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Evaluation of Saphenous Venipuncture and Modified Tail-clip Blood Collection in Mice

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The purpose of this study was to evaluate the effects of 2 methods of blood collection in unanesthetized mice. The saphenous venipuncture method was compared with a modified tail-clip technique that requires minimal restraint. Mice were evaluated through behavioral observation and plasma corticosterone levels. The results showed that the 2 methods produced similar corticosterone responses and that the tail-clip method produced fewer behavioral reactions. In addition, the effects of saphenous venipuncture method appeared to be dependent on the handler's technical expertise. When a series of 4 blood collections were performed over 1 wk, the 2 methods yielded similar corticosterone levels that did not increase over time. Some of the behavioral signs appeared to increase over the series of blood collections obtained by the saphenous venipuncture method. Serial complete blood counts showed that the tail vessels yielded higher total white blood cell, neutrophil, and lymphocyte counts than did the saphenous vein. Neither method appeared to cause stress-associated changes in the leukogram after serial blood collection. Overall, the effects of modified tail-clip method were similar to those of the saphenous venipuncture method in unanesthetized mice.

Abbreviation: EIA, enzyme immunoassay

Stress has been defined as a nonspecific response of the body to external stimuli.[33,40] The body reacts to stress with a number of physiologic responses that include increased blood pressure,[34] muscle tension,[32] anorexia,[20] changes in body weight,[11,12,22] increased plasma corticosterone levels,[3,15,31] and altered white blood cell counts.[8] In addition, stress evokes behavioral responses related to irritability[30] and depression.[23] Unfortunately, signs of stress have been noted in rodents during routine laboratory procedures[2] involving noise,[25] movement of cages,[5,9,37] and restraint.[11,16] Because stressors can have profound effects on the well-being of experimental subjects as well as on experimental results, minimizing stress wherever and whenever possible is of utmost importance.

During experimental studies, blood collection is a very common procedure performed on laboratory mice and can induce stress.[28] Although many methods for blood collection have been described,[21] each method requires a combination of stimuli that may cause anxiety or a moment of pain (or both). The stress induced by blood collection can be compounded when experimental design requires serial sample collection or if scientific concerns preclude the use of anesthetic agents. Saphenous venipuncture is well-described and has been advocated as a method for serial blood collection from unanesthetized mice.[13] A tail-clip method involving a tube restrainer has also been described for use in serial sampling.[24,36] However, whether repeated use of the tail-clip results in excessive trauma has been questioned,[6,17] and this method has been associated with transient increases in plasma corticosterone levels.[36,42] The stress induced by serial blood sampling with the tail-clip technique has not been compared directly with that from saphenous venipuncture.

The purpose of this study was to compare the effects of saphenous venipuncture and a modified tail-clip method of blood collection. The two techniques were compared by evaluation of plasma corticosterone concentrations and behavioral observations. The effect of the technical expertise of the handlers also was evaluated. In addition, changes in body weight and complete blood counts were used to compare the effect of serial blood collection. We hypothesized that responses elicited by the modified tail-clip and saphenous venipuncture methods of blood collection would not differ.

Materials and Methods

Study design. To evaluate responses to blood sampling, 40 µl of blood were drawn from individual mice either by the modified tail-clip or saphenous venipuncture method without anesthesia. Two handlers performed each method of blood collection, and handlers were matched by level of experience with the specific technique. Corticosterone levels and behavior were used in the evaluation. The white blood cell counts obtained at each site also were examined. For evaluation of chronic responses to repeated blood sampling, 1 handler performed blood collections on days 1, 3, 6, and 8. The response over time was evaluated by plasma corticosterone and behavioral observations as well as changes in white blood cell counts and body weights.

Animals. Specific pathogen-free, female ICR mice (22 to 25 g) were obtained from Harlan Sprague Dawley (Indianapolis, IN). The mice were housed in polycarbonate cages in a temperature-controlled room with a 12:12-h dark:light cycle. Water and food (Laboratory Rodent Diet 5001, PMI Nutrition International, Richmond, IN) were offered ad libitum. All animal care was in accordance with standards set forth in the *Guide for the Care and Use of Laboratory Animals*.[26] The University Committee on

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Use and Care of Animals approved all of the procedures used in this experiment.

Handlers. A total of 3 handlers performed all the procedures. One person was defined as the novice handler and had received the basic training required by the institution prior to handling mice. The novice handler then received instruction on both techniques of blood collection and observed while performing each technique. After the initial instruction, the novice was observed but did not receive additional coaching. This scenario mimicked the training given to new research staff members at this institution. Two handlers were defined as experienced handlers. Experienced handlers had performed a specific technique (the modified tail-clip or saphenous venipuncture) with proficiency at least once weekly for more than a year. For the first experiment, the novice and 1 experienced handler performed each technique. Serial blood samplings were performed by the novice handler.

Saphenous venipuncture technique. This technique was performed as previously described.[13] The handler encouraged the mouse to enter a restrainer (a perforated 50-ml centrifuge tube) while holding the base of the tail. A hindleg was extended, and the tarsal area of the hind leg was shaved. After preparation with 70% alcohol, petroleum jelly was applied over the area. Mild pressure was applied above the knee to occlude the saphenous vein, and the vein was penetrated with a 25-gauge needle. Blood was collected in a capillary pipette flushed with EDTA; two 20-µl increments were collected. Gentle pressure was applied to the site to stop the bleeding at the end of the procedure. For repeated sampling, the same site was used by disrupting the healing site. If blood could not be obtained from the original site, the opposite saphenous vein was used.

Modified tail-clip technique. In previous descriptions of this technique, the tail-clip method was performed on mice held in a restraint device.[36] Investigators at our institution frequently use a modified technique that involves minimal restraint; we used this modified technique in our study. For this modification, each mouse was placed on an unfamiliar surface (an overturned wire basket) and allowed to explore while the handler held the base of the tail (Figure 1). During the procedure, the handler moved with the animal and only limited movement if the mouse tried to leave the working surface. The distal 1 to 2 mm of the tail was clipped, and a capillary pipette flushed with EDTA was used to collect two 20-µl samples from the bleeding surface. Immediately after blood collection, styptic powder (Kwik-stop, ARC Laboratories, Atlanta GA) was applied to the tail. For repeated sampling, the surface of the original wound was disrupted; in general, removal of more tail was unnecessary.

Blood counts. For complete blood counts, EDTA-anticoagulated blood samples were analyzed within 5 min of blood collection by using an automated system (Hemavet Mascot Multispecies Hematology System counter, model 1500R, CDC Technologies, Oxford, CT).

Plasma. For sample dilution, 20 µl of anticoagulated blood was added immediately after collection to 180 µl of 3.38 mM EDTA in PBS. The diluted sample was centrifuged at 2000 × *g* for 5 min at 4 °C. The plasma was frozen at –20 °C until analysis of corticosterone levels.

Corticosterone immunoassay. Corticosterone in plasma samples (final dilution, 1:50) was measured by a competitive immunoassay (Correlate-EIA Corticosterone kit, Assay Designs, Ann Arbor, MI). The plate was read immediately at 405 nm on an ELISA plate reader (model Elx808, Bio-Tek Instruments, Winooski, VT).

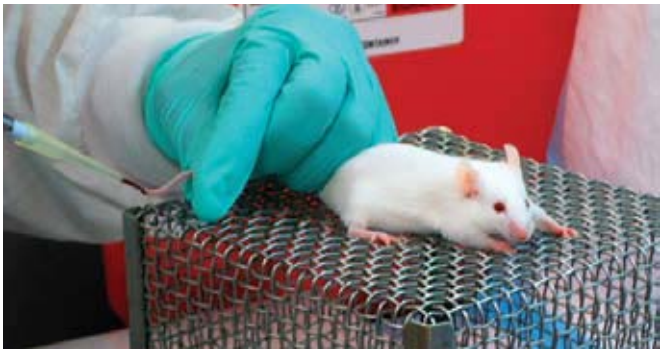

Figure 1. Modified tail-clip technique. Demonstration of the minimal restraint used during the modified tail-clip blood collection procedure.

Observation of animal behavior. An observer monitored behavior from the beginning of the period of restraint until the animal was released at the end of procedure. Individual mice were observed for the following signs: pulling, flinching, urination, defecation, aggression, and vocalization. Pulling was defined as an active, directed attempt to move away from the handler. Flinching was muscular movement in response to sharp penetration of the skin. Urination or defecation was recorded if the animal voided during the procedure. Vocalization was scored if the animal made an audible sound. Aggression was defined as movement directed toward the handler or an attempt to bite. The behavioral signs were scored as present or absent for each mouse. This scoring method did not account for multiple occurrences of the same behavior by an individual mouse. The number of mice demonstrating a specific behavior was recorded for comparison between techniques. Therefore, the maximal score for a given behavior would be equal to the number of mice in the group.

Body weight measurements. In the experiments evaluating serial blood collection, mice were weighed prior to the experiment and again on Day 8. The difference in body weight was recorded and was expressed as a percentage of the baseline weight. Another group of control mice (matched for age, sex, and starting weights) were weighed over 8 d but were not subjected to blood collection.

Statistics. Dependent variables were compared at day 1 and over time for the 2 techniques. Values for behavioral variables (for example, flinch) were expressed as the number and percentage of animals exhibiting the behavior. The Fisher Exact and Sign tests were used to evaluate behavioral parameters. Pearson's correlation test was used to analyze for the impact of procedure time on corticosterone levels for the two methods of blood collection. Values for continuous variables (for example, weight, corticosterone) were expressed as mean ± SEM. Independent-samples *t* tests were used to compare values of continuous variables on day 1 for each technique (saphenous venipuncture and modified tail-clip) performed by novice and experienced handlers. A 2-way repeated-measures analysis of variance was used to analyze data from serial blood collection, with posthoc tests comparing the 2 techniques on each day of blood collection by using Bonferroni adjustment for multiple comparisons. The Mann–Whitney nonparametric test was used to compare the change in body weight for the 2 techniques of blood collection. An alpha level of 0.05 was used for statistical tests. Statistical analyses were carried out by using SPSS 15.0 for Windows (SPSS, Chicago, IL).

Results

Comparison of sampling techniques. Saphenous venipuncture and modified tail-clip blood sampling methods were compared for several parameters, including plasma corticosterone levels and behavioral responses. For these comparisons, 2 groups of mice (n = 13 per group) underwent blood sampling by either technique (2 handlers, 1 experienced and 1 novice, per group, as defined in the Methods).

Because merely handling mice can alter corticosterone concentrations, the mean time from beginning of handling to end of blood collection was compared. The mean time for the modified tail-clip method (150 ± 11 s) was similar to that of the saphenous method (159 ± 32 s). During the saphenous method, the initial restraint and preparation required a longer period of time than for the modified tail-clip method. The modified tail-clip method required minimal preparation but a longer period of time to obtain the full volume of blood. The relationship of procedure time to corticosterone level was also examined. When values for all mice were compared, the correlation was quite small (r =0 .110) and was not significant (p=0.601). Then, the correlation between procedure time and corticosterone levels within each blood collection technique was examined separately. These results were not significant for saphenous venipuncture (r=0.290, p=0.337) or for the modified tail-clip method (r= -0.179, p=0.578).

Mean plasma corticosterone concentration did not differ between saphenous venipuncture and the modified tail-clip technique (Figure 2). The animals were monitored for flinching, vocalization, urination, defecation, pulling, and aggression. Because none of the animals showed aggression as defined prior to the study, this parameter was not reported for this part of the study. The number of animals exhibiting vocalization, urination, defecation, or flinching did not differ between sampling methods (Table 1). However, the number of mice that pulled away from the handler was significantly higher (P = 0.03) for the saphenous venipuncture method (53.8%) than the tail-clip method (7.7%).

When complete blood counts were compared (Table 2), the mean total white blood cell count obtained by the saphenous venipuncture method was significantly lower (P = 0.006) than the mean for the modified tail-clip method. This difference was primarily a function of neutrophils; lymphocyte, monocyte, eosinophil, and basophil counts did not differ for the 2 groups.

Effect of technical expertise. The data obtained for the 2 blood collection methods were reevaluated with attention to the experience of the handlers (novice, n = 8 mice; experienced, n = 5 mice).

Saphenous venipuncture method. The mean time to perform the procedure was significantly less (P = 0.023) for the experienced handler (81 ± 10 s) than for the novice handler (208 ± 44 s). Likewise, the mean plasma corticosterone level obtained by the experienced handler was significantly lower than that obtained by the novice handler (P = 0.023) (Figure 3 A). When individual behaviors were compared, fewer mice exhibited pulling, flinching, and defecation when the saphenous venipuncture was performed by an experienced handler than a novice (Table 3). However, the difference was only statistically significant for pulling behaviors in mice handled by the novice when compared with those of the experienced handler (P = 0.009) (Table 3).

Modified tail-clip method. As with the saphenous venipuncture method, the mean time for the procedure was significantly lower (P = 0.014) for the experienced handler (122 ± 4 s) than for the novice handler (170 ± 14 s). However, the mean plasma corticosterone obtained by the experienced handler (168 ± 27 ng/ml) did not significantly differ from that from the novice handler (110 ± 39 ng/ml; P = 0.309; Figure 3 B). The behaviors rarely were exhibited during the modified tail-clip method of blood collection, with the exception of defecation (Table 4). This behavior occurred more (P = 0.035) often when the novice handler was performing the procedure.

Effect of serial blood collection. To evaluate the effects of sequential collection, blood was obtained from unanesthetized mice by either saphenous venipuncture or modified tail-clip techniques (n = 8 mice per group) on days 1, 3, 6, and 8. When the time required for blood collection was averaged across all of the experimental days, the saphenous venipuncture

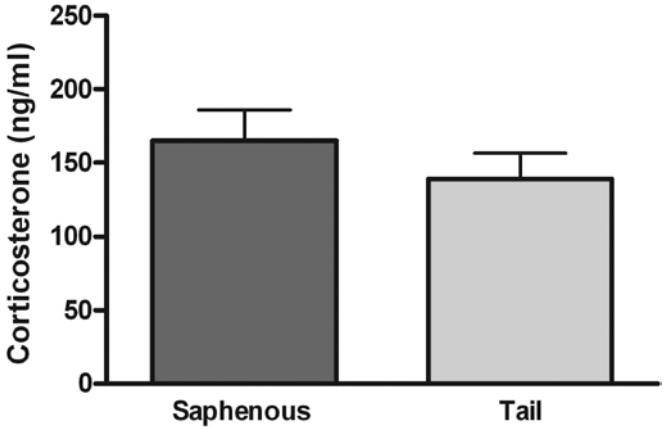

Figure 2. Effect of blood collection technique on plasma corticosterone levels. Blood was collected from mice by either saphenous venipuncture or the tail-clip technique. The average plasma corticosterone level for the saphenous venipuncture group did not differ from that of the tail-clip group. n = 13 per group.

Table 1. Behaviors noted during blood collection

	No. (%) of mice displaying the indicated behavior (n = 13 per group)	
Behavior noted	Saphenous	Tail-clip
Pulling	7 (53.8)	1 (7.7)[a]
Flinching	3 (23.1)	0 (0)
Vocalization	3 (23.1)	0 (0)
Urination	1 (7.7)	0 (0)
Defecation	9 (69.2)	10 (76.9)

[a]Value significantly (P < 0.05) different from that of mice undergoing blood collection by saphenous venipuncture.

Table 2. Comparison of complete blood counts

	Cell count x 10^3/µl (mean ± SEM)	
Cell type	Saphenous	Tail-clip
Total white blood cells	10.23 ± 0.69	14.58 ± 2.12[a]
Neutrophil	1.44 ± 0.17	3.15 ± 0.44[a]
Lymphocyte	8.31 ± 0.51	10.38 ± 1.26[a]
Monocyte	0.43 ± 0.04	1.00 ± 0.44
Eosinophil	0.04 ± 0.02	0.04 ± 0.01
Basophil	0.01 ± 0.01	0.01 ± 0.00

[a]Value is significantly (P < 0.05) different from that of mice undergoing blood collection by saphenous venipuncture.

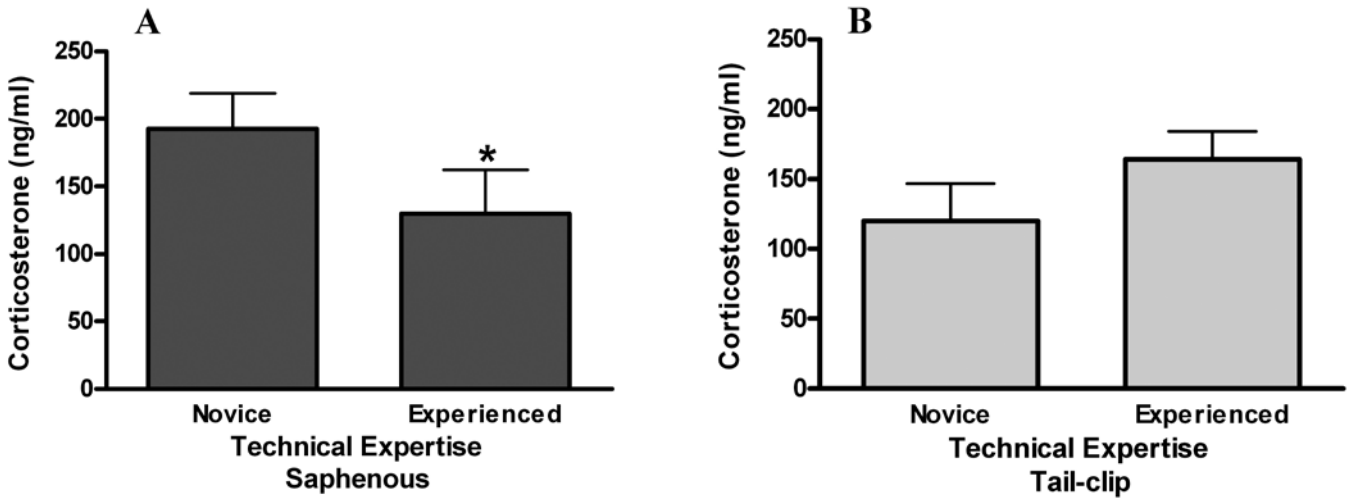

Figure 3. Effect of technical expertise on plasma corticosterone concentrations. To analyze the effect of technical expertise on corticosterone levels within each technique, an expert or novice handler collected blood from mice. (A) For the saphenous venipuncture group, the mean plasma concentration was significantly higher (*, $P < 0.05$) when a novice handler performed the blood collection. (B) However, the mean plasma level obtained by the tail-clip method was not statistically different between novice and experienced handlers. n = 5 to 8 per group.

Table 3. Effect of technical experience on behaviors noted during saphenous venipuncture

	No. (%) of mice displaying the indicated behavior	
Behavior noted	Experienced (n = 5)	Novice (n = 8)
Pulling	1 (20)	6 (75)[a]
Flinching	0 (0)	3 (37.5)
Vocalization	2 (40)	1 (12.5)
Urination	1 (20)	0 (0)
Defecation	3 (60)	6 (75)

[a]Value is significantly ($P < 0.05$) different from that of mice undergoing saphenous venipuncture by an experienced handler.

Table 4. Effect of technical experience on behaviors noted during blood collection by tail-clip technique

	No. (%) of mice displaying the indicated behavior	
Behavior noted	Experienced (n = 5)	Novice (n = 8)
Pulling	1 (20)	0 (0)
Flinching	0 (0)	0 (0)
Vocalization	0 (0)	0 (0)
Urination	0 (0)	0 (0)
Defecation	2 (40)	8 (100)[a]

[a]Value is significantly ($P < 0.05$) different from that of mice undergoing blood collection by tail clip by an experienced handler.

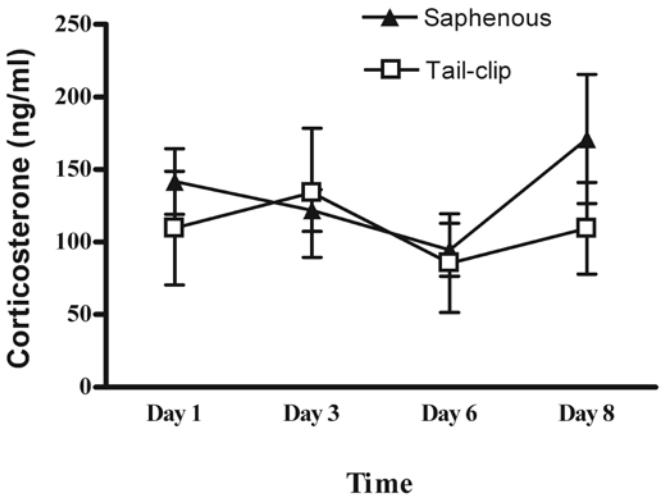

Figure 4. Effect of serial blood collection on plasma corticosterone levels. Blood samples were collected from mice on days 1, 3, 6, and 8. The daily mean plasma corticosterone concentrations obtained for the saphenous and the tail-clip groups did not differ significantly. n = 8 per group.

method required a significantly ($P = 0.043$) longer time (228 ±18 s) than did modified the modified tail-clip method (170 ±19 s). The mean corticosterone level across all days for the saphenous venipuncture method (132.4 ± 19.8 ng/ml) was not significantly different than that for the modified tail-clip method (109.7 ± 19.8 ng/ml). For the saphenous venipuncture technique, the mean corticosterone level did not differ ($P = 0.351$) across the 4d of blood collection (Figure 4). Likewise, corticosterone levels for serial blood samples obtained by the modified tail-clip method did not vary ($P = 0.737$) between the 4 days of collection (Figure 4). When all of the corticosterone levels were averaged for each day regardless of technique, there were no significant differences over time.

The saphenous venipuncture method was associated with a higher number (Sign test, $P = 0.0075$) of behavioral signs than was the modified tail-clip method (Table 5). The 2 groups did not differ with respect to urination, defecation, or vocalization on any given day of blood collection. However, on each day, mice exhibited pulling more frequently during the saphenous venipuncture technique than during the modified tail-clip method. These differences were significant on days 1 and 8 (day 1, $P = 0.007$; day 8, $P = 0.041$). For each technique, there was little change in the occurrence of pulling from day 1 to day 8. Although flinching was not observed as a response to the modified tail-clip method, mice that underwent the saphenous venipuncture method showed flinching, with significant differences between techniques detected on days 6 and 8 ($P = 0.007$ for both days). In addition, the observation of flinching during

Table 5. Effect of serial blood collection on behaviors noted

	No. (%) of mice displaying the indicated behavior (n = 8 per group)							
	Day 1		Day 3		Day 6		Day 8	
Behavior noted	Saphenous	Tail-clip	Saphenous	Tail-clip	Saphenous	Tail-clip	Saphenous	Tail-clip
Pulling	6 (75)	0 (0)[a]	5 (63)	1 (13)	6 (75)	2 (25)	7 (88)	2 (25)[a]
Flinching	3 (38)	0 (0)	4 (50)	0 (0)	6 (75)	0 (0)[a]	6 (75)	0 (0)[a]
Urination	0 (0)	0 (0)	0 (0)	0 (0)	2 (25)	0 (0)	2 (25)	0 (0)
Defecation	6 (75)	8 (100)	5 (63)	8 (100)	6 (75)	7 (88)	7 (88)	7 (88)
Vocalization	1 (13)	0 (0)	2 (25)	0 (0)	2 (25)	0 (0)	3 (38)	0 (0)
Aggression	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (25)	0 (0)

[a]Value is significantly ($P < 0.05$) different from that of mice undergoing saphenous venipuncture on the same day.

saphenous venipuncture increased from 37.5% to 75% from day 1 to day 8. Signs of aggressiveness were rare, and there were no significant differences between the groups on any given day. However, 2 mice in the saphenous venipuncture group displayed aggressive behavior on day 8.

Mean body weight on day 1 was compared with that on day 8. Saphenous venipuncture was associated with an average weight gain of 0.075 ± 0.481 g. However, this value included a 3% mean weight loss in 4 of the 8 mice that underwent saphenous venipuncture. Mice in the modified tail-clip group showed an average weight gain of 0.925 ± 0.180 g over the same period. In contrast to the saphenous venipuncture method, a weight gain over baseline occurred in all of the mice in this group. When the average weight changes were compared, they did not differ relative to blood collection method. An average gain of 1.5 ± 0.635 g occurred in all of the control mice over same experimental period. The mean weight change of the control mice was different ($P = 0.042$) from that of the saphenous venipuncture group but was comparable ($P = 0.142$) to that of the modified tail-clip group.

Mean total white blood cell counts from the tail vessels were significantly higher than those from the saphenous vein on days 1, 3, and 6 (day 1, $P = 0.003$; day 3, $P = 0.006$; day 6, $P = 0.016$; Figure 5 A). This increase was primarily due to differences in neutrophils and lymphocytes (Figure 5 B, C). However, the blood counts displayed no significant differences from day 1 to day 8 within the modified tail-clip and saphenous venipuncture groups. Neither technique indicated that a stress leukogram had developed during the use.

Discussion

Although it has been used for decades,[7,35] the tail-clip method of blood collection elicits concerns about unacceptable disfigurement and pain that affects animal well-being.[6,42] With refinements of the method, the concern about disfigurement was avoided completely by incising the tail vessels[6,17] or insertion of a catheter.[19] At our institution, we routinely use the tail-clip method with other modifications. In our modified tail-clip, a very small amount (1 to 2 mm) of the distal tail was removed. In addition, during the period of blood collection, the animal is allowed to move and naturally explore its surroundings, a feature that is in sharp contrast to previous studies reporting prolonged immobilization.[36] This modification appears to reduce the handling time for each mouse and may reduce the stress caused by restraint. However, exposing mice to an unfamiliar, open environment also might cause stress, and this possibility has not been evaluated. Therefore, we compared the modified tail-clip technique to a well-accepted method of blood collection routinely used at this institution: saphenous venipuncture.

The results of our study indicated that the modified tail-clip method compares favorably with saphenous venipuncture for collection of a small blood sample. Recently, histologic studies demonstrated that the tail has ossified vertebral bodies and innervation to the tip,[1] suggesting that tail-clipping would be painful. Long-term hyperalgesia after tail-tipping occurred in a model of amputation in which as much as 33% of the tail was removed.[42] However, tail-biopsy of about 4 mm without anesthesia caused only transient changes (2 h) in body-temperature and heart rate.[1] These results suggest a lack of long-term physiologic effects from removal of a small amount of tail tip. In our study, the modified tail-clip showed no difference in corticosterone levels and fewer behavioral signs of stress, compared with saphenous venipuncture. When both techniques for blood collection were used every other day for 1 wk, the results were similar. The mice subjected to the modified tail-clip method had fewer behavioral signs and similar corticosterone levels as the mice subjected to saphenous venipuncture. These results support earlier studies in which corticosterone levels after tail-clip compared satisfactorily with those after tail-nick in rats.[38] Certainly, blood collection procedures may cause stress, but the impact of the modified tail-clip appears to be comparable to that of other well-accepted methods.

The data also were analyzed with regard to the technical expertise of the handler. Previously, technical expertise had a significant impact on outcome after blood collection from the orbital sinus. However, this difference was related to the greater degree of trauma induced by inexperienced versus experienced handlers.[39] The effect of technical expertise during less-invasive blood collection methods had not been examined. In the current study, the mean time to perform saphenous venipuncture was longer and plasma corticosterone concentrations were significantly higher for a novice compared with an experienced handler; there was also a trend for more behavioral responses during blood collection by a novice handler. During blood collection by the modified tail-clip technique, plasma corticosterone levels and behavioral parameters appeared less dependent on the proficiency of the handler. The data collected reflect only a single handler in each group, and results might vary depending upon the individual.

For this study, we used plasma corticosterone levels as a key indicator of stress. Basal corticosterone levels follow circadian rhythms, and these basal levels may influence the magnitude of corticosterone release in response to stressful stimuli.[41] Therefore, we were careful to measure blood levels at the same time each day, near the trough of the diurnal variation. A previous study[36] demonstrated increased corticosterone levels after blood collection from the tail as compared with animals that had not been subjected to blood collection. In that report, corticosterone levels returned to normal within 24 h of the initial blood collection; there-

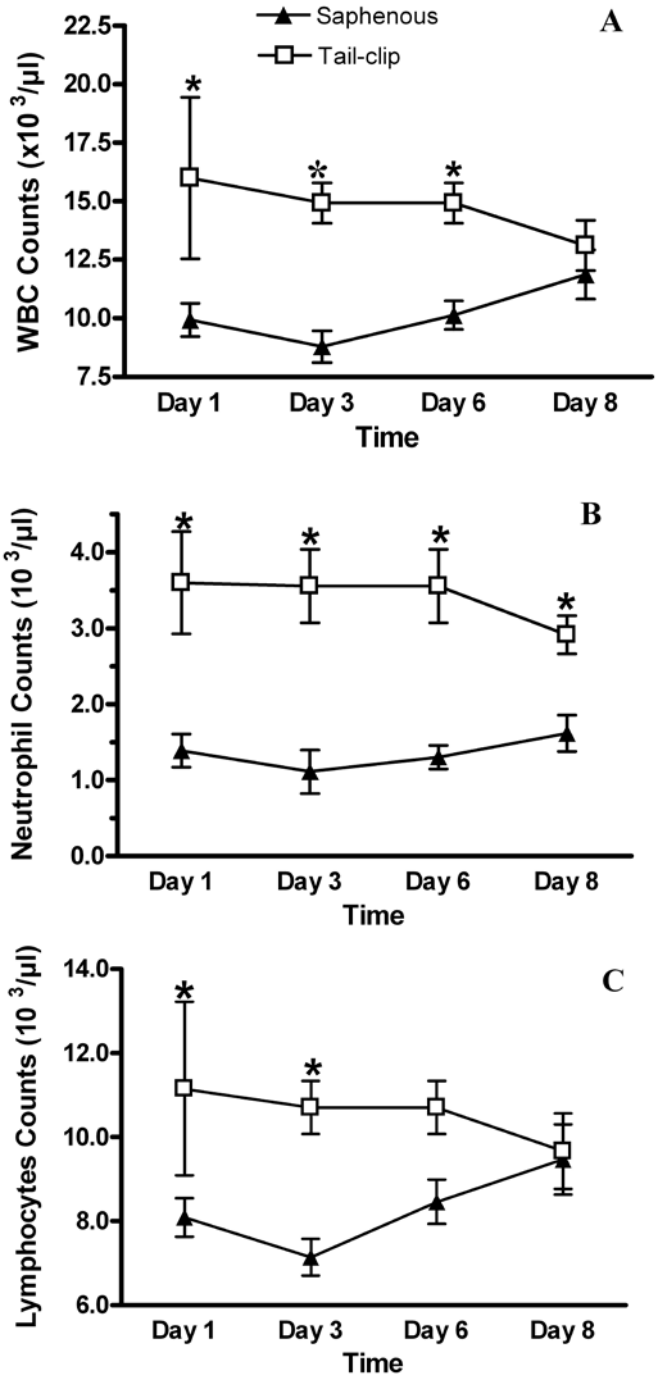

Figure 5. Effects of serial blood collection on complete blood counts. Complete white blood cell counts performed on serial blood samples revealed significant (*, $P < 0.05$) differences between the saphenous venipuncture and tail-clip methods for (A) White blood cell (WBC) counts, primarily because of increases in (B) neutrophil and (C) lymphocyte counts. However, for a given technique, there were no significant changes over time. n = 8 per group.

fore serial samples obtained every other day did not demonstrate any changes in corticosterone. The results of our study verified the results of that earlier evaluation of the tail-clip method and gave similar results for serial collection by the saphenous venipuncture method. Another study demonstrated that corticosterone levels correlate with the length of time required for blood collection.[18] In our study, previous experience by the handler affected the time required for blood collection. However, we did not find a distinct correlation between time and plasma corticosterone (Pearson's correlation r = 0.110; $P = 0.601$). This finding may be, in part, due to the brief time required for blood collection by either method used in this study. When blood is obtained in less than 2 to 3 min, the corticosterone levels measured have not peaked and may not reflect full activation of the hypothalamic–pituitary axis.[38] Therefore, it remains possible that the differences in corticosterone levels seen between the experienced and inexperienced handlers performing the saphenous venipuncture were due to duration of the procedure.

To further substantiate our comparisons, we opted to use behavioral observations. Various behavioral parameters have been described in the evaluation of pain and distress in rodents.[14] For the current study, we attempted to use simple and objective parameters that would not be subject to biased interpretation by the observer. However, observers indicated that aggressive behavior was difficult to assess during use of the saphenous venipuncture technique, because the mice were in a restraint tube. The observers also reported difficulty evaluating pulling during modified tail-clip blood collection. With that method, the handler moves with the animal as it ambulates and this characteristic may have made attempts to evade the handler less evident. For analysis of these data, each of the observed parameters was evaluated separately rather than by assigning a cumulative score for all of the behaviors in each mouse. This procedure was selected because some of the signs might indicate a more severe reaction (vocalization, aggression) than others (flinching, defecating).[14] Relatively few behavioral signs were exhibited by the mice subjected to the modified tail-clip method. However, this finding was similar to studies in which rats subjected to tail sectioning did not vocalize or struggle.[18] In addition, the animals in our study were allowed to explore their surroundings, which may have provided a stronger stimulus than the tail-clip.

Overall, the modified tail-clip method of blood collection elicited fewer behavioral signs than did the saphenous venipuncture method; however, we have not concluded that the saphenous venipuncture method is inferior to the modified tail-clip. In our study, performing the saphenous venipuncture technique may have been complicated because the operator was required to use a calibrated pipette in 1 hand to obtain an exact amount of blood. Saphenous venipuncture may be less demanding when the blood is collected in a tube, as previously described and very useful in most situations.[13] In addition, pulling behavior appeared to be a major determinant between the 2 techniques, but this parameter was somewhat more difficult to assess than the other behaviors. Finally, saphenous venipuncture may be more operator-dependent than the modified tail-clip method. More mice were assigned to the novice handler than the experienced handlers, and perhaps this factor affected the overall comparison of the 2 techniques. When these factors are taken into account, the 2 techniques appear comparable.

Although our study did not address the use of anesthesia, using topical analgesics or general anesthetics might further improve either or both blood collection techniques by reducing stress. The use of topical analgesics has been advocated.[24] The disadvantages of incorporating local analgesia may include increased procedure time and lack of impact on aversive factors other than pain; the use of general anesthetics may further avoid stress. However, the use of methoxyflurane and ether prolonged physiologic derangements after tail-biopsy.[1] Further research is needed to determine whether other currently available anesthetic agents would be beneficial for blood collection.

Complete blood counts may provide an indicator of stress in response to repeated stimuli. None of the animals developed

a classic stress leukogram, which is characterized by increased neutrophil counts accompanied by decreased numbers of lymphocytes and eosinophils. However, initial samples did demonstrate significant differences in total white blood cell and neutrophil counts obtained at the different sampling sites (tail versus saphenous vein). These results were in keeping with our previous findings[27] and those of others,[4,10,29] which showed that counts increase as the samples are derived from more peripheral sites. Although the exact physiologic explanation for this phenomenon is unknown, vascular stasis and the increased surface area of the capillary beds may account for this difference.[29]

In conclusion, the modified tail-clip and saphenous venipuncture methods of blood collection induced comparable responses in unanesthetized mice. However, each method has its own indications for use. Although the average blood collection times for the 2 methods were similar, the saphenous venipuncture method is faster than the tail-clip method when performed by an experienced person and generally yields a larger volume of blood. The tail-clip method appears to require less technical ability, but the lack of animal restraint may limit its use to tractable strains of mice. Regardless of which blood collection technique is chosen, maintaining consistency within a study is important, particularly when obtaining samples for measurements of glucocorticoids.

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