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INSECT BLOOD MEAL STUDIES USING RADIOSODIUM ^{24}Na AND ^{22}Na

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ABSTRACT. Rabbits were infused with 3.7 GBq (100 mCi) of [^{24}Na]Na ion in a 100-ml sodium carbonate solution. Beta particles were detected using a Tennelec Counting System®; background counts were 1.6 ± 1 counts per minute (cpm). Counts for one nanoliter of blood ranged from 22 to 30 cpm. Blood volumes on the mouthparts of tabanids following a 15-sec interrupted feeding were estimated to be 12.5 nl for *Tabanus fuscicostatus*, 10.8 nl for *T. nigrovittatus* and 6.12 nl for *Chrysops fuliginosus*. Estimates of the quantity of blood adhering to 22-gauge needles and insect pins (size 2) following a percutaneous intramuscular needle-stick were 8.8 ± 1.0 nl and 5.7 ± 1.8 nl, respectively. Mosquitoes, *Aedes aegypti* females, were fed to repletion with a ^{22}Na-artificial diet, and radioactivity was measured using a Packard Autogamma 5650. The estimated average blood meal size was 2.80 ± 0.94 µl.

INTRODUCTION

There are many reasons to determine the fate of vertebrate blood and its components after ingestion by a hematophagous arthropod. The potential of arthropods to transmit disease agents of man and other animals can depend on the amount of blood ingested. The economic impact of ectoparasites on animal production systems is also related to the volume of blood consumed by the arthropods. The amount of blood ingested can be directly related to fecundity (Colless and Chellapah 1960).

The size of a blood meal can be determined by a variety of techniques, but these methods are not without their drawbacks. Most of the precise techniques require sacrificing the arthropod and/or pooling samples: examples are enzyme-linked immunosorbent assay (ELISA) (Konishi and Yamanishi 1984, Foil et al. 1987), hemoglobin detection (Briegel et al. 1979), ion measurement (Fujisaki et al. 1987), protein determination (Bramhall et al. 1969) and radiotracer techniques (Bennett 1965, Redington and Hockmeyer 1976). The gravimetric technique (weighing the arthropod before and after feeding) is non-destructive (Yanovski and Ogston 1982), but immobilization of the arthropod for weighing prior to feeding can affect feeding behavior. Furthermore, some arthropods rapidly excrete water and salts as urine during feeding. If this process is ignored, results can vary dependent upon the amount excreted before the post-feeding weighing (Redington and Hockmeyer 1976).

Disease agents can be spread among animals by mechanical transmission. In this type of transmission, the agent is usually transferred between an infected and a susceptible host on or within the mouthparts of an arthropod during an interrupted feeding. There are many variables that control the probability of mechanical transmission (Foil 1989), but the amount of blood on the mouthparts is among the most important. The mouthparts of relatively large flies, e.g., horseflies, have been shown to have approximately 10 nl of blood residue per fly on the mouthparts following a feeding probe (Foil et al. 1987), but more accurate blood detection methods will be required to quantify the blood on the mouthparts of individual insects smaller than horseflies. This study was conducted to evaluate uses of ^{24}Na and ^{22}Na for detecting the residual volume of blood on the mouthparts of individual hematophagous insects and for estimating blood meal size of insects.

MATERIALS AND METHODS

Radiosodium ^{24}Na: ^{24}Na was produced in a research reactor at the Texas Engineering Experiment Station, Nuclear Science Center, Texas A&M University, College Station. It was calculated that 3.7 GBq (100 mCi) of activity would be necessary to achieve detection of 1-nl blood volumes from a 4.5-kg rabbit. At 1 MW power, the Na salts were placed in the reactor core at a position surrounded on 3 sides by nuclear fuel rods and on the 4th side by graphite. The thermal neutron flux was 2×10^{13} n-cm^{-2} sec^{-1}. The neutron flux coupled with the 2-h-long irradiation time necessitated quartz encapsulation of the Na salts.

The delivery of 3.7 GBq of ^{24}Na to Baton Rouge, LA, from College Station, TX (*ca.* 580 km), required producing 2.5 times the necessary amount of ^{24}Na due to the 15-h half-life of ^{24}Na. To obtain 3.7 GBq of ^{24}Na activity at 1000 h at the LSU laboratory, 0.6 g of NaCl (0.24 g Na) or 0.8 g of $NaHCO_3$ (0.22 g Na) was irradiated for 4 h between 1000 and 1400 h on the previous

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day, yielding 9.25 GBq (250 mCi) [^{24}Na]$NaHCO_3$ hydrogen or chloride carbonate at the end of neutron bombardment.

In a feasibility study, ^{24}Na was produced from NaCl. Intense gamma radiation produced by the $^{37}Cl(n,\gamma)^{38}Cl$ reaction delayed the packaging and shipment of the radioisotope from Texas. The beta radiation from ^{35}S from the $^{35}Cl(n,p)^{35}S$ reaction created potential contamination problems in the laboratory and delay of the reuse of tools and equipment due to the 87.2-day half-life of ^{35}S. Results reported in this paper are from ^{24}Na tracer produced from $NaHCO_3$, which produced no interfering radionuclides.

Radioisotope administration and blood collection: All animals were allowed water and food *ad libitum* and treated according to the policies set forth in the *NIH guide for the care and use of laboratory animals.* On July 12, 19 and 26, 1989, [^{24}Na]$NaHCO_3$ was injected into individual 4.5-kg rabbits. The irradiated quartz vial containing [^{24}Na]$NaHCO_3$ was positioned above 100 ml of physiological saline and crushed with pliers. The [^{24}Na]$NaHCO_3$ solution was mixed using a magnetic stirrer and drawn through a 0.45-μm filter (Costar, Cambridge, MA) by a peristaltic pump (Buchler Instruments), delivering 5 ml/min into the lateral vein of the right ear of a restrained rabbit. Injected rabbits were kept behind 5.1 cm of lead shielding to minimize radiation exposure to project personnel. Hourly blood samples were collected from the left ear by venipuncture using 22-gauge needles. Once a small droplet of blood appeared in the hub, the needle was removed and the droplet was used to charge a 1-μl capillary tube for direct counting and a 4-μl tube, which was used for serial 10-fold dilutions.

Tabanids, needles and pins: Tabanus fuscicostatus Hine females were collected at the Thistlethwaite Wildlife Management Area in St. Landry Parish, LA (Foil et al. 1990), and *T. nigrovittatus* Macquart females were collected in St. Mary Parish, LA, 2 days prior to the experiments using canopy traps (Hribar et al. 1991) baited with carbon dioxide. *Chrysops fuliginosus* Wiedemann were collected with entomological nets in St. Tammany Parish, Louisiana. Flies were transported and maintained in holding containers (Zyzak et al. 1989). Flies were released into screened cages and individually transferred in plastic cups to the shaved abdomen of control and injected ([^{24}Na]$NaHCO_3$) rabbits. Flies were allowed to feed on injected rabbits for 15 sec and then were transferred to a disposable petri dish by using wooden applicator sticks covered with Sticky Stuff® (Olson Products, P. O. Box 1043, Medina, OH 44258). The head was quickly removed from the thorax with a clean razor blade, and the mouthparts were subsequently dissected from the head; the fascicle, the palpi and the labium were included (Foil et al. 1987). Each dissection was done with separate scalpels to eliminate cross contamination. Intramuscular needle-stick of the abdominal muscles of an injected rabbit with 10 insect pins (size 2) and 10 needles (22 gauge) was conducted on July 12, 1990.

Five *T. fuscicostatus* females were allowed to engorge to repletion on an injected rabbit and were maintained individually in 5-ml (75 × 12 mm) plastic tubes stoppered with cotton for 1 h on July 26, 1989. Flies were then transferred to new vials, and the original plastic vials and insects were counted separately. The volume of blood ingested by *T. fuscicostatus* females was estimated by adding the counts of the flies to the counts of the original tubes.

Mosquitoes: Radiosodium ^{22}Na (Dupont, Boston, MA) was used in the *in vitro* feeding system; ^{22}Na is a positron emitter with a 1.27-MeV gamma ray of 100% intensity, annihilation energy of 0.511 MeV of 180% intensity and a 2.6-year half-life. A 10-μl aliquot of 37 MBg (1 mCi) [^{22}Na]NaCl from a 1-MC ^{22}NaCl per ml solution was added to an artificial blood solution (Kogan 1990) in a Falcon 3037 organ culture dish containing a stir bar. The 3.1-ml well of the dish was covered with an artificial membrane (Butler et al. 1984). The Falcon dish was placed at the bottom of a 300-ml cardboard container which had a clear plastic top and was on a magnetic stirrer. *Aedes aegypti* (Linn.) mosquitoes were transferred by groups of 5 to 10 females into this feeding chamber. Every hour, adenosine triphosphate (ATP) was added to the artificial blood solution at a rate of 1 μl of 1.0 mM concentration per ml of artificial medium (Kogan 1990). Engorged mosquitoes were aspirated individually into 5-ml plastic tubes, secured at the bottom 10% of the tube with a cotton plug and counted.

Detection: All blood dilution samples, the engorged flies and their vials containing urine were assayed for 1 min with a Packard Auto Gamma 5650® scintillation counter. Samples of blood dilutions in the 10^{-5}- and 10^{-6}-ml (10 and 1 nl) range were poured onto shallow stainless steel planchets (5.2-cm diam) with a detergent surfactant to assure even sample distribution. These blood samples, mouthparts, heads, needles and insect pins were counted for 1 min on a Tennelec α/β Counting System®, which analyzes the beta particle (E_{max} = 1.390 MeV; 100% intensity) of the decaying ^{24}Na (LB 5100, Tennelec, Inc., Oak Ridge, TN).

Dosimetry: Personnel were monitored for radiation exposure during experiments with radioisotope ^{24}Na by 3 independent personnel dosim-

Table 1. On different dates blood samples from 3 4.5-kg rabbits, each infused with 3.7 GBq of [^{24}Na]$NaHCO_3$, were assayed for radiotracer (^{24}Na) hourly from 2 to 7 hours. There were 3 replicates for each assay and all values were corrected for decay and background.

Date	Samples (*n*)	cpm/nl ± SD blood[1]	Range (cpm/nl)
July 12	6	24.3 ± 1.7	22.0–26.0
July 19	6	27.5 ± 1.9	25.0–29.6
July 26	5	26.0 ± 1.7	24.0–28.3

[1] Values averaged over the duration of the experiment.

eters plus frequent inspections by a radiation safety officer with a portable ion chamber. A pocket dosimeter was used to keep real-time records on exposure. When readings on this dosimeter reached 0.4 mGy (40 mrem), the worker was rotated out of the immediate field of exposure. Body badges were used to measure whole-body exposure and finger ring-badges were used to measure extremity doses (Tech/Ops Landauer, Inc., Glenwood, IL). Single ring-badges normally were worn according to handedness, but when ring-badges were worn by a person on both hands, the higher ring value was used in computing the dose. All dosimeters were evaluated weekly (Tech/Ops Landauer, Inc.) for radiation exposure.

RESULTS

Readings for the rabbit blood were consistent down to 1 nl when corrected for background and radioactive decay for the duration of the 3 experiments using [^{24}Na]$NaHCO_3$ (Table 1). The Tennelec Counting System had detection efficiency approaching 60% due to back scatter and low background (1.6 + 1.0 cpm) that allowed accurate low-level counting in short counting times.

Blood volumes on the mouthparts of tabanids following a 15-sec interrupted blood meal were estimated to be 12.5 ± 6.7 (SE) nl in 12 *T. fuscicostatus* females, 10.8 ± 4.3 nl in 10 *T. nigrovittatus* females and 6.12 ± 1.46 nl in 14 *C. fuliginosus* females. The heads of all the flies that fed 15 sec on injected rabbit had from 0.2 to 611.7 nl of blood, indicating that all specimens had ingested blood. Estimates of the quantity of blood adhering to 22-gauge needles and insect pins (size 2) following intramuscular needle-stick were 8.8 ± 1.0 nl and 5.7 ± 1.8 nl, respectively. Control mouthparts, pins and needles did not have above background counts.

The blood meal size estimates for the 5 flies fed to repletion and the percentage of the radiation excreted in the original tube (in parentheses) were 46.9 µl (38%), 50.8 µl (37%), 31.5 µl (24%), 42.4 µl (6%) and 46.8 µl (0.9%).

The ^{22}Na–artificial blood mixture had 41,799 cpm/µl. For 47 fed *Ae. aegypti,* the average blood meal size was estimated to be 2.80 ± 0.95 (SD) µl (range 1.19–4.71 µl).

Personnel dosimetry: The number of research personnel varied between 9 and 13 during the 3 experiments with ^{24}Na-injected rabbits. The average exposures and ranges (in parentheses) per person per experiment were 0.35 mGy (0.1–0.9) for whole-body and 6.30 mGy (0.30–34) for extremities. The highest whole-body exposure received was 0.9 mGy (90 mrem) and the highest extremity exposure was 34 mGy (3,400 mrem).

DISCUSSION

Previously, the most sensitive blood volume estimate method used to calculate blood residue on the mouthparts of *T. fuscicostatus* was the ELISA technique, which detected blood volume values of 10 ± 5 nl from pools of 10 mouthpart samples (Foil et al. 1987). The use of ^{24}Na allowed detection of the residual amount of blood on the mouthparts of individual tabanids. The major limitation of using ^{24}Na for detection of blood on mouthparts is the fact that sodium from the blood meal is transported into the hemolymph after feeding.

In trials of mechanical transmission of eastern equine encephalitis virus to chicks, no significant differences were found between insect pins and mosquitoes (Chamberlain and Sudia 1961). The residual amount of blood remaining on insect pins was found to be approximately 6 nl in our study. This value is over 1,000 times higher than previous estimates for mosquito mouthparts (between 10^{-9} and 10^{-10} ml; Miike 1987). Therefore, further research will be needed to determine the actual amount of blood transferred to a 2nd host by insect pins and mosquitoes. Our results indicate that studies comparing mechanical transmission trials using 22-gauge needles and horseflies would be warranted.

The range of estimated blood meal sizes for the horseflies fed to repletion on rabbits was 31.5–50.8 µl. These results are comparable to previous estimates of blood meal size (44.9 mg) for *T. fuscicostatus* females fed on cattle (Foil et al. 1990).

This technique of estimating the initial blood meal size for insects held alive without concerns about excretion may be extremely valuable for quantitative studies on the development or propagation of agents in insect vectors. The amount of ^{24}Na required to inject rabbits for studies on

blood ingestion and sodium excretion by blood-feeding insects would be 2 orders of magnitude less than that used in this study.

The Federal Code of Regulations (10CFR20) allows occupational exposure to radiation that does not exceed 5 cGy (5,000 mrem) per year or 1.25 cGy (1,250 mrem), whole body, per calendar quarter and 75 cGy (75,000 mrem) per year or 18.75 cGy (18,750 mrem), extremities, per calendar quarter. The 3 highest recorded whole-body exposures were 0.21 cGy (210 mrem), 0.19 cGy (190 mrem) and 0.18 cGy (180 mrem), which represent 17, 15 and 14%, respectively, of the quarterly Federal allowance.

The estimate of the average volume of artificial blood ingested by *Ae. aegypti* was 2.80 μl; Redington and Hockmeyer (1976) fed *Ae. aegypti* on a ^{144}Ce-labeled rhesus monkey and obtained an average blood meal estimate of 2.91 μl. As previously stated, the value of a nondestructive blood meal size estimate is critical to the evaluation of survival of agents ingested in arthropods. In addition to allowing use of totally *in vitro* systems, artificial blood can be screened for antimicrobial activity in advance of assays.

The use of ^{24}Na, under the proper management of personnel, for animal injections can be recommended when blood volume estimates in the nanoliter range are required. Radiosodium [^{24}Na]Na is an acceptable tracer to be used in *in vivo* systems because it has a short half-life (15 h) with a beta-ray radiation intensity of 100%. The short 15-h half-life of ^{24}Na eliminates disposal costs and allows the reuse of all equipment after one week. For comparison of *in vivo* to *in vitro* systems, ^{22}Na is commercially available and has a long half-life (2.6 years), allowing its use over an extended period.

Studies comparing blood residues following needle-stick of animals and membrane systems could be valuable in developing *in vitro* systems to measure iatrogenic transmission of agents. Due to post-prandial movement of sodium, studies to estimate blood meal residues on mouthparts are limited to probing insects. Blood meal volume estimates of live insects can be made at different times post-feeding without sacrificing specimens.

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REFERENCES CITED

Bennett, G. F. 1965. The effect of phosphorus^{32} on the fecundity of *Aedes aegypti* and its use in determining blood meal volumes. Mosq. News 25:465–469.

Bramhall, S., N. Noack and J. R. Loewenberg. 1969. A simple colorimetric method for determining protein. Ann. Biochem. 31:146–148.

Briegel, H., A. O. Lea and M. J. Klowden. 1979. Hemoglobimetry as a method for measuring blood meal sizes of mosquitoes (Diptera: Culicidae). J. Med. Entomol. 15:235–238.

Butler, J. F., W. R. Hess, R. G. Hendris and K. H. Holscher. 1984. *In vitro* feeding of *Ornithodoros* ticks for rearing and assessment of disease transmission. Med. Vet. Acarology 2:1075–1081.

Chamberlain, R. W. and W. D. Sudia. 1961. Mechanism of transmission of viruses by mosquitoes. Annu. Rev. Entomol. 6:371–390.

Colless, D. H. and W. T. Chellapah. 1960. Effects of body weight and size of bloodmeal upon egg production in *Aedes aegypti* (Linnaeus) (Diptera: Culicidae). Ann. Trop. Med. Parasitol. 54:475–482.

Foil, L. D. 1989. Tabanids as vectors of disease agents. Parasitol. Today 5:88–96.

Foil, L. D., D. J. Leprince and R. L. Byford. 1990. Sublethal effects and mortality of tabanids (Diptera: Tabanidae) induced by fenvalerate treatment of cattle. J. Entomol. Sci. 25:294–302.

Foil, L. D., W. V. Adams, J. M. McManus and C. J. Issel. 1987. Bloodmeal residues on mouthparts of *Tabanus fuscicostatus* (Diptera: Tabanidae) and the potential for mechanical transmission of pathogens. J. Med. Entomol. 24:613–616.

Fujisaki, K., T. Kamio, S. Kitaoka and T. Morii. 1987. Quantitation of the bloodmeal ingested by *Culicoides arakawae* (Diptera: Ceratopogonidae). J. Med. Entomol. 24:702–703.

Hribar, L. J., D. J. Leprince and L. D. Foil. 1991. Design for a canopy trap for collecting horse flies (Diptera: Tabanidae). J. Am. Mosq. Control Assoc. 7:657–659.

Kogan, P. H. 1990. Substitute blood meal for investigating and maintaining *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 27:709–712.

Konishi, E. and H. Yamanishi. 1984. Estimation of blood meal size of *Aedes albopictus* (Diptera: Culicidae) using enzyme-linked immunosorbent assay. J. Med. Entomol. 21:506–513.

Miike, L. 1987. Do insects transmit AIDS? Office of

Technology Assessment Staff Paper no. 1, U.S. Gov. Printing Office, Washington, DC.

Redington, B. C. and W. T. Hockmeyer. 1976. A method for estimating blood meal volume in *Aedes aegypti* using a radioisotope. J. Insect Physiol. 22: 961–966.

Yanovski, A. D. and C. W. Ogston. 1982. Sex differences in size of the blood meal in the bed bug *Cimex hemipterus* (Hemiptera: Cimicidae). J. Med. Entomol. 19:45–47.

Zyzak, M. D., D. J. Leprince, R. L. Byford and L. D. Foil. 1989. Toxicity of fenvalerate to five unfed species of tabanids and engorged *Tabanus fuscicostatus* Hine (Diptera: Tabanidae). J. Entomol. Sci. 24: 572–578.