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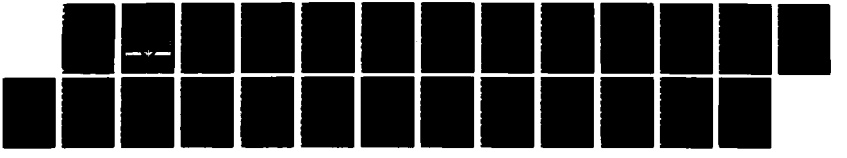

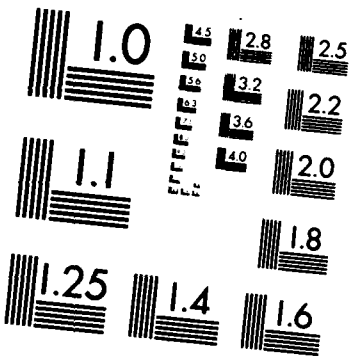

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RODENT TAIL VEINS: A CONVENIENT SITE FOR INTRAVENOUS INJECTION AND SAMPLING

US ARMY RESEARCH INSTITUTE
OF
ENVIRONMENTAL MEDICINE
Natick, Massachusetts

JUNE 1986

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MEDICAL RESEARCH & DEVELOPMENT COMMAND

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The tail veins of both rats and mice offer a convenient, easily accessible site for intravenous drug injection as well as the withdrawal of blood samples from rats. This site is also a good choice because of the minimal damage or trauma of the procedure to the animal. This report will discuss methodology for injection and sampling as well as some representative clinical values of samples.

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TECHNICAL REPORT

No. T10/86

RODENT TAIL VEINS: A CONVENIENT SITE FOR INTRAVENOUS INJECTION AND SAMPLING

by

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ABSTRACT

The tail veins of both rats and mice offer a convenient, easily accessible site for intravenous drug injections as well as the withdrawl of blood samples from rats. This site is also a good choice because of the minimal damage or trauma of the procedure to the animal. This report will discuss methodology for injection and sampling as well as some representative clinical values of samples.

INTRODUCTION

The rat and mouse are ideal laboratory experimental animals because of their relatively low cost and ease of maintenance. However, their small size and deep location of blood vessels makes blood sampling and intravenous injection difficult. Blood sampling in these small animals has sometimes involved drastic means such as decapitation, cardiac puncture through the chest wall, or orbital bleeding (inserting a needle or pipet along the medial orbit to the venous plexus posterior to the eye).Drug administration via a parenteral route is commonly achieved intramuscularly (im), intraperitoneally (ip), subcutaneously (sc), or less commonly intravenously (iv). Injection via the iv route is generally performed via a surgically implanted cannula located in the jugular vein (3). While these surgically cannulated animals do offer a less traumatic blood sampling procedure, they also represent a substantial investment in time for surgery and recovery.

We have found that the tail vein offers a convenient site for injection of drugs into rats and mice as well as for withdrawal of blood samples from rats. The tail vein site was chosen because of its accessibility and the minimal damage or trauma of the puncture to the animal. While the techniques described here require some practice, most people can become proficient in a relatively short period of time. The following report details our injection and blood sampling methods as well as some representative clinical values for these samples.

METHODS OF PERFORMING TAIL VEIN INJECTION AND SAMPLING

A. RATS

ANATOMY: The anatomy of the rat tail vasculature was well described by Thorington in 1966 (7). Two figures from this report have been redrawn here (figs. 1 and 2). Figure 1 is a midlength cross section of the tail. The two lateral veins are the veins of choice, because on the ventral side the artery is closer to the surface, and on the dorsal side the vein is small and has an irregular course due to the vertebrae below. Figure 2 is a dorsal view showing the proper position for the needle during either procedure. Note the high degree of anastomosing between vessels; this explains the blanching of the whole tail during an injection.

RESTRAINT: In order to inject into or sample from a tail vein, complete access to the tail is essential. We have developed a restraining cage (6) pictured in Figure 3 that: the rat will enter readily, is elevated so that soaking the tail in warm water is facilitated, and can easily be clamped to a working surface.

TECHNIQUE: A 22-G needle with a transparent hub (so that the first drop of blood withdrawn can be seen) on a 1cc TB syringe is the best size for injection and withdrawal. We have also used a 20-G catheter-needle with a stopcock attached for infusion over a longer period of time. Prior to

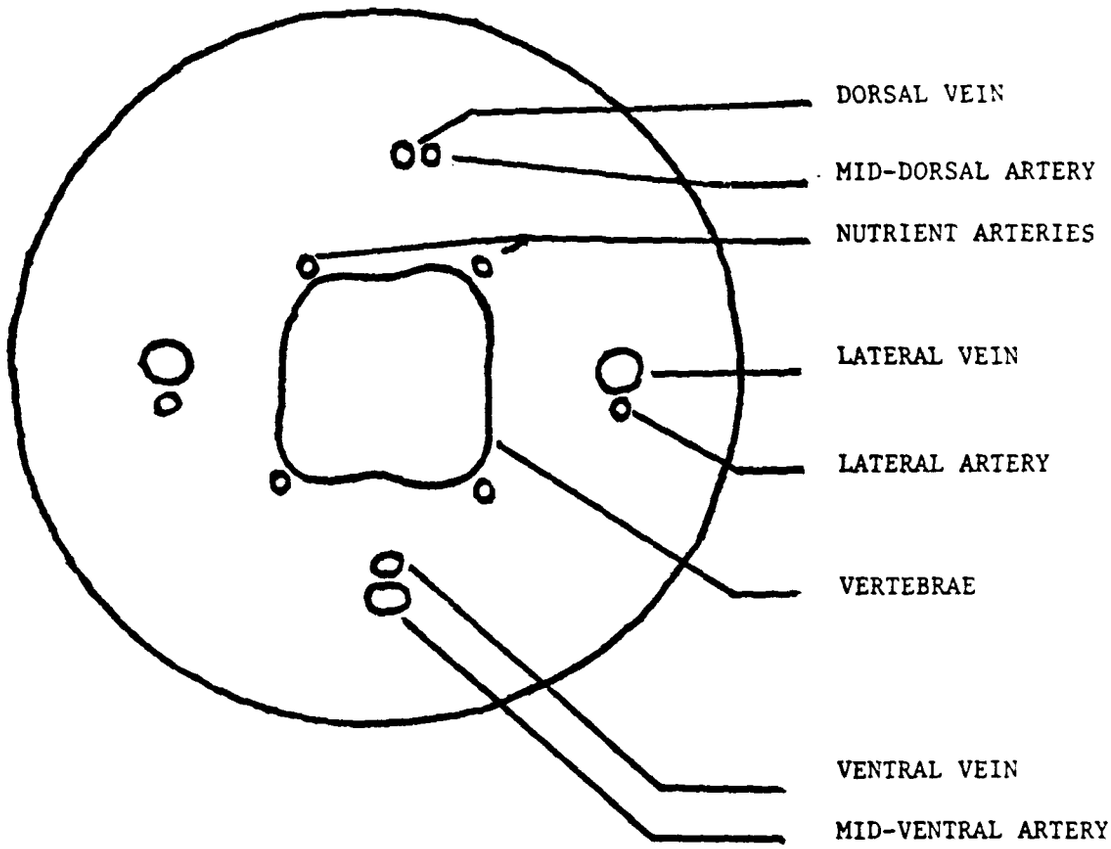
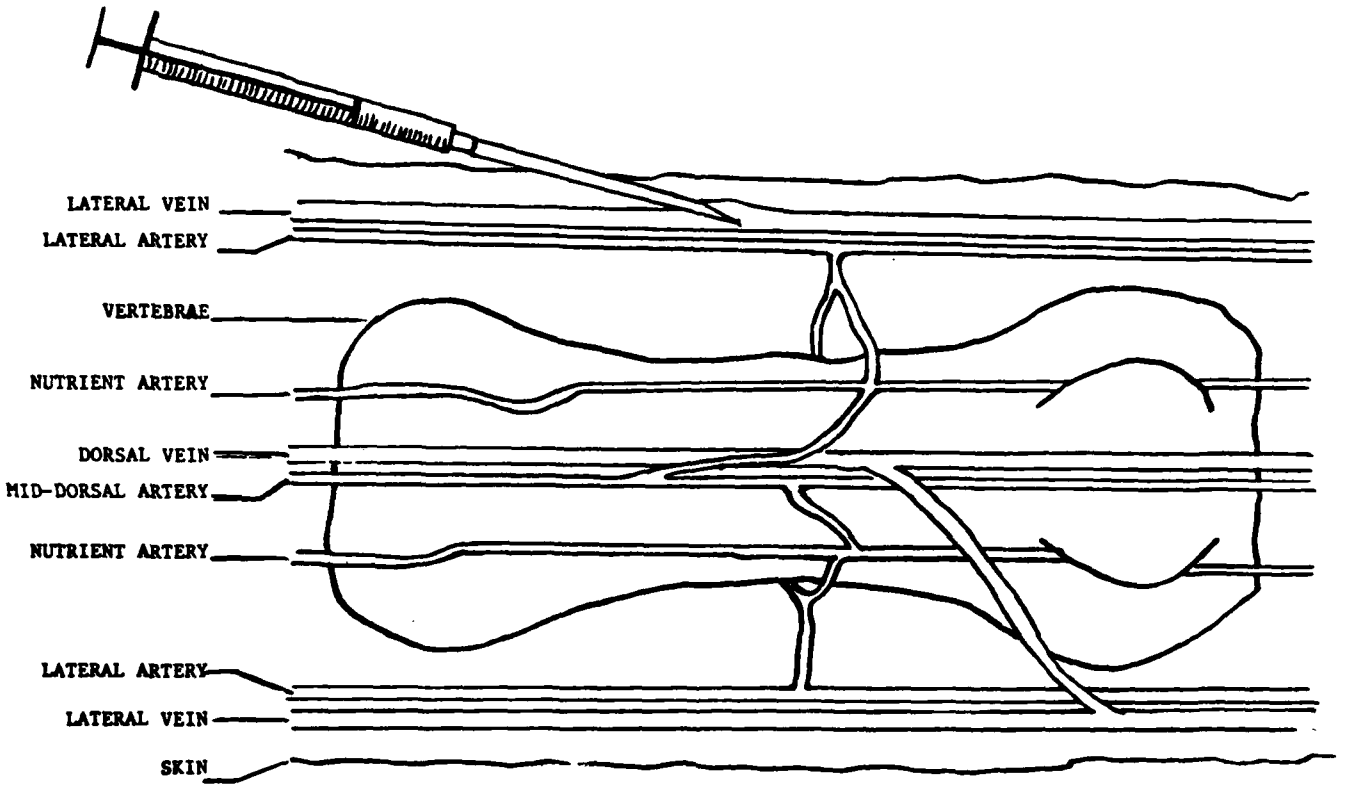


FIG. 1 AFTER THORINGTON (7)
DIAGRAMMATIC CROSS-SECTIONS OF THE CAUDAL VASCULARITY OF RATTUS

FIG. 2 SHOWING PROPER POSITIONING OF THE HYPODERMIC NEEDLE. AFTER THORINGTON (7)
DIAGRAMMATIC DORSAL VEIW OF THE ARTERIES AND VEINS NEAR THE MIDDLE OF THE TAIL OF RATTUS



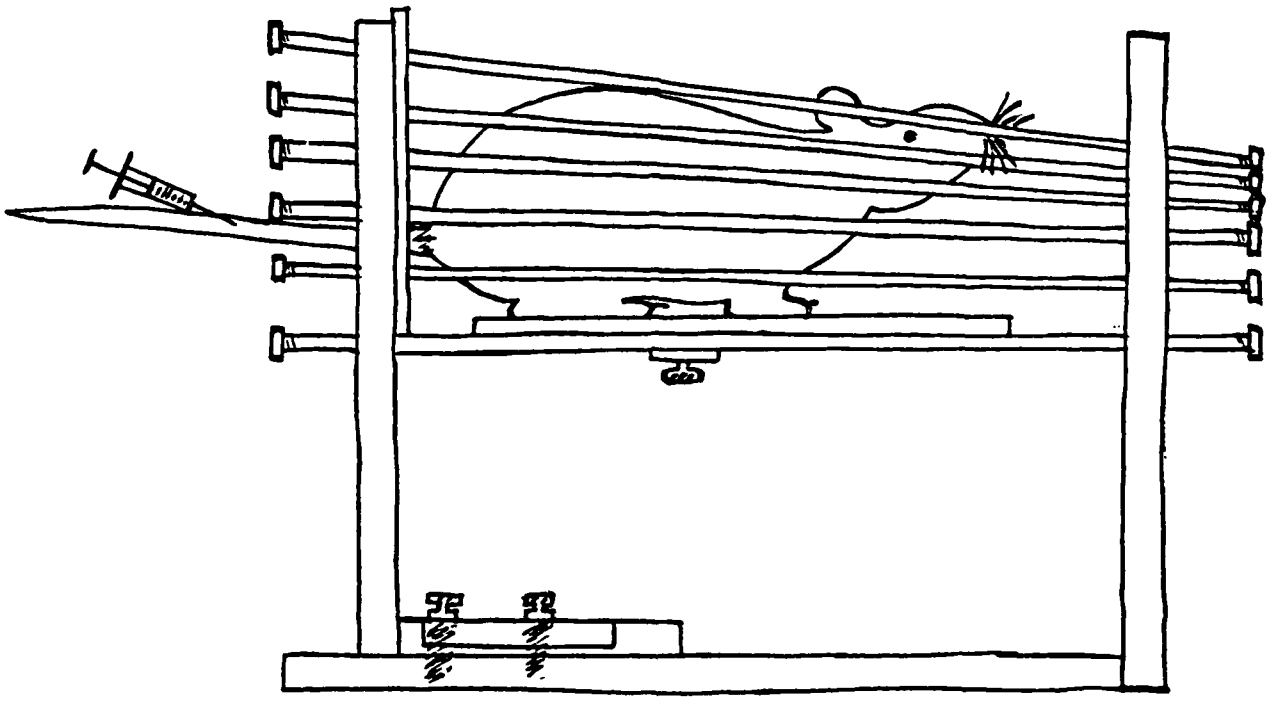


FIG. 3 RAT POSITIONED INSIDE OF A STAINLESS STEEL AND PLEXIGLASS RESTRAINING CAGE FOR INJECTION INTO A TAIL VEIN.

injection, the tail is warmed to dilate the blood vessels by soaking it in a warm (about 41°C) water bath for at least 2 minutes. The tail is dried quickly by wiping it (4x4 gauze works well) to prevent vasoconstriction due to evaporative cooling. The tail is then held taut with one hand while the needle is inserted, bevel up, into one of the 2 lateral tail veins with the other hand. The vein is very shallow so that the syringe should be almost parallel to the tail. Most first attempts are too deep. Also, the tail is covered with scaly skin so that penetration may require more force than expected. Pull back on the plunger, and if blood is seen in the hub of the needle, then it is in the lumen of the vessel. You may then inject slowly. If resistance or swelling at the site is noted, the needle is not in the vessel. Repeat attempts may be made by moving anteriorly. When the injection is complete, gentle pressure over the site will stop any bleeding. Hematoma is rare. A blood sample may be withdrawn following the above technique with the addition of a source of warmth in the form of a lamp with a 60 or 75 watt bulb (not a heat lamp) to help keep the veins dilated for the longer time required. Using this technique we have drawn samples of up to 1.5ml and then replaced that volume with serum or plasma, also via tail vein, with no problems.

B. MICE

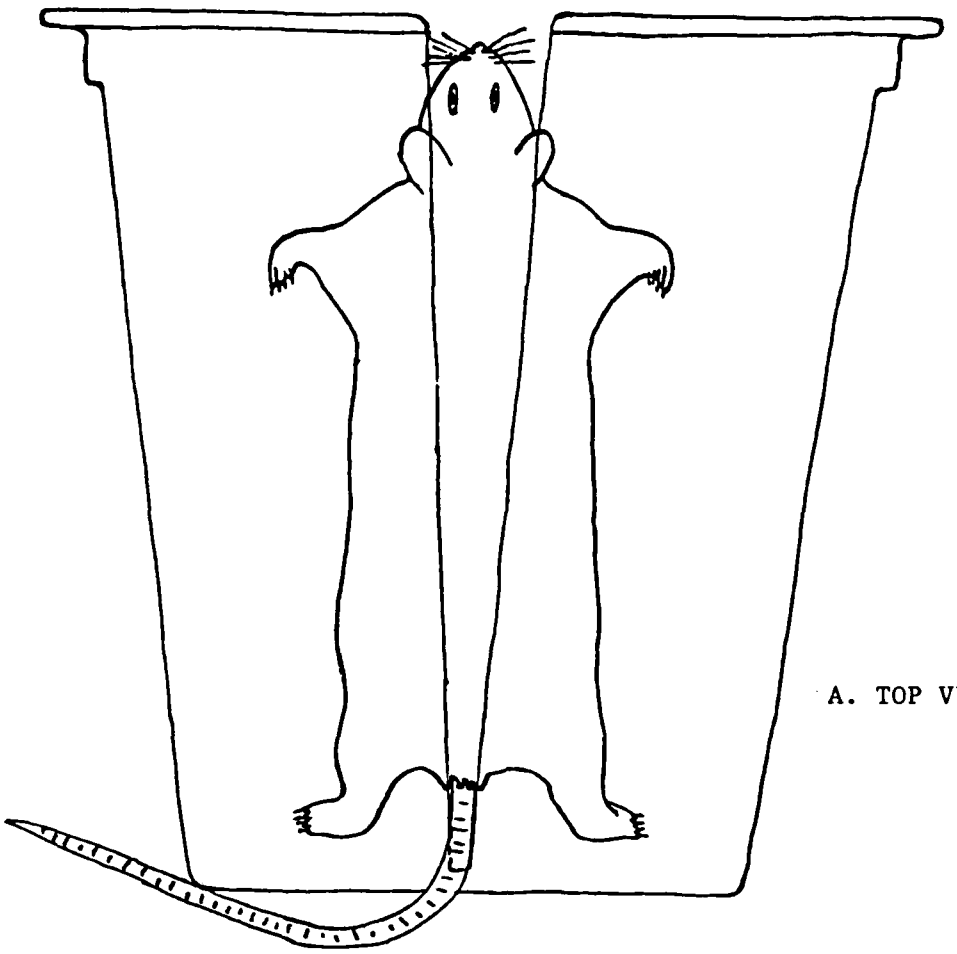
ANATOMY: The mouse tail vasculature is very similar to that of the rat (7); thus, for the purposes of this report, we can assume it is the same.

RESTRAINT: To restrain mice, we have cut a 400ml plastic beaker in half (fig. 4) longitudically; then, it was taped cut side down to the work surface and a slit was made in the top. After holding the mouse by the nap of the neck above a warm water bath to warm his tail, he can then be pulled by the tail backwards into this cup and held by maintaining the pull during the injection.

TECHNIQUE: A 27-G needle with a transparent hub on a 1cc TB syringe is optiomal. The injection procedure is similar to that described for the rat; however, there is no scaly covering of the tail. Therefore, the mouse's lateral tail veins are actually easier to see and inject into than the rat's. Blood sampling from the mouse tail vein is possible but not practicle, because the small size of the needle induces hemolysis and clotting.

DRUGS FOR INJECTION

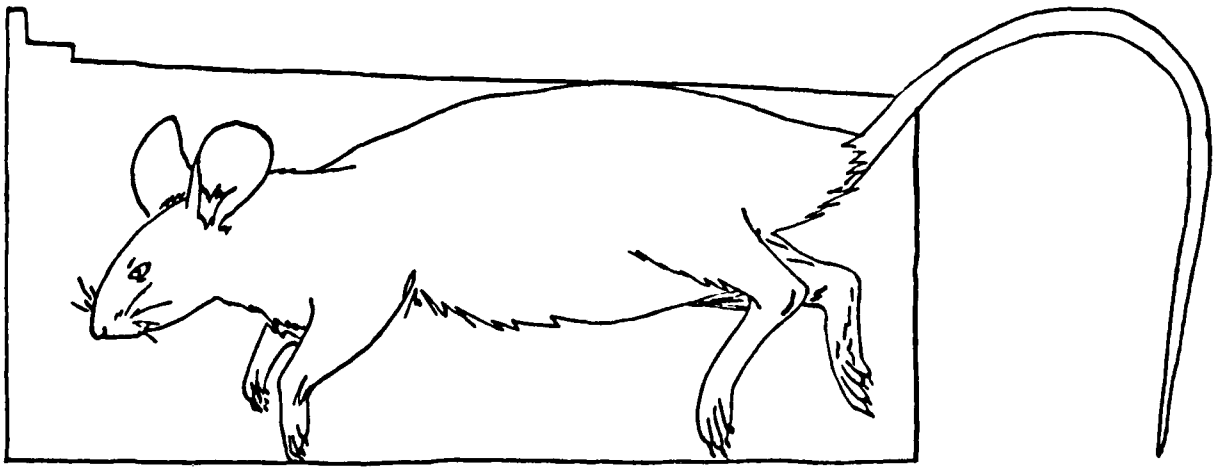
We have sucessfully injected aqueous solutions of many drugs via tail vein into the rat or mouse (2,4,5). Included among these are: atropine, chlorpromazine, 2-deoxy-D-glucose, dextrose, imipramine, physostigmine, pyridostigmine, saline, and scopolamine. However, caustic solutions such as sodium pentobarbital and diazepam must first be diluted because they damage the fragile vessel walls. While slightly more viscous solutions or suspensions such as serum, plasma or whole blood can also be injected via the tail vein, more viscous fluids such as carboxymethyl cellulose or packed cells are better administered via a cannulated larger vessel.



A. TOP VIEW

FIG. 4 MOUSE POSITIONED IN A RESTRAINING DEVICE USED FOR TAIL VEIN INJECTION. TRACTION ON THE TAIL KEEPS THE ANIMAL IN THE CAGE.

B. SIDE VIEW



CLINICAL BLOOD VALUES

Some clinical blood values for a sample obtained from the tail vein are indicative of the value for the whole animal; however, there are other values for which a tail vein sample is inappropriate. We took samples of blood from the proximal and distal 1/3 of the tail from the same 12 rats and determined that the mean and standard deviation for hematocrit (Hct) and total protein were identical (49% $\pm$ 2 and 7.2g $\pm$ 0.4 respectively) for both sites. Therefore, the sample site on the tail is immaterial for these variables. To test the reliability of tail vein values we compared them with values determined on samples taken from surgically implanted (3) jugular cannulae.

Both tail and jugular samples were drawn simultaneously from the same animals. Jugular cannulation was completed 3-5 days prior to sampling for adequate recovery. The cannulae were advanced to the right atrium (common cava); therefore, the sample was mixed central venous blood. Table 1 contains the results of these tests. Since we observed that the fed or fasted state of the animals affected the values at each site, feeding state is also noted in table 1. Hct and protein are both higher in the tail than the jugular sample in both fed and fasted states. Since rats are prandial drinkers, the higher values in the fasted state are indicative of the dehydration from fasting. The lower values for glucose in the fasted state are expected. Glucose in the fed state and lactate in the fasted state are lower in the tail than in the jugular samples, which may be explained by the proximity of the liver to each

Table 1

A Comparison of Values Determined on Blood Taken From the Tail and Jugular Cannulae of Fed and Fasted Rats

	Hct %	Total Protein g/dl	Glucose mg/dl	Lactate mg/dl	K+ mEq/L	Na+ mEq/L	CPK Iu/L	ALT (SGPT) Iu/L	Ast (SGOT) Iu/L
Fed Jugular	44 ±2	6.2 ±0.3	207 ±16	42 ±19	5.6 ±0.4	146 ±4	115 ±45	26 ±8	67 ±13
Fed Tail	48* ±2	7.1* ±0.4	150* ±19	32 ±13	6.1* ±0.4	147 ±3	78* ±21	30 ±10	70 ±6
Fasted Jugular	46+ ±2	6.4 ±0.4	127+ ±37	67+ ±29	5.1+ ±0.6	141+ ±2	92 ±67	22 ±5	74 ±13
Fasted Tail	50*+ ±2	7.1* ±0.4	116+ ±12	25* ±11	5.9* ±0.6	144*+ ±2	66 ±30	22+ ±7	51*+ ±12

Values are mean ± S.D. for 12 rats in each group.
* Significant difference between jugular and tail by Students "t" test $p<.05$.
+ Significant difference between fed and fasted by Students "t" test $p<.05$.

Table 2

The Effect of Heating on Tail and Jugular Hematocrits

	Jugular		Tail Vein		Wt. Loss
	Hct %	Protein g/DL	Hct %	Protein g/DL	Heating %
Fed Pre-Heat N=13	46 ±1	6.8 ±0.4	49* ±2	7.2* ±0.7	---
Fed Post-Heat N=13	52+ ±6	7.0 ±0.8	47*+ ±3	6.1*+ ±0.5	4.3 ±1.8

*Tail value significantly different from jugular ($p<.05$) by Student's "t" test.
+Pre value significantly different from post ($p<.05$) by Student's "t" test.

site and the principal fuel source in each state. K^+ is higher at the jugular site, but Na^+ is the same at both sites. For the enzymes CPK, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) site differences do not appear to be problematical. Current work with serum cholinesterase levels indicates that there is no difference in the values between the two sites at least in the fed state.

Much of our work has been on heat illness; therefore, we frequently subject animals to heat stress resulting in significant dehydration. The data in table 2 are from rats subjected to an ambient of 41.5°C until a 4.3% loss of body weight was achieved. This heat exposure caused a marked dehydration as indicated by central Hct and protein values. However, the decreased Hct and protein of simultaneously obtained tail samples would seem to indicate a hemodilution. Thus, whether blood samples were obtained from the jugular cannula or the tail vein under these conditions could lead to very different interpretations of the hydrational state on the animal.

DISCUSSION

Tail vein injection or blood sampling provides a quick and easy method of iv administration or sampling if care is taken in comparing the results with central samples. Unless significant dehydration is part of an experiment, tail vein hematocrit and protein values can be standardized by fixing the length of time and the temperature of the water during tail immersion; thus, these values will be consistent throughout an experiment. Injection into the

tail vein (2,4,5) may be the more useful technique, but we have also used tail blood sampling in our work (1,5).

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