin in liver golgi membranes (5). We therefore studied the effect of anesthesia on liver membrane receptors for insulin and glucagon. The present study sheds some light on the effect of anesthesia on metabolic and hormonal parameters. This in turn will help interpret the results of metabolic studies and will help identify a method of choice for euthanasia when measurements of specific metabolic and hormonal parameters are performed.

Material and Methods

Two separate experiments were carried out. In first experiment, 24 weanling male Sprague-Dawley rats weighing between 35-40 gm were fed ad libitum synthetic diets containing either 54% starch (12 rats) or 54% sucrose (12 rats) as carbohydrate along with 10% casein, 10% lactalbumin, 16% fat, 5.9% cellulose, 4% salt mix, 3.1% AIN-76 and 1% vitamin mix. The rats were housed in individual cages with a reverse dark-light cycle of 12 hours light. The rats were fed the diets for 6 weeks. At the end of the feeding period and after an overnight fast, 6 rats on each diet were euthanized by amytal, 100 mg/kg BW (sodium amobarbital, Eli Lilly and Co., Indianapolis, IN). Blood was collected from the abdominal aorta. The remaining rats were decapitated. In both cases blood was collected in tubes containing 10.5 mg EDTA and 1000 U Trasylol (BBA Pharmaceuticals, Inc., N.Y.). Plasma was separated, aliquoted and stored at -70°C until analyzed. Plasma was analysed enzymatically for glucose (11), triglyceride (12) and cholesterol (13) with the Centrifichem automated system (Baker Instrument, Pleasantville, N.Y.). Immunoreactive glucagon was measured using Unger's OIA antibody by the method previously described (14). Immunoreactive insulin was determined with a kit from Immunonuclear Corporation, Stillwater, MN (Cat. No. 0600). Rat insulin (gift from Eli Lilly and Co., Indianapolis, IN) was used as standard. Membranes were prepared from liver according to the procedure of Neville (15) and insulin and glucagon receptors were measured as previously described (16). Native porcine insulin and glucagon were gifts from Eli Lilly and Co., Indianapolis, IN. Hormone binding data were analyzed by Scatchard plots (17) and competition inhibition plots (18) to assess number and affinity of the receptors. Statistical analysis was carried out by randomized complete block analysis of variance (ANOVA) to evaluate the effects diet and method of euthanasia (19).

Since each anesthetic has a different effect, two anesthetics, Anytal and pentobarbital were used in the second experiment. Eighteen weanling male Sprague-Dawley rats weighing between 35-40 gm were fed laboratory chow diet (Ralston-Purina) for 4 weeks. After an overnight fast, 6 rats each were euthanized by either anytal (100mg/kg BW), pentobarbital (40 mg/kg BW) or decapitation. The collection of blood and the analytical procedures were performed as described for experiment 1.

Results

Table 1 shows the effect of dietary carbohydrates and method of euthanasia on plasma levels of glucose, triglyceride, cholesterol, insulin and glucagon binding to liver plasma membranes. Dietary carbohydrates had no significant effect on any of the plasma metabolites or hormones, but, dietary sucrose compared to starch significantly increased glucagon binding to liver plasma membranes. Sucrose fed rats also had larger liver than rats fed starch (data not shown). Compared to decapitation, the use of anesthesia significantly increased plasma glucose and insulin levels but
TABLE 1

<table>
<thead>
<tr>
<th>Euthanasia and Metabolic Parameters</th>
<th>Amynal starch</th>
<th>sucrose</th>
<th>Decapitation starch</th>
<th>sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>7.8 ± 0.4</td>
<td>8.9 ± 0.6</td>
<td>5.1 ± 0.3</td>
<td>5.1 ± 0.4</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>0.57 ± 0.04</td>
<td>0.58 ± 0.07</td>
<td>0.85 ± 0.12</td>
<td>0.94 ± 0.08</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>1.58 ± 0.17</td>
<td>1.47 ± 0.12</td>
<td>1.79 ± 0.13</td>
<td>1.78 ± 0.13</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>172 ± 27</td>
<td>136 ± 10</td>
<td>88 ± 4</td>
<td>102 ± 16</td>
</tr>
<tr>
<td>Glucagon, pmol/L</td>
<td>42.2 ± 2.6</td>
<td>43.8 ± 3.3</td>
<td>27.7 ± 1.5</td>
<td>34.7 ± 3.1</td>
</tr>
<tr>
<td>Insulin Binding #</td>
<td>4.2 ± 0.6</td>
<td>4.2 ± 0.3</td>
<td>5.3 ± 1.3</td>
<td>5.1 ± 0.6</td>
</tr>
<tr>
<td>Glucagon Binding #**</td>
<td>37.4 ± 2.8</td>
<td>43.2 ± 4.4</td>
<td>40.0 ± 1.7</td>
<td>42.3 ± 1.5</td>
</tr>
</tbody>
</table>

ANOVA
diet euthanasia

<table>
<thead>
<tr>
<th></th>
<th>NS</th>
<th>&lt;0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glucagon, pmol/L</td>
<td>NS</td>
<td>&lt;0.07</td>
</tr>
<tr>
<td>Insulin Binding #</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Glucagon Binding #**</td>
<td>p&lt;0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Mean ± SEM of 6 rats.
#binding/50 µg protein.
**binding/25 µg protein.

decreased plasma triglycerides. Although anesthetized rats had lower
concentrations of plasma cholesterol and glucagon, the values were not signifi-
cantly different from those rats that were decapitated. Plasma glucagon
and insulin and glucagon binding were not significantly affected by
anesthesia. When binding data were analyzed by Scatchard plots and
competition-inhibition plots, no significant differences were observed in
either receptor number or affinity for insulin and glucagon receptors between
rats euthanized by decapitation or anesthesia (data not shown). However,
sucrose fed rats, compared with those fed starch had higher number of glucagon
receptors in anesthetized as well as decapitated rats. Thus, increased
glucagon binding in sucrose fed rats is due to an increase in the number of
receptors.

In chow fed rats, no significant differences were observed in rats
euthanized by either amynal or pentobarbital (Table 2). Therefore, the data
from the two groups of rats were combined and compared with the data from the
decapitated rats (19). Anesthetized rats had significantly higher plasma
glucose and insulin but lower plasma triglycerides as compared to rats
sacrificed by decapitation. No significant differences were observed in
insulin or glucagon receptor numbers or affinity between any of the groups
(data not shown). Regardless of the nature of diet, similar differences were
observed in all parameters between anesthetized rats and decapitated rats.

In first experiment, 24
on 35-40 gm were fed ad libitum
rats) or 54% sucrose (12 rats)
albumin, 16% fat, 5.9%
in mix. The rats were housed
cle of 12 hours light. The
of the feeding period and
anesthetized by amynal, 100
Indianapolis, IN). Blood was
rats were decapitated. In
mg 10.5 µg EDTA and 1000 U
was separated, aliquoted
analyzed enzymatically for
with the Centrifichem
N.Y.). Immunoreactive
method previously
with a kit from
0600). Rat insulin (gift
ed as standard. Membranes were
Neville (15) and insulin and
scribed (16). Native porcine
Indianapolis, IN.
plots (17) and competition
ity of the receptors.
complete block analysis of
method of euthanasia (19).
two anesthetics, Amynal and
Eighteen weaning male
chow diet diet
fast, 6 rats each were
or barbital (40 mg/kg BW) or
analytical procedures were
Table 2
Effect of Mode of Euthanasia on Plasma Metabolites and Hormones Membranes in Laboratory Chow Fed Rats (Experiment 2)*.

<table>
<thead>
<tr>
<th>Euthanasia</th>
<th>Amytal</th>
<th>Pentobarbitol</th>
<th>Decapitation</th>
<th>ANOVA†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>8.7 ± 0.6</td>
<td>8.0 ± 0.5</td>
<td>6.6 ± 0.2</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>0.91 ± 0.14</td>
<td>0.86 ± 0.14</td>
<td>1.11 ± 0.12</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>1.97 ± 0.27</td>
<td>2.07 ± 0.20</td>
<td>2.05 ± 0.13</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>104 ± 9</td>
<td>113 ± 26</td>
<td>67 ± 14</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Glucagon, pmol/L</td>
<td>30.6 ± 3.2</td>
<td>31.7 ± 4.5</td>
<td>32.5 ± 4.7</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin Binding %</td>
<td>7.9 ± 1.2</td>
<td>10.7 ± 1.8</td>
<td>8.2 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Glucagon Binding %</td>
<td>30.4 ± 3.2</td>
<td>32.5 ± 6.1</td>
<td>29.5 ± 4.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Mean ± SEM of rats.
†Duncan's Multiple Range test showed no differences between rats sacrificed by amytal or pentobarbitol, therefore for ANOVA the two groups were combined and compared with decapitated rats.

Discussion
In the present study, significant differences were observed in several metabolic and hormonal parameters between rats euthanized by anesthesia compared to decapitated rats regardless of the type of diet.

Both amytal and pentobarbitol significantly increased plasma glucose levels. Penicaud et al. observed a transient hyperglycemia 3 minutes after treatment with pentobarbitol (6). The increase appeared to be due to the increased glucose production and the decreased glucose clearance. In anesthetized rats peripheral glucose utilization was decreased in brain and postural muscles but not in nonpostural muscles or adipose tissue in (6). It is important to note that in the present study increased plasma glucose occurred in spite of significantly elevated plasma insulin. It is possible that elevated glucose stimulated insulin secretion since insulin concentration decreases over time as seen with pentobarbitol anesthesia. However, a direct effect on pancreas cannot be ruled out. Increased glucose cannot be accounted for by changes in glucagon since glucagon was neither elevated in the present study nor decreased as observed in another study (6). Similarly, there were no significant changes in either insulin or glucagon receptors. It is possible that other counter regulatory hormones to insulin such as growth hormone, corticosterone or catecholamines could be responsible. A decrease in glucose utilization has also been observed in peripheral tissue (20) as well as in brain (21) with halothane anesthesia. Besides glucose, fructose-2,6-bisphosphate (22) and several other metabolites (23) are altered in liver and brain by use of anesthesia prior to decapitation.

In the present study, a decrease in plasma triglyceride was observed in anesthetized rats compared to decapitated rats. An increase in plasma insulin and no change in glucagon causes relative insulin excess which would favor lipogenesis thereby increasing plasma lipid level. A direct effect of anesthesia on lipolysis in adipose tissue cannot be ruled out. Besides pancreatic and adrenal hormones, lipolysis is also modulated by opioid peptides.
especially those derived from proopiomelanocortin. Thus, \( \beta \)-endorphin and lipotropin stimulate lipolysis (24). Decreased triglycerides could be due to increased lipolysis by these opioid peptides secreted from pituitary or hypothalamus under the direct stimulation by anesthetics. Pentobarbital has been shown to decrease the activity of enkephalin neurons in that the conversion of peptide met-enkephalin to tripeptide by enkephalinase is decreased by 60\% (4). However, we have not measured opioid peptides in the present study. Butler et al. (25) compared the effects of decapitation, pentobarbital and decapitation after anesthesia with CO\(_2\), methoxyflurane and ether on arachidonic acid metabolism in rat and rabbit aorta and observed significant differences among the different methods of euthanasia.

Nazian (7) studied the acute effect of several anesthetics including pentobarbital, halothane and ether on pituitary and adrenal reproductive hormones in intact and castrated male rats. All anesthetics produced some changes in one or more hormones in either intact or castrated rats. Even milder anesthetics like ketamine and halothane appear to alter follicle stimulating hormone, indicating that none of anesthetics was suitable for the study of any reproductive hormone. In proestrous rat pentobarbital imitatively stimulated prolactin secretion for 10-30 minutes, after that time, prolactin secretion was blocked (26). Others have also observed significant alteration in plasma levels of pituitary, thyroid, and gonadal hormones in animals euthanized after anesthesia (27, 28). Taborsky et al. (3) reported inhibition of cholinergic input to the pancreas in anesthetized rats thereby decreasing the secretion of pancreatic polypeptide. Similarly halothane has been reported to inhibit insulin release (8). Anesthetics have also been reported to decrease blood flow to some but not all organs and to decrease cardiac output (2). Thus anesthetics affect endocrine as well as non endocrine organs besides brain and CNS.

In the present study we did not observe any effect of pentobarbital or amytal on either insulin or glucagon binding to liver plasma membranes. In general, receptors are either up or down regulated by the plasma hormone levels (18). We have reported decreased insulin and glucagon receptors whenever the hormone levels are elevated (14). However, changes in receptors are not immediate and there is a substantial time lag. In humans, plasma insulin peaks 30-60 minutes after oral glucose load, but insulin binding changes only after 5 hours when plasma insulin concentration returns to fasting level (29). In rats glucagon binding to hepatocytes was decreased only in chronically injected (7 days) rats but not in 2 hour glucagon infused rats even though plasma glucagon levels were several fold higher in both treated groups as compared to controls (14). Thus, the lack of change in insulin or glucagon receptors in the present study was not surprising. Zarembska et al (30) have reported no effect of ether or ketamine anesthesia on rat uterine estrogen and progesterone receptors. However, chronic treatment with phenobarbital for more than 2 weeks has been shown to decrease both insulin as well epidermal growth factor receptors to liver microsomal and golgi fractions (5). The hormone concentrations were not measured, however, in that study. It is unclear whether changes in plasma hormone levels were responsible for the decreased binding.

The effects of carbon dioxide on metabolic and hormonal indices need to be investigated before it can be used for euthanasia. Carbon dioxide would alter the pH of blood and that may affect some metabolic indices. The acidic pH may also affect the secretion of some of the enzymes and/or hormones from different tissues. Carbon dioxide suffocation has been shown to affect arachidonic acid metabolism in animals (25) and substance P in plasma (31).

Thus, the results from these studies indicate that anesthetics affect metabolic and hormonal parameters. Anesthetics also result in changes in
circulation to most organs, which may impact on organ function. Further, different anesthetics have quantitatively and qualitatively different effects on some parameters and that same anesthetic may have quantitatively different effects on different parameters. It is clear that the use of anesthetics, either by injection or inhalation, is not a method of choice for studies involving metabolic and endocrine parameters. The use of anesthesia, however, does not appear to affect hormone receptor measurements. It is debatable whether decapitation produces any more pain and discomfort than injection of anesthesia, or the suffering during early period of inhalation (32-35).

Certainly, scientifically, decapitation would provide more uniform and reliable results.

Acknowledgments

The author thanks Kenneth Revett for his technical assistance.

References

action. Further, each different effect, antitatively different, use of anesthetics, choice for studies of anesthesia, however, presents. It is debatable comfort than injection of inhalation (32-35).Ide more uniform and reliable.

assistance.

rinal Exp 2 177-185 (1975).
D. LEBREC. Am J Physiol 269
X and R.L. GINGERICH, Am J
roc Natl Acad Sci, USA, 53
urr, Biochem Biophys Res
T. ISSAD and J. GIRARD, Am
(1988).

ND and P.C. FU, Clin Chem 26
SCANT, J Clin Invest 61
552 (1968).
.ES, L. Recant, J Nutr 117
(1949).
ome Receptors, M.Y. KALIMI
ESSON, J Comp Neurol 213

A. DUNAWAY, Lab Anim Sci 40
EANN and C.H. WEISS, Arch
73-77 (1986).
.W. GERRITY and W.B. CAMPBELL
d U. DOHLER, J Endocrinol 74