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EVALUATION OF A FIELD EXPEDIENT TECHNIQUE FOR SWEAT SAMPLE COLLECTION

U S ARMY RESEARCH INSTITUTE
OF
ENVIRONMENTAL MEDICINE
Natick, Massachusetts

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This series of laboratory experiments was conducted to evaluate a potential field expedient sweat collection technique (polyethylene arm bags) and to observe the sweat rate and electrolyte losses of human sweat glands during thermal stimulation. The development of the methodology in this report has resulted in a technique that offers: (1) a clean skin surface prior to sweat sample collection, (2) a known skin surface area inside the arm bag, (3) quantitative recovery (97.3%) of sweat electrolytes, and (4) sweat electrolyte concentrations

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which agree well with values reported by other laboratories. Polyethylene arm bags were found to induce changes in the local forearm microenvironment (when compared to OPEN arms); increases in skin temperature, relative humidity, sodium excretion, and potassium excretion were observed. In spite of these changes in local microenvironment, this technique is useful as a relative measure of day-to-day (or hour-by-hour) changes in sweat secretion. It offers advantages over other sweat collection techniques, such as large sample size, ease of use and field portability. Measurements such as sweat electrolyte losses during exercise in the heat, or sweat rates during heat acclimatization, are feasible with this technique.

TECHNICAL REPORT NO T2/85

EVALUATION OF A FIELD EXPEDIENT TECHNIQUE FOR SWEAT SAMPLE COLLECTION

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Abstract

This series of laboratory experiments was conducted to evaluate a potential field expedient sweat collection technique (polyethylene arm bags) and to observe the sweat rate and electrolyte losses of human sweat glands during thermal stimulation. The development of the methodology in this report has resulted in a technique that offers: (1) a clean skin surface prior to sweat sample collection, (2) a known skin surface area inside the arm bag, (3) quantitative recovery (97.3%) of sweat electrolytes, and (4) sweat electrolyte concentrations which agree well with values reported by other laboratories. Polyethylene arm bags were found to induce changes in the local forearm microenvironment (when compared to OPEN arms); increases in skin temperature, relative humidity, sodium excretion, and potassium excretion were observed. In spite of these changes in local microenvironment, this technique is useful as a relative measure of day-to-day (or hour-by-hour) changes in sweat secretion. It offers advantages over other sweat collection techniques, such as large sample size, ease of use and field portability. Measurements such as sweat electrolyte losses during exercise in the heat, or sweat rates during heat acclimatization, are feasible with this technique.

Introduction

Eccrine sweat glands have been studied for a variety of reasons, the most obvious of which is to measure the effects of sweat losses on body fluid balance. Because sweat contains small amounts of over 60 substances (such as minerals, vitamins, glucose, lactic acid), the nutritional impact of sweat losses on whole body nutrient balance continues to be of interest (Consolazio, et al. 1966). The effect of sweat evaporation on thermal balance has also been the purpose of many investigations. In addition, the function of sweat glands has been studied to resolve several questions: (a) why do the sweat glands freely secrete some substances and selectively secrete others? (b) what are the causes of sweat gland "fatigue"? (c) what are the effects of heat acclimatization on sweat rate and sweat electrolyte loss? (d) what stimuli initiate and maintain sweating? (e) what are sweat electrolyte losses and sweat rates during desert living?

The methods of sweat sampling are nearly as numerous as the number of investigators who have studied eccrine gland secretions. Since the pioneering studies on sweat gland function of the 1930s, six methods have been employed: filter paper/gauze collection, multi-site pipette collection, sweat gland cannulation, rubber gloves, arm bag technique, and whole body washdowns. All of these techniques are still used today, and there has been little impetus to standardize methods of sweat collection. No method has yet been

devised for the collection of sweat which has not altered the microenvironment of the collection site. Most of the above techniques are suspected of altering not only sweat rate but also sweat composition. Localized collections also may vary from site to site on the body (Kuno, 1956 (pp. 192, 294)).

Whole body washdowns are considered by some authors to be the best alternative method (Robinson, 1954; Vellar, 1969; Sohar et al. 1965). In situations such as desert maneuvers, however, whole body washdowns cannot be used. The time and logistical constraints of preparing the subject (10-30 minutes) and washing the body (5-15 minutes) do not lend this method to (a) repeated or continuous measurements (b) short-term collections, or (c) studies in which airborne dirt and contamination may enter samples. Clearly, there is a need for a sampling technique which will minimally affect the collection site, allow collection of relatively large aliquots of sweat (5-40 ml), be easy to use, and be field portable.

Rationale: Arm Bag Sweat Collection Technique

The arm bag sweat collection technique, as described by Mickelson and Keys (1943), underwent considerable evolution during 1951-1972. The majority of the 20 or more studies which have used this technique followed the simple design of: (1) securing an impermeable vapor barrier on the arm, (2) inducing sweat production, (3) opening the arm bag and (4) sampling the sweat via

pipette, pouring, or via spigot. In an analogous manner, rubber gloves, some with sleeves extending to the armpit, have also been used. However, use of the hand and axillary regions of the arm ignores sweat gland anatomy and distribution patterns. Apocrine sweat has an appearance, consistency,and composition which is quite different from eccrine sweat (Kuno, 1956, pp.45). Apocrine gland distribution is also quite different from eccrine gland distribution. Use of the hand and axillary regions of the arm also is confounded by the fact that three categories of eccrine glands are present: the glands of the palms respond only to emotional stimuli, the glands of the axillary region respond to both emotional and thermal stimuli, and those of the forearm and upper arm respond almost entirely to thermal stimuli (Montagna, pp.22, 1962). In the present investigation, the forearm region was used so that emotional (nervous) sweating would not confound measurements. The forearm region also contains a high density of sweat glands (Szabo, 1962, pp.1) when compared to other arm areas. In addition, forearm sweat is of clinical interest because sweat from this region is routinely sampled clinically for cystic fibrosis.

Therefore, the arm bag sweat collection technique apparently offers the following advantages over other sweat collection methods: repeated or continuous measures may be taken, sample size is relatively large, no sophisticated equipment or procedures are required, and materials are inexpensive and disposable.

Statement of the Problem and Six Subproblems

The purpose of this series of laboratory experiments was to evaluate a potential field expedient sweat collection technique (polyethylene arm bag) and to observe the responses of human sweat glands to thermal stimulation.

The approach was based on a stepwise resolution of the following subproblems, which ultimately determined the validity and usefulness of the technique:

1. What type of pre-trial forearm cleaning procedure is most effective?

2. Is the template technique accurate and/or reliable in measuring skin surface area enclosed by the arm bag?

3. Will this arm bag technique effectively recover all sweat electrolytes and allow accurate estimation of sweat volume?

4. Do significant differences exist in right arm vs left arm skin temperatures, sweat rates, and electrolyte losses (open and closed arms)?

5. What differences in skin temperature, % relative humidity, and electrolyte losses occur as a result of placing a vapor barrier (polyethylene bag) on the forearm?

6. How do arm bag sweat rates and sweat electrolyte losses compare to whole body values?

For the sake of clarity, the results and discussion of each subproblem are presented as a unit. The final sections summarize all findings and suggest applications of this sweat collection method.

Methods

Two definitions will be used throughout this report: OPEN - no arm bag used, CLOSED - subject's forearm covered by arm bag.

This project was conducted between January and March, 1984 in USARIEM environmental chambers 2,6A and 236C. All subjects were healthy males, free of obvious thermoregulatory defects and skin disorders which might have affected sweat production (e.g. dermatitis, miliaria rubra, sunburn).

All trials were conducted on a motorized treadmill (3.5 mph, 5% grade) on non-consecutive days, at 89.1±0.7°C WBGT (mean ± SE), 0.1 m/s wind velocity. OPEN trials under hot-dry conditions involved a relative humidity (%RH) of 33% while hot-wet trials were conducted at 84%RH.

Skin temperatures (Tsk) and rectal temperature (Tre) were measured using high precision platinum RTD (resistance thermometer device) sensors which were monitored via a Hewlett-Packard micro computer system (Matthew, 1982). The temperature sensor was inserted to a depth of 10 cm beyond the anal sphincter. Whole body sweat rate was measured using nude body weight differences (±50 g) corrected for water intake and by the absorption of sweat in clothing.

The arm bags used in this investigation were disposable, electrolyte-free, clear polyethylene bags open at one end (5 gauge, 0.0375 cm thick, dimensions: 12.5x8.75x40 cm). Bags were cut so

that both ends were open and were placed on the arm to coincide with marks on the wrist and elbow (approximate length: 16 cm).

Prior to arm bag placement, a template was temporarily taped to the arm (see Figure 1) and the circumference of eight consecutive arm segments (2 cm apart) were measured, to allow calculation of skin surface area under the bag. The ends of the template were used to draw rings around the wrist and elbow (using a permanent marking pen). In this manner, the skin surface area under the arm bag was known prior to each OPEN and CLOSED trial.

The only difference between OPEN and CLOSED trials was the arm bag. In OPEN trials (Figure 2), one absorbent cotton wrist band was placed at the wrist (A) and distal to the elbow (B) template marks. Wrist band A was used to keep forearm sweat above the wrist. Wrist band A was rinsed along with the forearm, to collect sweat electrolytes. Wrist band B was placed above the elbow and was used to keep upper arm and axillary sweat out of the forearm area. Wrist band B was not rinsed with the forearm skin.

During CLOSED trials (Figure 3) , Tsk was measured inside the bag by two RTDs, one on the skin surface and one 1 cm above the skin surface (midway between the template lines on the lateral aspect of the forearm). Skin temperatures outside the bag were measured on the back of the hand and on the lateral biceps midway between the shoulder and elbow joints. Platinum RTDs were secured with velcro straps and rubber bands. This same skin RTD placement

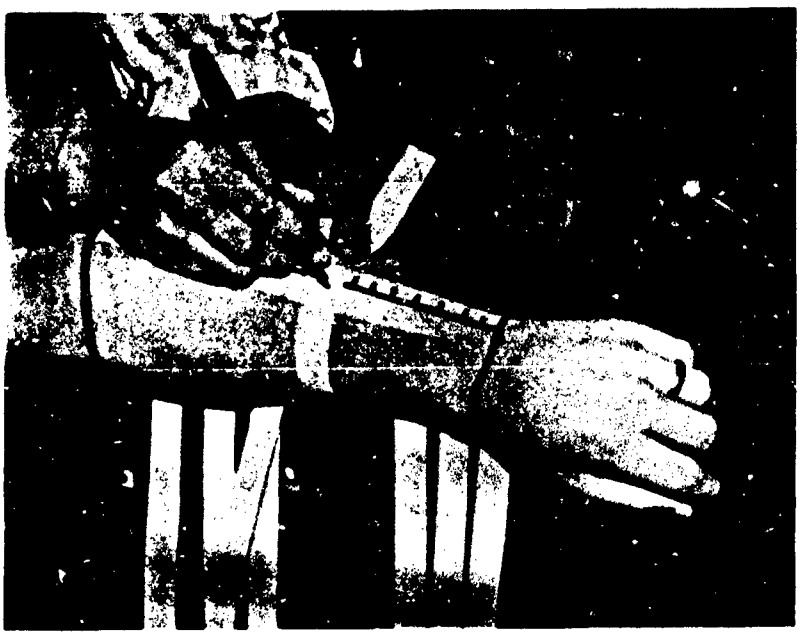


FIGURE 1 - PRETRIAL MEASUREMENT OF ARM SURFACE AREA.

FIGURE 2 - OPEN ARM WITHOUT ARM BAG. ARROWS MARK LOCATIONS OF RTD SKIN THERMISTERS.

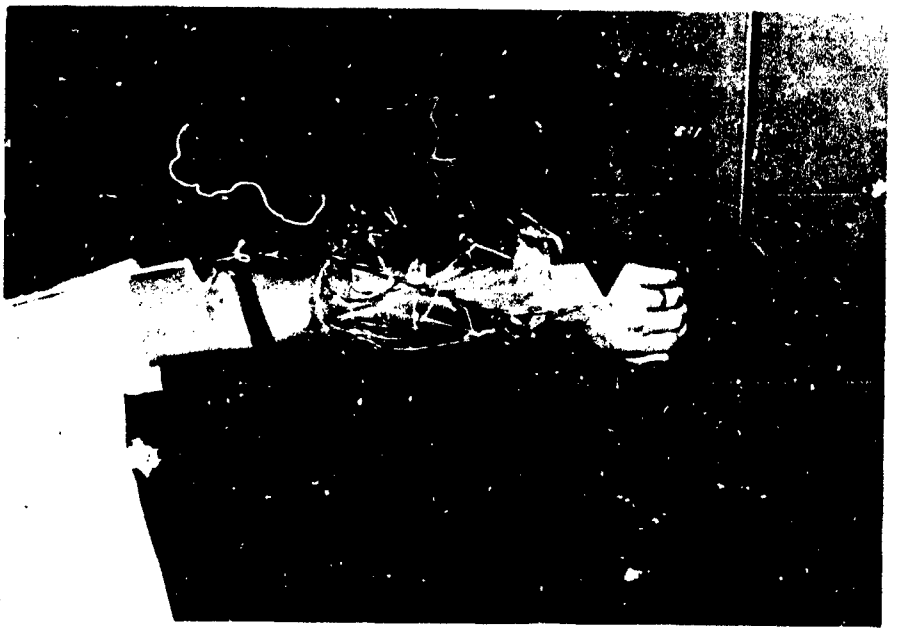


FIGURE 3 - CLOSED ARM WITH POLYETHYLENE ARM BAG. ARROWS MARK LOCATIONS OF RTD SKIN THERMISTERS.

was used during trials which involved no arm bags (OPEN). The relative humidity inside the arm bag was measured by a portable humidity meter (HUMICAP, Helsinki, Finland), accurate tc $\pm$3%RH

The calculation of the sweat volume (Sv) in the bag (see Subproblem 3) was performed using the following formula:

$$Sv = \frac{[Ctev\ (Ev - Ss)] + [(Ssv)\ (Sc)]}{Sc - Ctev}$$

The derivation of this formula and an explanation of the variables appear in Appendix A. Only deionized (<10 megaOhm) water was used during cleaning and washing procedures. Samples were centrifuged and analyzed by flame photometry for sweat sodium (Na^+) and potassium (K^+).

The total mEq of Na^+ or K^+ lost during the 30 minute trials was determined using the sweat volume and sweat concentration in the bag. Sweat electrolyte losses are expressed as either $mEq/m^2/hr$ (whole body measurements) or $mEq/cm^2/min$ (forearm measurements).

Results & Discussion

SUBPROBLEM 1: WHAT TYPE OF PRE-TRIAL FOREARM CLEANING PROCEDURE IS MOST EFFECTIVE?

Subjects had taken morning showers prior to testing and arms were relatively clean prior to beginning. Electrolyte-free towels and deionized water (DW) were used throughout.

Several pre-trial arm cleaning procedures were tested. The

most effective of these was:

1. Rinse area from elbow to wrist with at least two distilled water (DW) washings (1 liter minimum).

2. Lightly scrub same area with an electrolyte-free 10 cm x 10 cm gauze pad which has been wet with DW.

3. Repeat #1.

Following this procedure, no measurable electrolytes were recovered during five trial wash-downs inside arm bags.

Because the lower limits of flame photometry sensitivity fall within the range of arm bag wash concentrations, the volume of DW had to be determined which would (a) adequately rinse the forearm (post-trial) and (b) provide mesurable electrolyte concentrations following 30-45 min trials in the heat chamber. Six wash volumes (range: 25-300 ml) were tested, using the 12.5x8.75x40 cm polyethylene bag (see methods). It was found that a volume of 75 ml DW was the smallest volume which met criteria a and b above.

SUBPROBLEM 2: IS THE TEMPLATE TECHNIQUE ACCURATE AND/OR RELIABLE IN MEASURING SKIN SURFACE AREA ENCLOSED BY THE ARM BAG?

The skin surface area (SA) to be tested was measured before each trial, using the technique illustrated in Figure 1 (See methods). Reproducibility of measurements was the primary goal, although ease of template production was also considered.

The simplest and most acceptable method of measuring skin SA

involved templates printed on a Hewlett Packard plotter (model 9872C), using graphics plotter paper (PN 9230-0518). The surface of the arm was viewed as a series of adjoining cylinders which increased in circumference from the wrist to the elbow. Because the wrist and elbow were marked with a ring (see methods), the boundaries of the arm bag were clearly delineated. The SA measurements from day-to-day varied less than 2%, although movements of the template proximal or distal to the original sits resulted in considerably larger variations.

The sampling of forearm sweat involves only a portion of total skin SA, and this must be recognized as a limitation of this technique. However, sweat capsules, and collections by filter paper, pipette, or rubber gloves also share this limitation. Collins & Weiner (1962) reported that arm surface area represented 4.7% of the total body surface area (TBSA). Weiner (1945) later reported that the entire arm SA ranged from 5.9-11.9% of the TBSA. Data from the present study (ASA, TBSA, and the percent of TBSA which the arm represents) are presented in Table 1. The skin SA inside the arm bags represented 2.6-3.2% of TBSA observed.

SUBPROBLEM 3: WILL THIS ARM BAG TECHNIQUE EFFECTIVELY RECOVER ALL SWEAT ELECTROLYTES AND ACCURATELY CALCULATE SWEAT VOLUME?

The obvious problem with any sweat collection technique is that evaporative loss of water begins as soon as the sweat is exposed to

TABLE 1

Relationship between arm and body surface area

Subjects	Number of Measurements *	TBSA(m^2)**	ASA(cm^2)	$\frac{ASA}{TBSA} \times 100\%$
A	10	2.16	567.5	2.6
B	14	1.67	541.1	3.2
C	16	1.77	519.2	2.9
D	14	1.68	469.5	2.8
E	14	2.06	608.9	3.0

ASA - Arm surface area

TBSA - Total body surface area (1 m^2 = 10,000 cm^2)

* - Right and left arms used

** - DuBois, 1915

air. Basic biophysical principles remind us that this problem is magnified as wind velocity, Tsk and ambient temperature increase or as ambient humidity decreases.

Previous use of the arm bag technique involved a variety of materials, sites and sampling methods (see Rationale). During the course of the present investigation, the following sweat collection technique was developed:

1. Measure arm surface area and draw the boundaries at the wrist and elbow with marking pen.

2. Place bag on forearm and close ends with elastic or rubber bands in an attempt to prevent evaporation of sweat and to recover all sweat electrolytes.

3. Perform 30-45 min exercise in heat chamber. This produces 11.0-43.3 ml sweat in the bag (depending on the subject involved).

4. Following exercise, mix the sweat and water vapor in the bag thoroughly, to allow equilibration.

5. Using a syringe and needle, remove a measured aliquot of sweat (1-2 ml). Close the needle hole with surgical tape. Analyze the sweat aliquot by flame photometry to determine Na^+ and K^+ concentration.

6. Using large syringe inject 75ml of DW through the bag wall. Seal the opening with tape.

7. Wash the forearm thoroughly. Be certain to wash the inside wall of bag also.

8. Using a second syringe and needle, remove a measured aliquot of wash water (1-2 ml).

9. Using sweat electrolytes as a dilutional marker, calculate sweat volume. Use the formula described in Appendix A. Calculations may be done using Na^+, K^+, or both.

To our knowledge, no other arm bag measurements have utilized steps 5-9. The three advantages which make this arm bag technique superior to other sweat collection techniques are: (a) essentially no loss of sweat by evaporation, (b) the arm is cleaned prior to trials, and (c) the wash-down is conducted inside the bag so that contamination is minimal. In the desert, for example, blowing sand might contaminate sweat samples.

To evaluate our arm bag sweat collection technique, a synthetic sweat mixture (50 mEq NaCl/liter) was injected into arm bags. Subjects rested at 22^0C during 16 trials (using 10 ml and 25 ml injections). When the volume of synthetic sweat was calculated, 97.3 $\pm$ 0.9% (mean $\pm$ SE) of the synthetic mixture had been recovered. Having used all the other sweat collection techniques, the authors believe that this recovery rate is as high as any other sweat collection technique available. The most likely reason for the 2.7% loss of sample was insufficient mixing of the bag contents (step #4 above) because human skin has been shown to be impermeable to electrolytes (Whitehouse & Ramage, 1933)

A comparison of sodium concentrations of the samples collected

during the present investigation with the arm bag techniques of previous investigations appears in Table 2. Our data compare well with literature values.

SUBPROBLEM 4: DO SIGNIFICANT DIFFERENCES EXIST IN RIGHT ARM VS LEFT ARM SKIN TEMPERATURES, SWEAT RATES, AND SWEAT ELECTROLYTE LOSSES (OPEN AND CLOSED ARMS)?

There is evidence which indicates that there may be measurable differences in right vs left arm sweat parameters. If this were true, a potentially large source of error might be introduced during arm bag sweat collections. Kraning (1983), for example, has observed right vs left arm differences in sweat gland density (number of glands/cm^2). In addition, Jacob et al. (1981) have reported large right vs left arm differences in sweat excretion of zinc. Therefore, sweat rate, sweat Na^+, and sweat K^+ losses were measured for both arms during all OPEN and CLOSED trials.

Sweating in this investigation was stimulated by three inputs: core temperature, work intensity, and skin temperature. The first two of these factors were assumed to stimulate both arms equally. However, if the skin temperature of one arm was not equal to the other (due to variable vasoconstriction or variable blood flow) it is conceivable that a different sweat rate would result in each arm. During bilateral arm observations (OPEN vs CLOSED), temperatures were monitored on the wrist, forearm and upper arm.

TABLE 2

Sweat Sodium Concentrations
Measured in Arm Bag Collections

Investigation	Sweat Sodium Concentration Range (mEq/l)
Present Investigation	33-116
Kleeman, Bass, Quinn, 1946	19-94
Locke, 1951	8-62
Van Heyningen, Weiner, 1952	82-114
Sohar et al, 1965	10-42
Vellar, 1969	25-75
Cade et al., 1971	37-114

Skin temperature data from OPEN (N=17) and CLOSED (n=5) trials appear in Table 3.

These data demonstrate that right forearm and left forearm skin temperatures were only slightly different at the end of CLOSED trials (Δ = 0.08°C) and OPEN trials (Δ = 0.13°C). Nadel and colleagues (1971) have demonstrated that both regional and total body sweating are primarily functions of internal temperature, as modified by peripheral temperature. The internal temperature is nearly ten times more important than skin temperature in determining this central drive to sweating. Nadel, in a 1971 publication, presents a figure which illustrates that a forearm skin temperature difference of 0.13°C, as seen in the OPEN trial (Table 3), would result in a right vs left arm sweat rate difference of only 1.6 ml per 30 min trial (rate = 0.1 mg/cm^2/min). This 1.6 ml calculated difference is greater than the actual differences between arms, and indicates that skin temperature differences between arms may account for the right vs left arm differences seen in Table 5.

Right arm vs left arm sweat rate and sweat electrolyte data appear in Table 4. Values are presented for CLOSED trials (n=16) and for OPEN trials (n=8, hot-dry vs hot-wet). Clearly, this statistical analysis shows no right arm vs left arm differences which were significant at the $p<0.05$ level. The sweat rate data of Fox et al (1967) support these results.

TABLE 3

OPEN Trials vs CLOSED Trials:
Skin and Rectal Temperatures

Trial	Location	# of Trials	Temperature at Onset of 30 Min. Trial (°C)		Temperature at End of 30 Min. Trial (°C)	
			Left Arm	Right Arm	Left Arm	Right Arm
Arm Bag (CLOSED)	Wrist	5	35.17±.36	35.02±.50	36.14±.37	36.14±.41
	Forearm (in bag)	5	36.00±.30	36.08±.27	38.04±.17	37.96±.21
	Upper Arm	5	35.38±.36	35.47±.37	36.69±.37	36.85±.42
	Rectal	5	37.39±.14		38.25±.18	
No Arm Bag (OPEN)	Wrist	17	33.60±.35	33.62±.34	35.95±.13	35.85±.13
	Forearm (no bags)	17	34.23±.14	34.31±.17	36.31±.09	36.18±.11
	Upper Arm	17	34.60±.17	34.08±.27	36.80±.08	36.08±.22
	Rectal	17	37.39±.06		38.27±.05	

TABLE 4

Right Arm vs Left Arm Comparison:

Sweat Electrolytes and Sweat Rates in OPEN and CLOSED Trials (mean ± SE)

Trial	Number of Trials	Measurement (unit)	Right Arm	Left Arm	Probability Level
Arm Bag CLOSED	10	Na+ Excretion (mEq/m^2/hr)	42.9±4.6	44.9±6.6	NS
		K+ Excretion (mEq/m^2/hr)	4.1±0.4	4.2±0.5	NS
		Na+ Concentration (mEq/l)	70.7±7.1	70.3±6.8	NS
		K+ Concentration (mEq/l)	6.5±0.4	6.7±0.4	NS
		Sweat Volume: Na+ calc. (mg)	18.7±1.9	19.1±2.7	NS
		Sweat Rate: Na+ calc. (mg/cm^2/min)	1.1±0.1	1.0±0.1	NS
		Sweat Volume: K+ calc. (mg)	18.1±1.9	18.7±2.5	NS
		Sweat Rate: K+ Calc. (mg/cm^2/min)	1.0±0.1	1.0±0.1	NS
No Arm Bag OPEN Hot-Dry	8	Na+ Excretion (mEq/m^2/hr)	28.3±2.3	27.8±2.4	NS
		K+ Excretion (mEq/m^2/hr)	3.4±0.2	3.4±0.2	NS
No Arm Bag OPEN Hot-Wet	8	Na+ Excretion (mEq/m^2/hr)	32.0±4.0	30.3±3.8	NS
		K+ Excretion (mEq/m^2/hr)	2.7±0.2	2.6±0.2	NS

NS = not significant at $p<.05$ level

Hot-Dry: mean WBGT of 89.1°F, 33% RH

Hot-Wet: mean WBGT of 89.1°F, 84% RH

TABLE 5

Right Arm vs Left Arm Comparison:
Arm Bag Sweat Rates of Three Selected Subjects

Subject	Date	Right Arm Calculated Sweat Rate (ml/trial)	Left Arm Calculated Sweat Rate (ml/trial)	Difference Between Arms (ml/trial)	Per Cent Difference
B	19 Jan	25.2	23.7	1.5	5.9
	24 Jan	26.9	23.1	3.8	14.1
C	12 Jan	16.4	11.2	5.2	31.7
	17 Jan	13.8	6.9	6.9	50.0
E	12 Jan	15.6	25.7	10.1	39.3
	2 Feb	15.5	25.7	10.2	39.7

Although the data in Table 4 may represent the right arm vs left arm comparisons of a group of subjects, interchanging the right and left arm data of one individual is not recommended. Selected subjects in this experiment consistently demonstrated that one arm produced more sweat than the other (see Table 5). We recommend that the same arm be used for repeated measures and that one arm not be used as a control for the other.

SUBPROBLEM 5: WHAT DIFFERENCES IN SKIN TEMPERATURE, % RELATIVE HUMIDITY, AND ELECTROLYTE LOSSES OCCUR AS A RESULT OF PLACING A VAPOR BARRIER (POLYETHYLENE BAG) ON THE FOREARM?

This problem was investigated by comparing OPEN trials to CLOSED trials. The only difference between those two treatments was that the arm bag was worn during CLOSED trials; pre-trial preparation, ambient conditions and post-trial washdowns were otherwise identical.

The relative humidity values (Figure 4) inside an arm bag vs an open arm (at ambient %RH) clearly demonstrate that polyethylene arm bags altered the local conditions above the forearm skin surface, during the pre-exercise and 30 minute exercise trials.

The forearm skin temperature differences between OPEN and CLOSED trials (Table 6) were significantly different at the onset (and at the end) of 30 minute trials. CLOSED trials resulted in forearm Tsk which were 1.72-1.78^{0}C greater than on OPEN forearms.

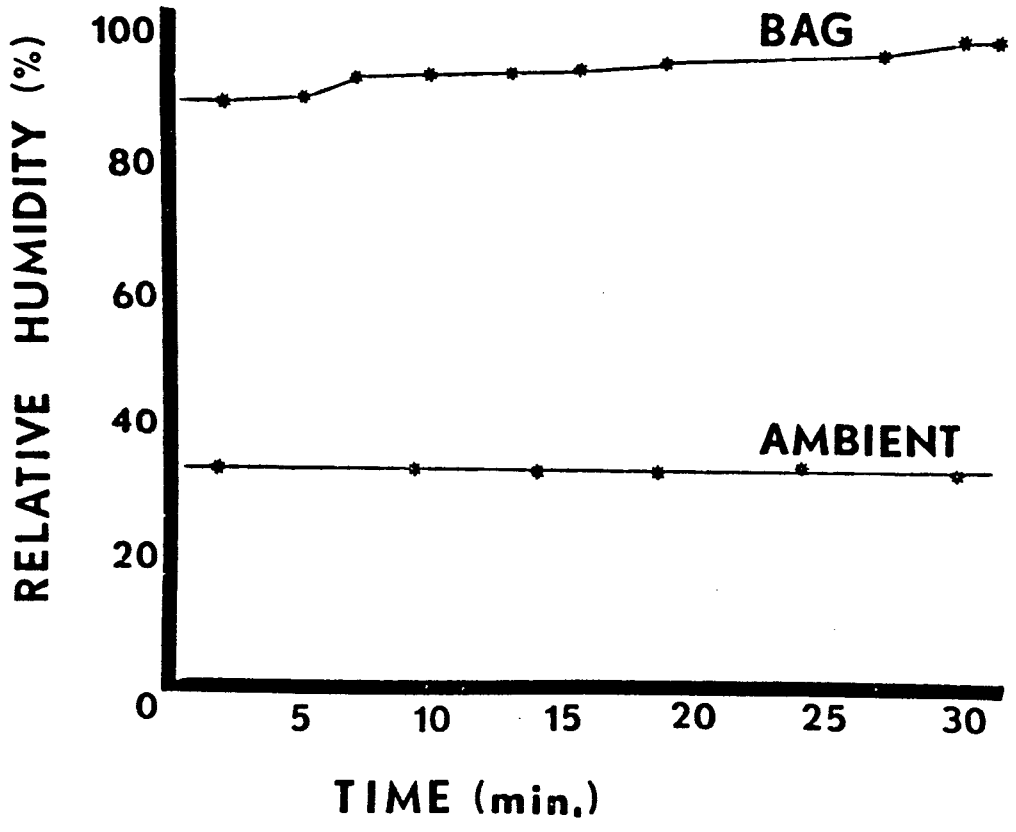


FIGURE 4 - AMBIENT AND ARM BAG RELATIVE HUMIDITY UNDER HOT-DRY CONDITIONS.

TABLE 6

OPEN Trials vs CLOSED Trials:

Forearm Skin and Rectal Temperature

Trial	Location	Temperature at Onset of 30 min Trial (°C)	Temperature at End of 30 Min Trial (°C)
Arm Bag (CLOSED) 5 Trials	Right Forearm	36.08 ± .27	37.96 ± .21
	Left Forearm	36.00 ± .30	38.04 ± .17
	Rectal	37.39 ± .14	38.25 ± .18
No Arm Bag (OPEN) 17 Trials	Right Forearm	34.31 ± .17	36.18 ± .11
	Left Forearm	34.28 ± .14	36.31 ± .09
	Rectal	37.39 ± .06	38.27 ± .05
Mean Difference Between CLOSED and OPEN Trials	Right Forearm	1.77 ($p<.001$)	1.78 ($p<.001$)
	Left Forearm	1.72 ($p<.001$)	1.73 ($p<.001$)
	Rectal	0.00 (NS)	0.02 (NS)

NS = Not Significant

Sweat electrolyte losses also were very different. Sweat Na^+ and K^+ losses (Table 7) were significantly greater in CLOSED trials than OPEN trials ($p<0.001$ and $p<0.002$, respectively).

Thus, the polyethylene arm bags used in this investigation did alter skin temperature, sweat electrolyte losses and the relative humidity of the forearm microenvironment. It is assumed that influences on sweat gland activity (such as adrenal cortical activity, state of acclimatization, and dietary salt content) were constant between trials. Collins and Weiner (1962) demonstrated that hidromeiosis (formerly called sweat gland "fatigue") occurred inside an arm bag after 80 minutes of sweat collection. Trials shorter than 80 minuces showed no indication of hidromeiosis.

Bass, Mager, and Barrueto (1959) reported that a skin temperature increase inside an arm bag led to increased excretion of Na+ and K+, while the increased humidity in the bag caused a decrease of Na+ and K+. Thus, the net result of temperature and humidity increases is an algebraic summation of forces acting in opposite directions. Unfortunately, this study by Bass et al.(1959) contained two serious flaws: right arm values were used as a control for left arm data (see Table 5 above), and hand sweat was included in all experiments (see Rationale, para. 1).

A complete description of the mechanisms which caused the OPEN vs CLOSED differences is beyond the scope of this investigation.

TABLE 7

OPEN vs CLOSED Trials:
Sodium and Potassium Excretion

Electrolyte	Arm	Amount Excreted* ($mEq/m^2/hr$)		Mean Closed vs Open % Difference	Probability Level
		No Arm Bag (OPEN) N=8 Trials	Arm Bag (CLOSED) N=10 Trials		
Sodium	R	28.3±2.3	42.9±4.6	34.0	p<.001
	L	27.8±2.4	44.9±6.6	38.0	p<.001
Potassium	R	3.4 ±0.2	4.1 ±0.4	17.0	p<.002
	L	3.4 ±0.2	4.2 ±0.5	19.0	p<.002

* Both conducted under hot-dry conditions (WBGT = 89.1°C, 33% RH)

Nevertheless, four hypotheses may be advanced. First, increased sweat production in the arm bag (due to increased Tsk) may have produced a greater variation in [Na+]/ml or [K+]/ml. Second, Tsk may have altered electrolytes, independent of sweat rates. Third, a high relative humidity may have altered excretion of certain electrolytes. Fourth, water may have been reabsorbed into the skin (Webb et al. 1957), causing increased electrolyte concentration in sweat.

SUBPROBLEM 6: HOW DO ARM BAG SWEAT RATES AND SWEAT ELECTROLYTE LOSSES COMPARE TO WHOLE BODY VALUES?

Forearm sweat rate and whole body sweat rate comparisons here are straightforward because these two measurements were taken simultaneously during all CLOSED trials.

Figure 5 illustrates the relationship observed between whole body and arm bag sweat rates which have been normalized per unit of surface area (DuBois, 1915) and per unit of time. The correlation coefficient of $r = +0.83$ indicates that the measurement of arm bag sweat is strongly related ($p<0.001$) to whole body sweat rate by the regression equation $Y = 276.9X + 447$, where Y is whole body sweat rate ($g/m^2/hr$) and X is arm bag sweat rate ($mg/cm^2/min$).

Whole body sweat electrolyte losses were not measured in the present investigation, but the work of Kleeman, Bass, and Quinn (1953) sheds light on the relationship of arm bag electrolyte

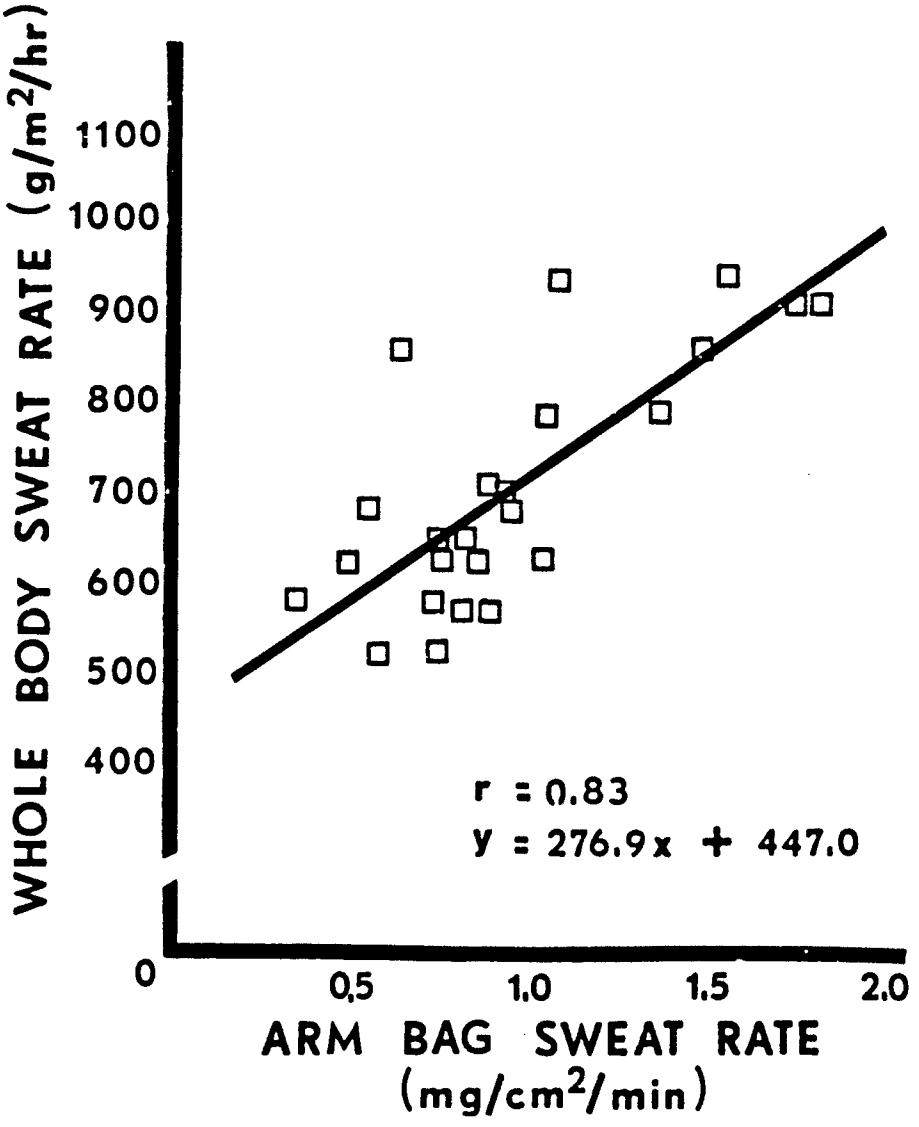


FIGURE 5 - WHOLE BODY SWEAT RATE VS ARM BAG SWEAT RATE, AS MEASURED IN THIS INVESTIGATION.

concentration to whole body electrolyte concentration. Their work demonstrated that both sodium and chloride concentrations in total body sweat losses could be well predicted from arm bag Na^+ and Cl^- concentrations. They reported that the correlation coefficient between arm bag Na+ concentration and total body Na+ concentration was r = +0.90 (Figure 6); for K+ this correlation coefficient was r = +0.42 (not shown). The fifteen data points used in Figure 6 have ranges of 13.8-60.0 mEq Na+/l (total body) and 19.0-94.6 mEq Na+/l (arm bag).

Further support for a strong correlation between arm bag and whole body values is found in the work of Costa et al. (1969); they examined localized sweat samples (gauze pads), arm bag samples and total body losses of sweat sodium. They found that arm bag samples predicted total body sodium losses (r = +0.83) better than any single gauze pad site.

Figure 7 compares the arm bag Na+ values from the present investigation to Na+ values from several other studies. These Na+ concentrations show that arm bag sweat Na+ values tend to be slightly higher than whole body sweat Na+ values. This is probably due to alterations in the local microenvironment (Subproblem 5) and is in agreement with Table 7 above, which compares CLOSED vs OPEN arm trials.

It is unlikely, however, that predictions of whole body values from arm bag values will be accurate because: (a) only 2.6-3.2% of

SODIUM ION REGRESSION LINE

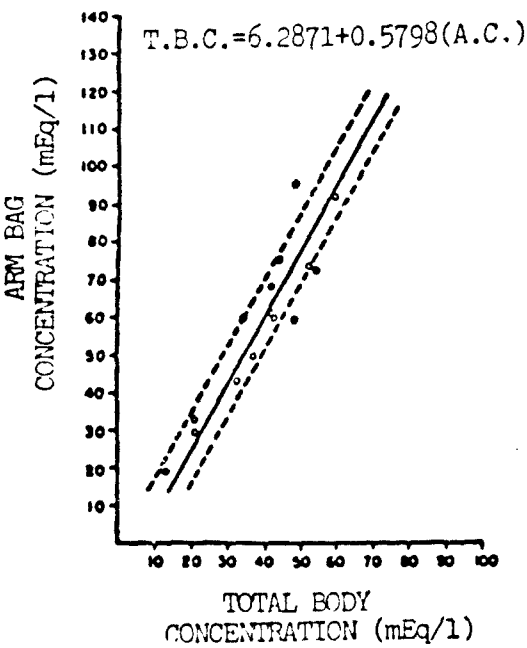
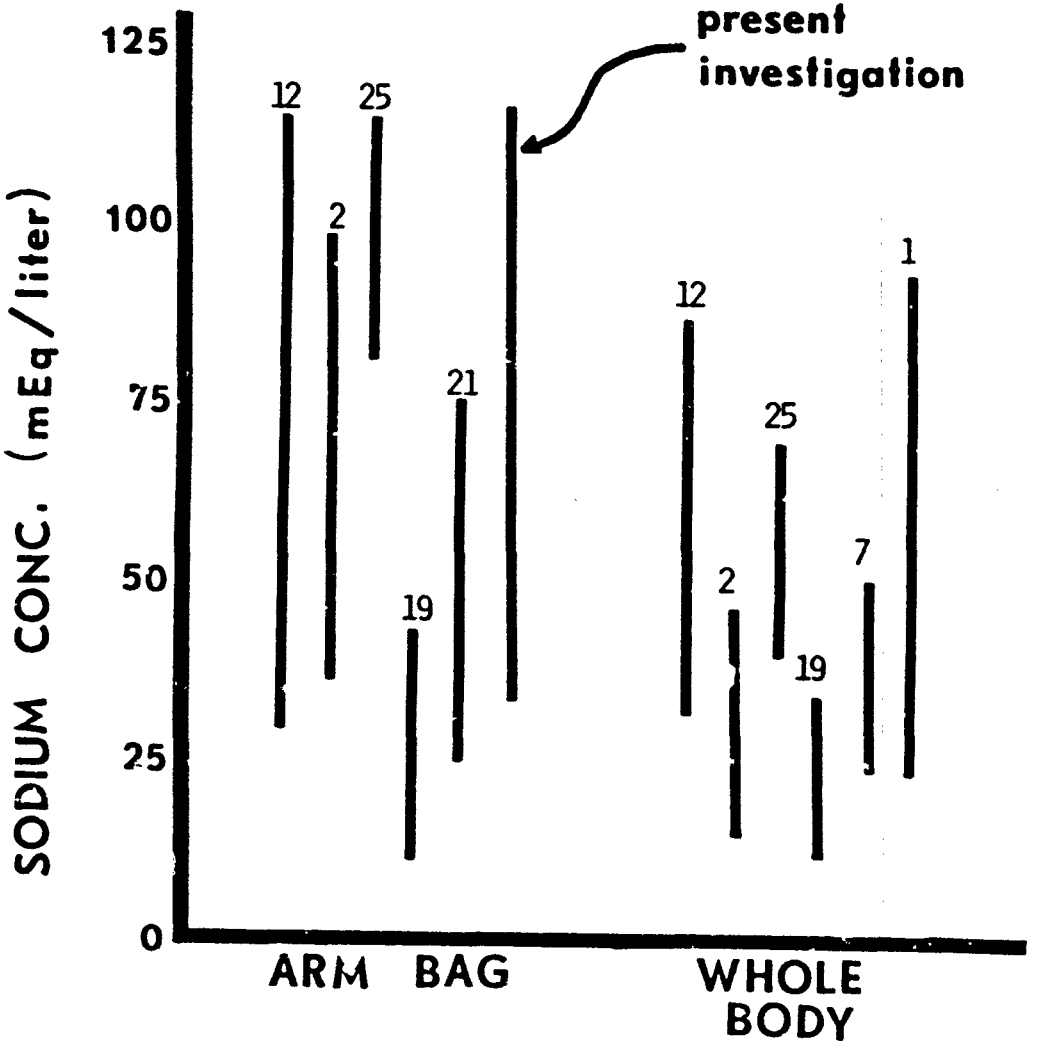


FIGURE 6 - ARM BAG VS TOTAL BODY SODIUM CONCENTRATION. REPRODUCED FROM THE JOURNAL OF GENERAL PHYSIOLOGY, 1953, VOLUME 32, PP. 736-745 BY COPYRIGHT PERMISSION OF THE ROCKERFELLER UNIVERSITY PRESS. SEE REF. NO. 12.



(NUMBERS ABOVE VERTICAL BARS CORRESPOND TO THE REFERENCE CITATIONS IN THE BIBLIOGRAPHY OF THIS REPORT.)

FIGURE 7 - SODIUM CONCENTRATIONS OF SWEAT PREVALENT IN THE LITERATURE.

the total skin surface area (Table 1) was inside the arm bag, (b) the sweat volume in the bag accounted for only 2.5% of the total body sweat loss, and (c) localized sweat rate differs at different sites on the body (Kuno, 1956, pp.192,294). Other sweat collection techniques (e.g. filter paper, sweat capsules, direct pipette collection) also share these disadvantages.

This is not meant to infer, however, that the arm bag sweat rate measurements presented in this report are inaccurate. Indeed, the literature demonstrates that they are in good agreement with other techniques. For example, Verde, Shephard, Corey, and Moore (1982) reported local sweat rates of 1.29-2.16 mg/cm^2/min, using a gauze pad technique. Gonzalez, Pandolf and Gagge (1974) reported localized sweat rates of 0.2-1.1 mg/cm^2/min, using a resistance hygrometry sweat capsule. These values compare very well with those of the present investigation (range: 0.3-1.6 mg/cm^2/min; Table 4, Figure 5).

Summary of Findings

The purpose of this series of experiments was to evaluate the use of polyethylene arm bags as a potential field expedient sweat collection technique and to observe the accompanying response of sweat glands to thermal stress. Six subproblems were defined. The following conclusions are numbered according to the appropriate subproblems:

1. A method has been described which cleans the forearm skin surface prior to testing and insures that no measurable electrolytes are found.
2. A method has been described which measures skin surface area with a day-to-day variance of less than 2%.
3. The forearm sweat collection technique demonstrated that 97.3% of a synthetic sweat mixture was recovered. Sweat Na^+ concentrations measured in these experiments were similar to values of arm bag sweat Na^+ observed in other laboratories.
4. A right arm vs left arm statistical comparison showed no statistical differences in forearm skin temperatures (Table 6), sweat rates (Table 5), Na^+ excretion or K^+ excretion (Table 4). Yet, because selected individuals demonstrated that the sweat rate of one arm may greatly exceed the other (Table 5), it is recommended that arm bag data from one arm not be used as a control for the other. It is further recommended that the same arm be used during repeated measures.

5. The placing of a polyethylene arm bag on the forearm of these subjects resulted in higher skin temperatures($p<.001$), higher relative humidity, greater Na^+ excretion ($p<.001$), and greater K^+ excretion ($p<.002$), when compared to OPEN trials. Four hypotheses were presented to explain electrolyte excretion differences.

6. Arm bag sweat rate is strongly correlated with total body sweat rate ($r = +0.83$), and arm bag sweat Na^+ concentration is strongly correlated with total body Na^+ concentration ($r = +0.90$). Arm bag sweat Na^+ tends to be somewhat higher than whole body Na^+.

Recommendations Regarding The Arm Bag Technique

1. We recommend that the arm be cleaned thoroughly prior to testing and that the skin surface area inside the bag be carefully measured.

2. As a result of the following:

   a. 97.3% of synthetic sweat sample was recovered
   b. arm bag sweat rate is proportional to whole body sweat rate (Fig. 5)
   c. arm bag sweat Na^+ concentration is proportional to whole body sweat Na+ concentration (Fig. 6)
   d. polyethylene arm bags increase skin temperature, Na^+ execretion, K^+ excretion and % RH,

we recommend that this technique be used as a relative measure of sweat rate and sweat Na^+ loss, but that it should not be used to

project absolute values for whole body sweat losses within (and between) subjects. This technique appears to be most useful in measuring the day-to-day or hour-by-hour changes of a subject.

3. During testing, we recommend that the sweat collection technique described in Subproblem 3 be followed closely.

4. Because the polyethylene arm bag increases the humidity of the forearm microenvironment (Figure 4), this technique may not be appropriate for certain situations in hot-dry environments.

5. When repeated measures are done, the same arm should be used during each test.

6. One arm should not be used as a control for the other.

7. Research from other laboratories indicates that arm bag trials longer than 80 minutes may be influenced by hidromeiosis.

8. Although this technique offers the advantages listed below, further tests should be conducted to evaluate arm bags in the field and to suggest modifications.

Military Relevance and Positive Features of this technique:

The arm bag sweat collection technique offers the following positive features:

1. The forearm is one of the few open skin areas for soldiers dressed in the Battle Dress Uniform. This technique, therefore, is appropriate for measuring mineral losses via sweating or for monitoring heat acclimatization in the field.

2. By nature, polyethylene arm bags resemble other vapor barrier/high humidity situations experienced during combat (e.g. MOPP IV configuration, closed-hatch tanks). Future research may indicate that arm bag specimens are representative of whole body sweat losses in these situations.

3. The arm is cleaned prior to testing and airborne contamination is minimal, a feature which is very attractive when considering blowing sand experienced during desert field maneuvers.

4. Although a flame photometer is required to analyze Na^+ and K^+ concentrations, samples are withdrawn by syringe and may be transported in the syringe or transferred to airtight containers.

5. Evaporative water losses are essentially nonexistent.

6. By using Na^+ or K^+ as a dilutional marker during the washdown and by thoroughly mixing the bag contents, the problem of losing a portion of the sweat sample in corners of the bag (or on the inner bag surface) is overcome. Previous arm bag methods have poured sweat into test tubes, thereby losing an unknown amount of sample.

7. The sample size collected during a 30 minute exercise bout is large (11.0-43.3 ml).

8. Collection materials are inexpensive, portable and disposable (bag, rubber bands, syringes, sample vials).

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Appendix A

This appendix describes the derivation of the formula used to calculate sweat volume in the Methods section (also see Subproblem 3). This calculation of sweat volume (Sv) may be done using either Na^+ or K^+ as a dilutional marker.

These variables are used in all equations:

Sv = volume of sweat (ml) produced inside arm bag during treadmill trials

Sc = concentration of Na^+ or K^+ (mEq/ml) in sweat

Sav = sweat aliquot volume (ml) removed for flame photometry analysis

Wv = volume (ml) of deionized water used to wash arm

Cv = combined volume (ml) of deionized water and sweat inside arm bag

Ccv = concentration of combined volume (Cv)

Equation 1: $Cv = Wv + Sv - Sav$

Equation 2: $$Sv = \frac{(Cv)\ (Ccv)}{Sc} + Sav$$

Substitute expression Cv into equation 2.

Equation 3: $$Sv = \frac{(Wv + Sv - Sav)\ (Ccv)}{S_c} + (Sav)$$

The objective of the following manipulations is to derive an expression which eliminates Sv from the right side of Equation 3.

Multiply both sides of Equation 3 by (Sc).

Equation 4: $[(Sv)(Sc)] = [(Wv + Sv - Sav)(Ccv)] + [(Sav)(Sc)]$

Equation 5: $[(Sv)(Sc)] =$
$[(Ccv)(Wv)] + [(Ccv)(Sv)] - [(Ccv)(Sav)] + [(Sav)(Sc)]$

Equation 6: $[(Sv)(Sc)] - [(Ccv)(Sv)] =$
$[(Ccv)(Wv)] - [(Ccv)(Sav)] + [(Sav)(Sc)]$

Factor the expression (Sc-Ccv).

Equation 7: $Sv(Sc-Ccv) = [(Ccv)(Wv-Sav)] + [(Sav)(Sc)]$

Equation 8: $Sv = \frac{[(Ccv)(Wv-Sav)] + [(Sav)(Sc)]}{Sc - Ccv}$ (SOLUTION)

Assumptions implicit in this derivation:

1. No loss of liquid sweat occurs at either the junction of

the bag and skin, or due to polyethylene bag permeability.

2. Any loss of vaporized sweat is inconsequential, relative to Sv.
3. The wash solution reaches a uniform concentration equilibrium inside the arm bag, prior to sampling of Sav.
4. Sweat production during the sampling procedure is inconsequential, when compared to Sv.

Disclaimers

The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as official department of the Army position, policy, or decision, unless so designated by other official documentation.

Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRDC Regulation 70-25 on Use of Volunteers in Research.

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