INTRODUCTION

The purpose of this paper is to review the literature on the effects of acute and chronic blood loss and techniques of blood sampling in laboratory animals. When possible, recommendations regarding limits on single and multiple blood samplings in laboratory animals are offered.

Although blood sampling is a common laboratory procedure, it is nonetheless problematic, especially for techniques that attempt to set up protocols for chronic blood taking. In a letter to The American Journal of Physiology, Giner et al. (1987) announced their failure to reproduce an experimental protocol on rats that involved repeated blood sampling by means of an arterial catheter described in an earlier article in the same publication (Burt et al., 1980). Giner and associates attempted the described procedure on eight rats with no success. They then modified the technique by increasing the heparin concentrations, by using larger catheters, by flushing the cannula frequently, and finally by using a Teflon cannula. They succeeded in obtaining blood samples, but not without causing renal or intestinal infarctions and ischemia to the hind limbs. According to the authors, "after three months of work in attempting to emulate Burt’s work and the death of a large number of rats, we were left with an excellent model for arterial embolism."

Giner’s letter highlights a problem basic to many vascular sampling techniques: their successful execution requires considerable skill. The difference between one experimenter’s success with a technique and another’s failure may have as much to do with differences in the experimenter’s technical skill as with any flaw inherent in the technique.

The questionable reproducibility of the many techniques, for whatever reasons, is not the only problem facing the researcher making decisions about blood sampling. Another problem concerns the amount of blood that can be withdrawn from laboratory animals without causing injury or stress, either in a single sampling or in multiple samplings. Most facilities have their own rules of thumb. For example, many animal care and use committees recommend a limit of 1 percent of the total body weight for a single sampling. However, such guidelines may be based more on custom than on scientific studies describing blood volume losses that cause minor to significant physiological and psychological stress.

The issue is complicated further by the problems of judging an animal’s psychophysiological state. Analogies from human experience may be of limited help. For instance, after a loss of 15-20 percent of the total blood volume, a man or woman will probably feel nausea, experience dizziness, have blurred vision, and is likely to faint. However, laboratory animals have not been reported to lose consciousness after a blood loss of 15-20 percent. Nevertheless, laboratory animals may conceivably experience some symptoms of the vasovagal response, such as nausea. At this point we do not know.

EFFECTS OF BLOOD LOSS AND BLOOD SAMPLING ON RESEARCH DATA

In this section we look at a number of experimental variables associated with blood sampling and blood loss. Each of the variables has the potential simultaneously to affect research data and to cause distress in research animals (Rose, 1987).
Overview of Physiological Responses to Hemorrhage

With minor blood losses, animals may be asymptomatic. With losses of approximately 10 percent or less of the total blood volume, baroreceptor-initiated reflexes cause release of cholinergics from the adrenal medulla and sympathetic nerve endings. As a result, (1) heart rate increases, (2) arteriolar beds in muscle and skin constrict, and (3) veins and venous reservoirs constrict. Thus arterial pressure, venous return, and cardiac output are maintained or minimally affected. Slower-acting compensatory mechanisms that help replace lost volume include secretion of antidiuretic hormone and activation of the renin-angiotensin aldosterone system.

With moderate blood losses the animal will suffer drops in arterial pressure and cardiac output despite compensatory mechanisms. Losses of approximately 15–20 percent of the total blood volume cause massive cholinergic release with tachycardia and intense arteriolar constriction with further redistribution of blood away from the gut and skin. Venous constriction partially sustains venous return, and mobilization of interstitial fluid to the intravascular compartment over time restores some of the lost fluid volume. Anaerobic glycolysis occurs due to the lack of oxygen. The increased plasma lactate causes metabolic acidosis, and a compensatory tachypnea ensues.

With further blood losses, drops in cardiac output, blood pressure, and tissue perfusion become life threatening. Tissue anoxia, hypercapnia, and acidosis can lead to widespread cell injury and irreversible tissue damage, organ compromise, and death. With severe blood losses, cardiac function is limited by decreased cardiac perfusion as well as myocardial depressant factor, which is released by the poorly perfused pancreas. Late in shock, decreased perfusion of the medullary vasomotor center causes diminished compensatory reflexes.

Researchers should plan and execute each sampling protocol with an appreciation for the stresses associated with blood loss to the animal and do whatever they can to minimize the animal’s reaction to the stress. Careful planning and control of blood sampling and all experimental variables associated with it should not only improve the state of the animal, but also minimize confounding influences on research data.

Dodds (1987) has four suggestions to researchers wishing to minimize changes in blood components while sampling blood: (1) select a more tractable species or calm individuals, (2) precondition the animals to adapt them to handlers and procedures, (3) use a sampling technique that minimizes changes in blood values, and (4) note and control the use of anesthetics, because these may change blood values. Because all of these suggestions are associated with stress reduction, following them will benefit the animal as well as the scientific results.

Manipulation and Acclimatization

Not only is the trauma of blood loss stressful to the animal, but manipulation alone is stressful. Handling for as little as five seconds has been shown to cause significant increases in corticosterone levels (Seggie and Brown, 1975). Besch and Chou (1971) reported that decreases in plasma glucose levels are directly related to handling time. (Plasma glucose changes reflect stress because stress-released epinephrine raises blood sugar by its hyperglycemic action on the liver.) Mattheij and van Pijkeren (1977) observed that simply handling rats and placing them in an empty jar for 45 seconds or transferring them to another cage in the same room induced significant increases in prolactin levels.

Few studies specifically contrast stress levels of tamed, acclimated animals undergoing blood sampling with those of untamed, unacclimated animals. However, several studies have described how stress experienced by untamed or unacclimated animals confounds experimental results. For example, responses to toxins are adversely affected if the animals are not acclimated to experimental conditions (Damon et al., 1986); likewise, animals not tamed by handling react to euthanasia with significant changes in blood values that are not seen in tamed animals (Uphouse et al., 1982). Researchers who wish to reduce the stress of blood sampling, therefore, will need to habituate the animals to gentle handling and must reduce handling time as much as possible. Furthermore, they must consider the time required for various sampling techniques as an important element in deciding which is least stressful.

Puncture

All blood-sampling techniques are invasive. Vessel puncture, appendage amputation (tail transection, toe clipping), or vessel incision all presumably cause at least some pain and stress if used without anesthesia. One study of humans suggests that stress caused by venipuncture accompanied by minor blood volume sampling is not sufficient to change prolactin levels, at least in pregnant women (Ferriani and De sa Silva, 1985). However, in a study on rhesus monkeys by Heindon et al. (1984), stresses associated with capture and venipuncture were shown to increase levels of growth hormone (GH) and cortisol, but not testosterone or prolactin. Another study, by Calligaris and
Taleisnik (1983), used heart puncture by itself as a stressor to measure prolactin release under the influence of variables such as time of day.

Anesthesia

Often, anesthesia can increase the stress of a sampling procedure. Wiersma reported that taking blood samples from an atrial cannula in the rat was more stressful when light ether was used because prolactin levels were significantly elevated only when ether was used (Wiersma and Kastelijn, 1985). Mattheij and van Pijkeren (1977) observed an increase in prolactin levels after a 45-second ether stress.

Furthermore, some anesthesias cause more stress than others. Rats subjected to a 2.5-2.7 ml/100 g hemorrhage over 10 minutes had a much lower survival rate when anesthetized with intramuscular sodium pentobarbital than with inhalant methoxyflurane (Yale and Torhorst, 1972). Upton and Morgan (1975) found little difference in blood parameters from the effects of ether, pentobarbitone sodium, and fentanyl-droperidol while obtaining cardiac blood, although the use of manual restraint significantly increased blood acidity and raised the levels of hemoglobin, hematocrit, and plasma protein, presumably because of stress. Lawson and Gala (1974) observed prolonged increases in plasma prolactin following ether and intraperitoneal injection of sodium pentobarbitone, but no change in plasma prolactin following ketamine or intraarterial sodium pentobarbitone. (Refer also to “Mortality” on page 13 for references to studies on anesthesia effects in pigs.)

Catheter

Sampling blood through an indwelling catheter is generally considered to be less stressful and to cause fewer changes in blood variables than sampling with a needle and syringe (Flynn and Guilloud, 1988). Catheter sampling is particularly useful in studies requiring multiple blood collections. However, some research indicates that indwelling catheters can affect basal levels of hormones. For instance, Fagin et al. (1983) reported that individually housed rats with external carotid artery cannulas had slightly elevated morning levels of plasma adrenocorticotropic hormone and corticosterone. After intravenous (IV) injection of saline in rats via femoral artery or vein cannulas, Lestage et al. (1985) found that plasma corticosterone levels were 2.4-fold lower than in restrained rats, but levels still indicated moderate stress.

Richman et al. (1980) noted that platelet counts fell 64 percent in dogs after 48 hours of catheterization with Swan-Ganz pulmonary artery catheters.

Rate of Bleeding

With a slower rate of sampling, a greater volume can be removed without stressing the animal. In fact, Jain (1986) reported that a 50-percent blood volume loss, if slow, will not be accompanied by clinical signs of shock. Mattheij and van Pijkeren (1977) observed that a loss of up to 3.0 ml withdrawn over five hours from rats weighing at least 400 g, or roughly 13 percent of the total blood volume, did not cause a change in serum prolactin.

BIOLOGICAL EFFECTS OF A SINGLE HEMORRHAGE

Problems in Estimating Blood Volumes and Losses

We recommend estimating blood volumes based on body weight to determine guidelines for blood-sampling volumes in research animals (Table 1). This is obviously more practical than measuring the blood volume of each individual animal, but there are limits to making such estimates. For instance, a blood volume estimate for a single species may not reflect differences among individual breeds or variations due to age, size, or illness. According to standard texts, estimates of blood volume based on surface area or lean body mass tend to be closer to actual determined volumes, but are impractical for standard laboratory purposes.

Our discussions of the literature report the percentage of total blood removed from an animal when the data have been provided in the original source. If such data have not been provided, we have estimated percentage of blood volume removed based on body weight. However, sometimes only absolute volumes of blood removed are reported. In such cases it is impossible to know what percentage of total blood volumes in research animals (Table 1). This is obviously more practical than measuring the blood volume of each individual animal, but there are limits to making such estimates. For instance, a blood volume estimate for a single species may not reflect differences among individual breeds or variations due to age, size, or illness. According to standard texts, estimates of blood volume based on surface area or lean body mass tend to be closer to actual determined volumes, but are impractical for standard laboratory purposes.

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<table>
<thead>
<tr>
<th>Species</th>
<th>Whole Blood Volume (ml/kg)</th>
<th>% Body Weight</th>
<th>Plasma Volume (ml/kg)</th>
<th>Approximate Absolute Blood Volumes of Animals of Described Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>86</td>
<td>8.6</td>
<td>50</td>
<td>14-kg (30 lb) dog: 1200 ml</td>
</tr>
<tr>
<td>Cat</td>
<td>56 (47-66)</td>
<td>41 (35-52)</td>
<td>4.5-kg (10 lb) cat: 252 ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>67*</td>
<td>48*</td>
<td>4.5-kg (10 lb) cat: 300 ml*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>43*</td>
<td>4.3*</td>
<td>4.5-kg (10 lb) cat: 194 ml*</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>64 (58-70)</td>
<td>6.4</td>
<td>40</td>
<td>300-g rat: 20 ml</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>75 (67-92)</td>
<td>7.5</td>
<td>40</td>
<td>90-g guinea pig: 68 ml</td>
</tr>
<tr>
<td>Rabbit</td>
<td>56 (44-70)</td>
<td>5.5</td>
<td>39 (28-51)</td>
<td>3.2-kg rabbit: 180 ml</td>
</tr>
</tbody>
</table>

NOTE: All values from Altman and Dittmer (1974) unless otherwise specified.

* Jain (1986).

A Breznock and Strack (1982).
volume was removed from an animal. Also, most of the data we found were obtained from studies on rats and dogs. There were very few data on effects of blood loss in the rabbit or mouse.

Significant differences in blood volumes exist between animals of the same species. Courtice (1943), using the Evans-blue dye method, observed the mean total blood volume of a group of mongrels to be 79 ml/kg and the mean total blood volume of a group of greyhounds to be 114 ml/kg. This represents a 35 ml/kg difference.

A hypothetical example based on these observations demonstrates the need to err on the side of caution when determining limits of nonstressful blood-sampling volumes. If, for instance, researchers working with mongrels had records only of the total blood volume of greyhounds and thought they were removing 20 percent of blood volume from the mongrels based on the greyhound data, they would actually be removing 22.8 ml/kg, or about 30 percent, of the mongrels’ blood volume.

Discrepancies in the literature on the average blood volumes of animals are common. One reason may involve the technique used to measure blood volume. The International Committee on Standardization in Haematology recommends that investigators determine blood volume by measuring red cell volume using sodium radiochromate or sodium pertechtate as a red cell label and measuring plasma volume using human serum labeled with radioiodine as a plasma label (Jain, 1986). According to one researcher, the Evans-blue dye (T-1824) method, the most common of the early methods, overestimates blood volume by as much as 10 percent (Linderkamp et al., 1977). Consequently, blood volumes determined using the Evans-blue dye method may tend to be higher than those in studies that use labeling techniques.

Evidence from the literature seems to confirm this reported difference. For instance, the average blood volume of rabbits in two studies using the Evans-blue dye method is 70 ml/kg (Courtice, 1943) and 69.8 ml/kg (Aikawa, 1950). Armin et al. (1952) used a labeling technique and reported a blood volume of 57.3 ml/kg for albino rabbits and 64.7 ml/kg for brown rabbits.

Breznock and Strack (1982) concluded that radiolabeling techniques cannot be used to interpret blood volumes of nonsplenectomized cats with accuracy because labeled red blood cells are sequestered within the spleen. By measuring blood volumes in splenectomized cats, the investigators concluded that true blood volumes are probably closer to 4 percent of body weight rather than 6–7 percent as is commonly reported in the literature.

Sato et al. (1985) found significant differences in the bleeding volume (percentage of total blood volume) resulting in similar mortality rates in rats of different sizes. Larger rats had lower percentage blood volumes than smaller rats (Table 2). The mortality rates for different sized rats are given in Table 3.

Researchers should keep in mind that many variables in addition to loss of blood volume affect an animal’s response to blood sampling. These include the rate of blood loss, the site and technique of withdrawal, the skill of the bleeder, the use and type of anesthesia, the age and sex of the animal, and its nutritional and health status. To give one example, if an animal is obese, the researcher should lower an estimate of blood volume and base it on the animal’s expected normal weight.

There is obviously great potential for error in determining blood volumes, which leads to serious discrepancies in the literature. These are further reasons why investigators should be cautious when determining limits on blood-sampling volumes based on estimates from standardized tables. When possible, the investigator should use the lower, more prudent limits.

**Massive Blood Loss**

**Sustained Hypotension**

A frequently studied model of hemorrhagic shock has been the standardized Wiggers model of hemorrhage (Wiggers, 1950). In this model, the anesthetized

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### Table 2

<table>
<thead>
<tr>
<th>Body Weight (g)</th>
<th>Total Blood Volume (ml)</th>
<th>% Body Weight (ml/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>17.4</td>
<td>6.97</td>
</tr>
<tr>
<td>300</td>
<td>19.5</td>
<td>6.51</td>
</tr>
<tr>
<td>350</td>
<td>21.1</td>
<td>6.04</td>
</tr>
<tr>
<td>400</td>
<td>22.3</td>
<td>5.58</td>
</tr>
</tbody>
</table>


### Table 3

<table>
<thead>
<tr>
<th>Body Weight (g)</th>
<th>Total Blood Volume Removed (ml) per 100 g Body Weight, LD&lt;sub&gt;50&lt;/sub&gt;</th>
<th>% Total Blood Volume Removed, LD&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>2.7</td>
<td>39</td>
</tr>
<tr>
<td>300</td>
<td>2.6</td>
<td>40</td>
</tr>
<tr>
<td>350</td>
<td>2.5</td>
<td>42</td>
</tr>
<tr>
<td>400</td>
<td>2.4</td>
<td>43</td>
</tr>
</tbody>
</table>

animal is rapidly bled to maintain extremely low blood pressures. Bassin et al. (1971) argued that the Wiggers dog model is inferior because it poorly resembles clinical hemorrhage, and Loegering and Carr (1978) reported that sustaining blood pressure at 40-45 mm Hg by bleeding rats causes a more severe shock state than reducing blood by a fixed volume. We mention this model of hemorrhage in passing because of its previous popularity, but we see little relevance in this branch of blood loss research for the purposes of this paper.

Absolute Volume Removal

Various experiments have focused on different parameters to evaluate the effects of blood loss in humans and animals. In the following section, discussions of blood loss are organized according to these various parameters. Table 4 summarizes results from the literature on blood losses of greater than 30 percent of total blood volume.

<table>
<thead>
<tr>
<th>TABLE 4 Effects on Animals and Humans of Blood Loss Greater than 30 Percent of Total Blood Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Rat</td>
</tr>
<tr>
<td>Rat</td>
</tr>
<tr>
<td>Rat (germ-free)</td>
</tr>
<tr>
<td>Rat (germ-free)</td>
</tr>
<tr>
<td>Rabbit</td>
</tr>
<tr>
<td>Dog</td>
</tr>
<tr>
<td>Dog</td>
</tr>
<tr>
<td>Dog</td>
</tr>
<tr>
<td>Swine (unanesthetized)</td>
</tr>
<tr>
<td>Swine (unanesthetized)</td>
</tr>
<tr>
<td>Human</td>
</tr>
</tbody>
</table>

Data not available.

Estimated values based on Table 1 and/or experimental data.
Unfortunately, the data published on these experiments do not include total blood loss and body weight, so we cannot know what percentage of blood these animals lost.

Furneaux (1969) observed that arterial pressure in dogs who were bled 46 percent of their total volume fell to 29.5 percent of the control just after the hemorrhage, rose to 45.3 percent of the control after two hours, and then fell again as death approached. All dogs in this study died.

According to one human study, when a drop in blood pressure of 20 mm Hg or more corresponds with a pulse greater than 100, blood loss is estimated to be at least 30 percent of the total blood volume (Tovey and Lennon, 1962).

**Residual Blood Volume** A number of blood loss studies in dogs have focused on residual blood volume, that is, the volume of blood remaining one to two hours after hemorrhage. This value takes into account the body’s ability to respond to blood volume loss by mobilizing reserve blood from the spleen and fluid from the interstitium. One study of dogs showed a 50-percent survival rate associated with a residual blood volume of 62.6 percent of the prehemorrhagic blood volume and an 80-percent survival rate associated with a residual blood volume of 70.3 ml per kg of body weight, or about 72 percent of the prehemorrhagic volume (Wang et al., 1947).

Walcott (1945) showed a residual blood volume of 60 percent to be the irreducible minimum compatible with survival. Each dog in Walcott’s study underwent a single massive bleeding with a loss of about one-half its total blood volume. In the group that survived, residual blood volumes one hour after hemorrhage averaged 65.5 percent of the prehemorrhagic volume, and in the group that died, residual blood volumes averaged 57 percent of the prehemorrhagic volume. Thus, survival in this study correlated with the individual’s ability to mobilize reserve plasma and cell volumes.

Another study, done on splenectomized dogs, showed a 50-percent survival rate to occur at a residual blood volume of 66 percent (Rawson et al., 1959). All dogs with residual volumes greater than 74 percent survived, and all dogs with residual volumes of less than 62 percent died. Seven of 13 animals within the 62- to 74-percent range died. Incidentally, only in some species does the spleen serve as a blood storage organ; in others, such as mice and rats, the situation would be more comparable to splenectomized dogs.

**Mortality** One study on germ-free rats focused on critical bleeding volumes, that is, the critical blood volume loss below which mortality increased sharply (Yale and Torhorst, 1972). The critical bleeding volume for blood withdrawn at a steady rate over 10 minutes was 2.6 ml per 100 g or, by our calculations, roughly 40 percent of total blood volume. For blood withdrawn over 60 minutes, the critical bleeding volume was 3.0 ml per 100 g, or roughly 47 percent of the total blood volume. This study concluded that rate of blood loss as well as amount of blood loss affects mortality.

**Blood Values** In dogs, a hemorrhage equal to 1/30 of the dog’s body weight, or according to the authors a 43.6-percent blood loss, caused increased activity of aminotransferases and phosphatases, changes that are due to hypotension and impaired tissue and organ metabolism, and a transient decrease in blood prothrombin levels (Glowinski et al., 1972). Parenthetically, if we assume the total blood volume of a dog to be 8.6 percent of body weight (Table 1), a loss of blood equivalent to 1/30 of the body weight would be 38 percent of the total blood volume, a 5.6-percent difference from the authors’ calculation.

**Moderate or Small Blood Losses**

Table 5 summarizes results from the literature on blood losses of 30 percent of total blood volume or less. Like the last section, this one is organized according to the various research parameters used to evaluate blood loss. None of the studies described in this section concern sustained hypotension; all deal with absolute volume removal.

**Cardiac Output**

Saperstein et al. (1960) reported that a hemorrhage of 10 ml/kg in rats caused a 50-percent reduction in cardiac output. By our calculations, this loss is roughly 15 percent of the total blood volume.

Ploucha and Fink (1986) contrasted the effects of a 4-ml hemorrhage on 309-g rats and 233-g chickens. This loss, about 20 percent of the rat’s volume by our calculations, reduced cardiac output in the rats by 43 percent, but reduced cardiac output in the smaller chickens by only 4 percent. The authors explain the chicken’s superior ability to endure massive hemorrhage by its ability to mobilize extravascular fluids twice as rapidly as the rat. This occurs despite the fact that hemorrhage decreases, rather than increases, peripheral resistance in the chicken.

**Arterial Blood Pressure**

In men who were bled 15 percent of their total blood volume, arterial pressure dropped from an average of 92 mm Hg before hemorrhage to 76 mm Hg one minute after hemorrhage. Arterial pressure then rose again to
82 mm Hg after 90 minutes (Skillman et al., 1971). Ploucha and Fink (1986) reported that in rats, a 4-
ml hemorrhage, roughly 20 percent of the total blood volume by our calculations, decreases arterial pressure
by 25 percent. According to Furneaux (1969), dogs that were bled 27 percent of their total blood volume showed a return of arterial pressure to normal in less than two hours.

### TABLE 5 Effects on Animals and Humans of Blood Loss Less than 30 Percent of Total Blood Volume

<table>
<thead>
<tr>
<th>Species</th>
<th>Volume Removed</th>
<th>% Total Blood Volume</th>
<th>Sample Site and Method</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>20 ml</td>
<td>30%</td>
<td>Inferior vena cava puncture</td>
<td>Severe gastric necrosis</td>
<td>Menguy et al., 1974</td>
</tr>
<tr>
<td>Rat</td>
<td>1–2 ml at 1 ml/7.5 min</td>
<td>5–11</td>
<td>Ischiatic artery catheter</td>
<td>No change in plasma corticosterone/prolactin</td>
<td>Wiersma and Kastelijn, 1985</td>
</tr>
<tr>
<td>Rat</td>
<td>3 ml at 1 ml/7.5 min</td>
<td>16%</td>
<td>Arterial catheter</td>
<td>Increased plasma corticosterone</td>
<td>Wiersma and Kastelijn, 1985</td>
</tr>
<tr>
<td>Rat</td>
<td>...</td>
<td>...</td>
<td>Inferior vena cava puncture</td>
<td>50% reduction in cardiac output</td>
<td>Saperstein et al., 1960</td>
</tr>
<tr>
<td>Rat</td>
<td>3 ml over 5 h</td>
<td>13%</td>
<td>Arterial catheter</td>
<td>No change in plasma prolactin</td>
<td>Mattheij and van Pijkeren, 1977</td>
</tr>
<tr>
<td>Rat</td>
<td>1.2 ml over 20 min</td>
<td>6–7</td>
<td>Arterial catheter</td>
<td>Significant change in plasma prolactin; no change when volume replaced by saline</td>
<td>Lawson and Gala, 1974</td>
</tr>
<tr>
<td>Rat (unanesthetized)</td>
<td>4 ml</td>
<td>20%</td>
<td>Femoral artery catheter</td>
<td>Mean art. press. decr. 25%, cardiac output decr. 43%, total peripheral resistance decr. 65%</td>
<td>Ploucha and Fink, 1986</td>
</tr>
<tr>
<td>Chicken (unanesthetized)</td>
<td>4 ml</td>
<td>...</td>
<td>Ischiatic artery catheter</td>
<td>Mean art. press. decr. 15%, cardiac output decr. 4%, peripheral resistance decr. 13%</td>
<td>Ploucha and Fink, 1986</td>
</tr>
<tr>
<td>Dog (unanesthetized)</td>
<td>26 ml/kg</td>
<td>30%</td>
<td>Tygon catheter in jugular vein</td>
<td>Sustained decr. in mean art. press. of 23 mm Hg, heart rate incr. by 88/ min, cardiac output fell</td>
<td>Vatner, 1974</td>
</tr>
<tr>
<td>Dog</td>
<td>...</td>
<td>10</td>
<td>Arterial and venous pressures returned to normal within 2 h</td>
<td>Arterial and venous pressures returned to normal within 2 h</td>
<td>Furneaux, 1969</td>
</tr>
<tr>
<td>Dog</td>
<td>...</td>
<td>27</td>
<td>Arterial and venous pressures normal within 24 h, blood volume normal after 90 min</td>
<td>Arterial and venous pressures normal within 24 h, blood volume normal after 90 min</td>
<td>Furneaux, 1969</td>
</tr>
<tr>
<td>Swine</td>
<td>Bled over 30 min; after 2 h, lost volume reinfused</td>
<td>10</td>
<td>Superior vena cava catheter</td>
<td>17% mortality; 66% mortality; 66% mortality</td>
<td>Hobler and Napodano, 1974</td>
</tr>
<tr>
<td>Swine</td>
<td>15 ml/kg, rapid withdrawal</td>
<td>20</td>
<td>Superior vena cava catheter</td>
<td>No signif. change in capillary pressure to any tissue except stomach</td>
<td>Simon and Olsen, 1969</td>
</tr>
<tr>
<td>Baboon (unanesthetized)</td>
<td>14 ml/kg</td>
<td>...</td>
<td>Tygon catheter in jugular vein</td>
<td>Decrease in mean arterial pressure of 23 mm Hg; heart rate rose by 67/min</td>
<td>Vatner, 1974</td>
</tr>
<tr>
<td>Human (males)</td>
<td>800 ml average over 15.5 min</td>
<td>15</td>
<td>“Venous removal”</td>
<td>Signif. decr. in cardiac index, stroke vol., left ventric. work, art. press., central venous press.</td>
<td>Skillman et al., 1971</td>
</tr>
</tbody>
</table>

*Data not available.
*Estimated values based on Table 1 and/or experimental data.
Ulcers

In studies of hemorrhage in rats, Menguy et al. (1974) reported that a hemorrhage equal to 2 percent of the animal’s body weight caused severe gastric epithelial necrosis within 15 minutes and gross erosions within 45 minutes. By our estimate, assuming the total blood volume of a 300-g rat to be about 20 ml, a 2-percent loss of body weight, or about 6 ml of blood, is about 30 percent of the total blood volume.

Vasovagal Response

Many studies of blood loss in humans focus on the vasovagal fainting response (Barcroft et al., 1941; Ebert et al., 1941; Grindon, 1982; Howarth and Sharpey-Schafer, 1947; Poles and Boycott, 1942), although the literature does not address whether this response is important to nonhuman animals or even occurs in them.

Blood Values

Attempts to measure a distress response in animals have been frustrated by the lack of a single predictable measure of stress. The three systems activated in response to stress—behavior, autonomic nervous system, and neuroendocrine system—all have been evaluated for use as measures of the stress response. Unfortunately, indicators of an autonomic response, such as plasma catecholamines, fluctuate too rapidly and unpredictably to be of much help.

Behavioral responses are complex and vary from one species to another, although studies of changes in grooming and exploratory behaviors indicate that they might be useful as distress indicators. Antin et al. (1975) reported that grooming behavior is a normal and predictable sequel of feeding. More recently, Barclay et al. (1988) reported that they have developed a reproducible and sensitive index of an animal’s reaction to various experimental procedures based on exploratory behavior. They demonstrated that both mice and rats explore a new cage and that the amount of exploratory behavior is reasonably predictable in the absence of disturbance. The state of the animal following a particular procedure can then be assessed by observing the departure (either excitation or depression) from the normal levels of exploratory behavior. The method is sufficiently sensitive to discriminate between the handling of mice by experienced or inexperienced handlers. However, injections of non-irritant substances in modest amounts did not change the behavioral response. Although the authors did not assess the effects of various blood-sampling techniques or regimens, the experiment would be an easy one to do.

At present, a popular indicator of stress is fluctuation in plasma concentrations of adrenal and hypophyseal hormones. Unfortunately, not all stresses initiate a change in corticosteroid levels (Breazile, 1987; Moberg, 1987), so these hormones may be overused as indicators. However, a change does occur predictably in response to certain stressors, such as injury, physical restraint, and electric shock (Moberg, 1987). Krulich et al. (1974) reported that of a variety of hormone serum concentrations responsive to stress, including prolactin, GH, luteinizing hormone, and follicle-stimulating hormone, prolactin concentrations are most susceptible, because a significant response is consistently observed, even following very mild stimulation.

In one study, corticosterone and prolactin changes appeared after roughly 15 or 16 percent of total blood loss. The study concluded that rats bled 1 or 2 ml at a rate of 1 ml per 7.5 minutes from an atrial cannula (shown in the study to be a stress-free method of blood sampling) were not stressed by the blood loss. However, those bled 3 or more ml at the same rate did experience stress, because corticosterone levels increased significantly as one reached and exceeded the 3-ml level (Wiersma and Kastelijn, 1986). The study offered no data on percentage of blood volume removed. The rats used in the study ranged in weight from 180 to 430 g; therefore based on Table 1, we estimate that a 3-ml blood loss would equate with a 9- to 23-percent reduction of total blood volume, or a mean value of 16 percent.

In another study, stress indicators appeared after as little as a 6- to 7-percent blood loss. Lawson and Gala (1974) reported significant responses of plasma prolactin concentrations after removal of only 1.2 ml of blood from 225- to 300-g rats, or 6-7 percent of total blood volume, within 20 minutes, and no changes in prolactin concentrations when the lost blood was replaced by saline. In the study, an indwelling arterial catheter was used to eliminate stresses associated with sampling technique.

Hematocrit has not proven to be a helpful indicator of blood loss or physiological stress. Although a low initial hematocrit predisposes an animal to early onset of irreversible shock (Crowell et al., 1958), hematocrit may not accurately reflect true red cell losses for up to 72 hours because plasma and red cell volumes are reduced proportionally during hemorrhage (Wintrobe et al., 1981).

Some studies of humans donating a standard 400-ml unit (about 8 percent of total blood volume) have focused on length and magnitude of depletion of critical factors. One study of iron deficiency concluded that, after a single 400-ml (8 percent) loss, normal dietary intake of iron may not return iron values to normal
until after 4 months in men and 8–12 months in women (Finch, 1972). Another study reported that hemoglobin concentration is lowest one to two weeks after donation and reaches predonation levels three to four weeks after donation (Wadsworth, 1955).

Physical Performance

A 1982 article by Grindon surveys studies on blood donation in humans. One study cited by Grindon on physical performance following blood donation in humans indicated a deterioration in performance lasting a few days, particularly for activities requiring sustained endurance. According to a second study cited by Grindon, maximum oxygen uptake levels, five days after bleeding, were only 6 percent lower than predonation levels, which is probably due to the improved oxygen unloading of hemoglobin. A third study concluded that performance and endurance levels completely return to normal only after 28 days.

Mortality

Surprisingly, in studies on anesthetized pigs, a 20- to 30-percent blood loss is associated with significant mortality. In one study, a 30-percent blood loss caused 66 percent mortality (Hobler and Napodano, 1974). In the same study, a 20-percent loss also caused 66 percent mortality, while a 10-percent loss caused 17 percent mortality. These results should be interpreted in the light of comparative studies of hemorrhage in anesthetized and unanesthetized pigs. One such study demonstrated that a 20-percent blood loss in a pentobar-anesthetized pig has biological effects similar to a 40-percent blood loss in a nonanesthetized pig (Simon and Olsen, 1969). In none of the studies on animals other than pigs was mortality a factor with volume losses of 30 percent or less.

Biotransformation

Dogs bled as little as 5 ml/kg, or about 6 percent of their total volume, showed a measurable decrease in the rate at which IV-administered hexobarbital was biotransformed (Cumming et al., 1971). This effect is due to decreased perfusion of the liver.

Recommendations for Single Blood Samples

From the literature on acute blood loss, one can suggest that a 30- to 40-percent loss is too great: a 40-percent blood loss is, at least for germ-free rats, the critical bleeding volume (causing 50 percent mortality), and a 30- to 40-percent loss corresponds with hemorrhagic shock. Because of a lack of data on stress indicators below a 30-percent blood loss, we cannot make absolute recommendations about smaller losses. However, in one study, corticosterone levels may be elevated in rats that undergo approximately a 16-percent blood loss, while in another study, levels rise after as little as a 6-percent blood loss. We tentatively recommend that investigators limit blood sampling to 15 percent of total blood volume for a single sampling and provide special justification for taking volumes greater than 15 percent.

The Department of Laboratory Animal Medicine at Cornell University recommends a 15- to 20-percent limit with a 30-day recovery period. Given the absence of reliable data in the literature, there is some danger that the 20-percent limit may be too high because of the potential errors described above in estimating blood volume based on body weight.

How do our recommended limits compare with other rules of thumb currently used by investigators? One commonly used rule of thumb is referred to as the 10 percent–10 percent rule. This approach assumes that a safe sampling volume is 10 percent of the total blood volume, which is estimated to be 10 percent of the animal’s body weight. On the surface, the approach seems more conservative than our own because it recommends a 10-percent sampling limit. Actually, the approach significantly overestimates the blood volume of laboratory animals, so that calculated bleeding volumes based on the 10 percent–10 percent rule are equal to or greater than volumes calculated using blood volume tables and our own 15-percent maximum sampling volume. For example, according to the 10 percent–10 percent rule, an investigator may take 2.5 ml of blood in one sampling from a 250-g rat. Actually, a rat’s blood volume is closer to 6 percent of its body weight (Table 1) than 10 percent. Applying our own 15 percent maximum recommended bleeding volume, an investigator may take 2.4 ml from a 250-g rat, a volume slightly less than the one reached with the 10 percent–10 percent rule.

Investigators might consider replacing lost blood volume with saline to reduce the stress of blood volume loss. Lawson and Gala (1974) reported that plasma prolactin concentrations in ovariectomized rats decreased significantly after removal of only 1.2 ml of blood within 20 minutes. However, when the lost blood was replaced with saline, no change in prolactin levels occurred. Also, Rochae Silva et al. (1987) found that severe hemorrhagic shock (44.5 ml/kg, or about 50 percent of total blood volume) was reversed by intravenous administration of a small volume (4 ml/kg) of NaCl solution.
BIOLOGICAL EFFECTS OF MULTIPLE HEMORRHAGES

The data on multiple hemorrhages or samplings are more sketchy, and thus recommendations for multiple samplings are more difficult to offer.

Single Day

Agrelo and Miliozzi (1974) reported taking 6 ml of blood, roughly 50 percent of the total blood volume of a 175-g rat, over five to six hours in 1-ml samplings. The authors reported no changes in biodegradation of drugs if each 1-ml blood sample is replaced by blood or saline. They also noted that their use of a caudal artery cannula caused no tail necrosis. Upton (1975), using a jugular canula, reported taking 15 blood samples of 0.2 ml, or 3 ml total, over six hours from rats without the rats showing ill effects.

More Than One Day

Cardy and Warner (1979) reported that a monthly sampling of 1.25-2.0 ml of blood from young mature Fischer 344 rats caused no effect on common blood values (hemoglobin, hematocrit, red blood cell count, mean corpuscular volume [MCV], mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration [MCHC], total leukocyte count). However, the protocol caused a decrease in the rate of body weight gain. This difference was noted as early as 3 weeks after the study began, was apparent in both sexes, and persisted throughout the 23-week study. In the protocol, each rat was bled by orbital puncture less than 1 ml every two weeks. An average of 1.6 ml was taken from each rat per month. This volume constitutes roughly 25 percent of the total blood volume at the beginning of the study, when all rats weighed about 100 g, and constitutes 7 percent of the total blood volume of males (350 g) and 13 percent of the total blood volume of females (200 g) at the study’s end. The authors concluded that “the regular withdrawal of amounts of blood small enough not to induce abnormal hematologic values can affect other parameters of physiologic status.”

In one study of repeated blood withdrawal, six 4-ml samples of blood were taken from rats at one- or two-week intervals (Wiersma and Kastelijn, 1986). Blood parameters in the first group (weekly blood withdrawal) were significantly affected, while those in the second were not. In the rats in the first group, hemoglobin and MCHC values decreased steadily while blood sedimentation values and osmotic resistance increased steadily. It should be noted that some of the indicators used in this study, such as MCHC and hemoglobin, are relatively insensitive indicators of stress. The researchers provided no data on percentage of blood volume lost, so we can only estimate from the average weights of the rats (225 g for females and 375 g for males) that between 17 and 28 percent of the blood volume, or a median value of 23 percent, was removed.

A study on rabbits by Nerenberg et al. (1978) focused on the effects of chronic bleeding on several blood components. For a period of eight weeks, rabbits in the study were bled 1.5 percent of their body weight three times a week, or about 50 percent of their total blood volume per week. Parameters were measured at the end of the 8-week period and again after a 45-day rest period. At the end of the experimental period, red blood cell, hemoglobin, and hematocrit levels were low. After the rest period, all of these parameters were higher than before the bleedings were begun, which suggests overcompensation. Based on MCV and MCHC values, all rabbits bled according to this protocol developed macrocytic hypochromic anemia. Nevertheless, the authors recommended the protocol for the production of large amounts of antisera.

A study of women who donated a standard unit (about 400 ml, or 8 percent of total blood volume) every two months without iron therapy found that, after four donations, one-third of the women had no stainable iron in the marrow—that is, their iron stores had been depleted (Lieden, 1975).

One study that looked at hemoglobin as an indicator of blood status concluded that improved oxygen unloading by hemoglobin in the weeks following bleeding more than compensates for the reduced hemoglobin concentration (Edwards and Cannon, 1972). This improved oxygen unloading seems to be due to the increased number of young erythrocytes following bleeding. However, it is uncertain from this study of men who had donated 250 ml weekly for two weeks or a single 500-ml unit, what effect multiple bleedings beyond a period of a few weeks would have on oxygen unloading and hemoglobin concentrations.

Recommendations for Multiple Samplings

We can offer very little in the way of conclusive recommendations for multiple bleedings. The Wiersma study made it clear that a loss of about 20 percent or slightly more per week is too much, but studies of acute bleeding discussed in the previous section suggest that these limits may be too great for even a single sampling. The monthly sampling study by Cardy and Warner (1979) resulted in significant growth depression, although it is difficult to identify this effect with a specific percentage of blood volume removal because
the animals were gaining weight throughout the study. During the initial stages, approximately 25 percent of the blood volume was being removed. Cornell University recommends a 10-percent weekly limit, but perhaps a 7.5-percent weekly limit would be more prudent given the evidence that hemoglobin concentrations may take many weeks to return to normal following a loss as small as 8 percent. In written correspondence, W. J. Dodds (New York State Department of Health, personal communication, 1988) concurred with a 7.5-percent weekly limit. Dodds' own research on platelet and coagulation requires measurement of very sensitive blood parameters, so that stress and physiological variables must be controlled. Her own bleeding limits are 10 percent of total blood volume followed by a rest of at least two weeks.

**SAMPLING TECHNIQUES**

A 1983 article, with over 500 references, reviews the literature from 1952 to 1982 on techniques for vascular access in the rat (Cocchetto and Bjornsson, 1983). The article describes 39 procedures for arterial samplings from 7 sites, 81 procedures for venous sampling from 19 sites, and 4 “miscellaneous” procedures that yield a mixture of arterial and venous blood. The article includes expected blood yields from some of the more common procedures. For instance, retroorbital plexus collection is described as yielding about 0.5 ml of venous blood, tail amputation as yielding up to 4 ml of mixed venous-arterial blood, and cardiac puncture as yielding up to 5 ml of mixed blood.

Our intention in writing this section was not to sift through the vast body of literature on sampling techniques for the purpose of offering absolute recommendations on which technique to use on each animal. Researchers have at least as many reasons for collecting blood as there are sampling techniques, and each technique is more suited to some purposes than others. Furthermore, in the judgment of some researchers whose opinions we solicited, sampling techniques considered stressful by some (e.g., retroorbital puncture) in fact cause minimal stress in the hands of technically adept handlers.

The brief survey of sampling techniques below aims to report facts and opinions regarding sampling techniques, particularly those concerned with stresses to animals. We hope it will serve as a helpful resource.

**Rats**

In the rat, many techniques are used for sampling blood. The three most common are retroorbital plexus puncture, tail amputation, and heart puncture.

Retroorbital plexus puncture yields an average of 0.5 ml of venous blood in the rat (Cocchetto and Bjornsson, 1983). Grice (1964) described a procedure for bleeding the medial canthus that yields 1-2 ml of blood. When the technique was first described, yields of up to 1 ml were sampled from the anterior (medial) canthus (Stone, 1954). According to Sorg and Buckner (1964), sampling from the posterior (lateral) canthus causes fewer nose bleeds and less trauma to the eye and is useful for repeated samplings in rats, mice, guinea pigs, rabbits, and hamsters. However, these same authors reported success in taking up to 8 ml of blood from a rat with retroorbital plexus puncture without killing the rat. Even in a 400-g rat, 8 ml of blood—about 36 percent of the total blood volume—exceeds limits we would recommend for a single nonterminal bleeding.

Descriptions of the retroorbital puncture method commonly include the following steps: making the eye protrude by retracting the skin adjacent to the eye, causing constriction of venous return by applying thumb pressure behind the angle of the jaw, and inserting a pipette gently through the canthus into the retroorbital plexus, gently rotating the pipette as it is advanced (Kraus, 1980). However, it has been reported that these steps are sometimes not practiced, and instead the pipette is more or less inserted behind the orbit and scraped through the orbital plexus, a procedure that probably causes serious hemorrhage but is likely to yield larger blood volumes.

An article by Timm (1979) challenges the conventional assumption that the rat has an orbital plexus. Instead, Timm’s study suggests that rats have a venous plexus. The paper has several implications for orbital bleeding:

- The medial canthus is not a proper site for orbital bleeding because it is occupied largely by the Harderian gland and has only small anastomotic veins.
- The area ventral to the orbit is also not an appropriate site since it has a variable anastomotic vein.
- The ophthalmic venous plexus is difficult to reach with a capillary tube because it lies deep in the orbit.
- The area dorsal to the orbit is the most appropriate site for sampling because it is occupied by the caudal-dorsal anastomotic vein. The article describes a procedure for sampling from this site.

Using cardiac puncture, Burhoe was able to collect 5 ml of blood from rats weekly for three months (Burhoe, 1940). Another study reported 12 percent fatality for heart puncture (Stuhlman et al., 1972). Incidentally, it is becoming a standard requirement of many institutional animal care and use committees that cardiac puncture be used under anesthesia and
only as a terminal sampling procedure in all laboratory species.

Bober (1988) noted that tail amputation has “fallen from favor due to the traumatic nature of the procedure.” Bober described a technique for sampling from 3 to 6 ml of arterial tail blood under general anesthesia using a 22-gauge syringe.

Golba et al. (1974) concluded that sampling blood from the retroorbital plexus of the rat is much more stressful than sampling blood by tail amputation. Retroorbital sampling, but not tail amputation, caused a significant decrease in white blood cell count for several weeks. According to the authors, the decreased white blood cell count indicates a general adaptation stress response. This response to internal and external factors results in lymph system atrophy and decreased mitotic activity in bone marrow.

In another study comparing stresses of different sampling techniques, Horton et al. (1986) observed serum creatine kinase to be significantly higher following retroorbital plexus puncture but not following heart puncture. The increase in creatine kinase levels could be due to either tissue destruction or stress.

According to the studies above, retroorbital plexus puncture seems to be the most stressful of the three techniques discussed, although the high rate of fatality reported for heart puncture does not recommend that technique for nonterminal procedure either. Also, repeated samplings from the retroorbital plexus should be avoided because they cause local tissue damage involving the Harderian gland (Canadian Council on Animal Care, 1984).

Toe clipping under anesthesia has been used to obtain small yields of blood, up to 0.3 ml, in the rat. However, these procedures are distasteful to many workers.

Mice

In the mouse, retroorbital sinus puncture and heart puncture are the most common sampling techniques, although success has been reported with other methods, including severing brachial vessels, jugular vein, carotids, femoral vessels, and abdominal aorta, as well as venipuncture of the caudal vena cava and tail vessels. Cardiac puncture in the mouse yields variable blood volumes, and the blood is likely to be hemolyzed and contaminated by tissue fluids (Adeghe and Cohen, 1986). Anesthesia is required for cardiac puncture (Moreland, 1965).

Retroorbital sinus puncture can yield up to 1 ml of venous blood in the mouse (Moreland, 1965). Adeghe and Cohen (1986) reported that many trials are required for acquisition of an appropriate level of skill, that the blood is likely to be hemolyzed, and that “the procedure is distasteful to many workers.”

Cate (1969) described an orbital sinus sampling technique for unanesthetized mice that yields about 50 percent of total blood volume. The procedure was developed as an alternative to standard orbital sinus sampling techniques, which typically yield small blood volumes. The described technique, which involves inserting polyethylene tubing in the medial canthus, yields about 3 percent of total body weight, or 50 percent of total blood volume, in less than one minute.

Two recent letters to the ACLAM Newsletter argued that orbital bleeding in the mouse is obviously painful, and therefore, the technique should be accompanied by anesthesia (Letcher, 1987) or avoided altogether (Farnell, 1987).

Rabbits

In rabbits, the most common sites for blood sampling are the marginal ear vein, the central artery of the ear, and the heart.

Marginal ear venipuncture frequently is followed by vascular spasm (Fick and Schalm, 1986; Tillman and Norman, 1983). Incision of the marginal ear vein or the central artery of the ear can yield up to 50 ml of blood (Moreland, 1965). According to Moreland, anesthesia is not required for either procedure. It has been fairly common to swab the marginal ear vein with xylene to facilitate blood flow and collection, but xylene can cause serious inflammation if not carefully washed off. The policy set by the Department of Laboratory Animal Medicine at Tufts/New England Medical Center is to give the rabbit a dose of acepromazine IV in the marginal ear vein and collect the blood from the central ear artery (M. Ellenberger, personal communication, 1989). Grice (1964) reported that 25 ml of blood, about 14 percent of total blood volume, can be readily obtained from one auricular artery.

Hoppe et al. (1969) described a technique for bleeding the marginal ear vein that can yield from 30 to 50 ml of blood (roughly 17–28 percent of total blood volume) in four or five minutes. The technique, which uses a vacuum reservoir and centrifuge tube to increase sampling volume, is recommended for repeated samplings. In the author’s words, “the technique of bleeding rabbits described here does not endanger the life of the donor provided the animal is not excessively exsanguinated.”

Lumsden et al. (1974) described a modified technique for orbital sinus sampling in the rabbit. Rather than using as a sampling site the medial or lateral canthus, which failed to yield consistent samples, the authors...
described a technique involving capillary tube insertion through the conjunctiva dorsal to the orbit. Studies showed that weekly blood withdrawal over a 13-week period caused some transient subconjunctival hemorrhage and some degenerative changes in retroorbital muscles. However, the authors concluded that the technique, which requires neither topical nor general anesthesia, results in minimal discomfort, is "as humane as some other methods used for sampling the rabbit," and can yield 6 ml in 15–30 seconds.

Heart puncture in the rabbit carries risks of hemo-pericardium and cardiac tamponade (Fick and Schalm, 1986; Tillman and Norman, 1983). According to Moreland (1965), heart puncture should always be performed under anesthesia.

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Commentary: H. Richard Adams

The article by McGuill and Rowan provides a good overview of what seems to represent, at first glance, a rather straightforward and biologically unambiguous topic about blood sampling in laboratory animals. However, as is quickly emphasized, the issue of sampling for hematologic assay in biomedical research is far from simplistic and demands greater consideration than just the dexterity of venipuncture technicians.

One aspect of particular importance is the underlying theme that blood-sampling techniques can evoke rather substantial biologic reactions in the experimental animal. Such reactions alter the basal homeostasis of the subject and thereby comprise yet another variable in a research protocol. The sympathetic division of the autonomic nervous system is especially reactive to stressful environmental stimuli, and its activation during blood sampling can rapidly modify cardiovascular dynamics and also influence metabolic functions of other tissues and organ systems. Blood and its various components themselves can be altered by blood collection procedures and associated paraphernalia, whether sampling is acute or chronic. Some of the resulting influences may well alter the precise hematologic variable undergoing study, some represent general systemic stress factors that seem mostly unavoidable owing to the fact that blood sampling is inherently invasive and traumatic, some are simply not known or recognized, and some can be catastrophic to the experimental study. Indeed, McGuill and Rowan poignantly point out that one attempt to develop an arterial catheter for chronic blood sampling in rats actually yielded "an excellent model for arterial embolism" with intestinal and renal infarctions! The importance of such confounding influences to certain studies, especially if they remain unrecognized and uncontrolled, can hardly be overemphasized.

Many blood-sampling factors now are known to hold real potential for altering hematologic and other phys...
iorologic variables. These factors include methods of physical restraint, types of chemical restraint drugs, blood volume removed, subject experience, and investigator experience. These and other related and unrelated influences are addressed by McGuill and Rowan. A strength of their review is that it tabulates a selected sampling of a broad topic entailing even more complexity than could be assembled in this setting. Another value of this article is that it incorporates related animal care and welfare issues the biomedical community must consider. Thus, on the one hand, this article is an appropriate starting point for investigators and laboratory animal veterinarians needing a literature resource for information about blood-sampling technology and its affiliated intricacies.

On the other hand, this review should not be misconstrued as a definitive treatise or policy directive about precise blood-sampling techniques and resulting biologic reactions. The authors make no such claim, and it would be a mistake for readers to rely on such an assumption. This topic is too complex to justify such a universal application, as revealed by the paper itself. The sections dealing with hemorrhagic shock, for instance, are superficial relative to the data base in this field, and they actually are somewhat tangential to the main points of the article. Readers could be mislead by the comment that severe hemorrhagic shock evoked by removal of 50 percent of total blood volume in dogs is reversible by a small volume (4 ml/kg) of “NaCl solution.” The study in question actually used a highly hyperosmolar solution of NaCl. Responses of the sympathetic nervous system to hemorrhage or other stressors are mediated not by “cholinergics,” but by the adrenergic mediators norepinephrine and epinephrine. Review/advisory committees or consultants could find themselves in a rather untenable position if they relied solely on this article for detailed information about saline resuscitation or nervous system responses to hemorrhage. These points reflect subsidiary elements of the entirety of this article, but they illustrate two important caveats:

1. Judiciousness should be employed when single literature sources are used to build specific policy dealing with complex biologic problems.
2. Hemorrhage and blood-sampling procedures do not constitute a trivial biologic problem.

The influence of chemical restraint during hematologic sampling is introduced relatively early in the paper. Yet in later discussions of hematologic variables, the reader is not informed if the data originated from anesthetized, sedated, or conscious animals. Acclimation to handling and environmental conditions are addressed as important issues in all species, and yet, later comments about pertinent data in the literature do not distinguish between trained versus naive subjects. These limitations should not be criticized as weaknesses unique to this review. Rather, they serve to substantiate the dearth of information available from some of the original reports and the resulting lack of historical background on precise biologic consequences of blood loss and sampling. In relevant literature about hematologic sampling in the past, details that are now deemed important were not always recognized as salient features at that time. It seems quite likely that currently undiscovered sequelae of blood loss will turn out to be important considerations in the future.

In summary, the article by McGuill and Rowan contains important generic and starting-point information about blood-sampling techniques, as well as relevant guidelines about total blood volumes versus volumes withdrawn for hematologic samples in different species. This information should guide investigators about this issue, but it does not absolve them from the responsibility to validate and justify the appropriateness of their own hematologic assay procedures relative to their own particular experimental objectives.