ELEMENTS AS MARKERS IN STUDIES OF HOST SELECTION AND DISPERSAL BY ARTHROPOD PARASITES OF VERTEBRATE HOSTS

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ABSTRACT

We review the development and application of elemental marking in circulating blood of vertebrate hosts as a method for studying feeding behavior, host selection and dispersal of their arthropod parasites. The alkali metals rubidium and cesium which do not normally contaminate parasites such as mosquitoes may be readily detected in these insects when they imbibe blood from previously inoculated host animals. Thus, marking experiments determine which vertebrates serve as hosts for a particular insect. By uniquely marking hosts, each with a different alkali metal, mosquitoes that parasitize more than one host in a single evening may be identified. In a different experimental context, elements of the lanthanide series permanently mark adult and immature stages of tsetse when they are directly applied to the cuticle of these insects. Herein, we note that elemental markers remain largely unexploited in published studies of medically important arthropods, and that most papers focus on the development of technique. Yet simple experiments that incorporate elemental marking incisively determine hosts and their utilization.

INTRODUCTION

The idea that relatively uncommon alkali metal elements could be administered to vertebrate hosts, and then be used to identify organisms which have imbibed blood from these hosts, derives from field studies of the dispersal of herbivorous insects that originated from plants experimentally contaminated with rubidium (Berry et al. 1972, Stimmann 1974, Van Steenwyk et al. 1978a, 1978b). This simple paradigm finds experimental application in diverse biological interactions of host organisms and their parasites or herbivores.

The vertebrate source of blood in the gut of a parasitic arthropod usually may be identified by various serological means (Washino and Tempelis 1983). Yet these techniques often fail to differentiate blood antigens taken from closely related hosts (Boreham and Garret-Jones 1973, Boreham and Lenahan 1976). However, an elemental marker administered to particular host animals and later assayed in locally collected parasites could resolve which host these arthropods tend to parasitize.

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Additionally, injecting different elements into each host could determine if individual arthropods parasitized more than one host. Such multiple feeding behavior would greatly increase the rate arthropod-borne pathogens spread in a vertebrate host population (Klowden 1988). Thus, elemental marking could provide elusive data which are critical to understanding the dynamics of pathogen transmission and perpetuation (Scott and Grumstrup-Scott 1988, Scott et al. 1983).

Herein we review those studies incorporating elemental marking in animal-parasite systems and describe how such techniques were developed and applied. In particular we attempt to show how elemental marking may augment studies of host association and pathogen transmission.

DEVELOPMENT OF MARKING WITH ALKALI METALS

To identify suitable materials to mark the blood of host animals and, therefore, insects which imbibe that blood, Kimsey and Kimsey (1984) screened various compounds and label carriers and selected the element rubidium, which had been previously used to mark plant feeding insects in the field (Van Steenwyk et al. 1978a, 1978b). Accordingly, Kimsey and Kimsey (1984) determined: (1) the largest intraperitoneal (i.p.) dose of isotonic rubidium administered to Swiss Webster mice, *Mus musculus* L., Rhode Island Red chicks, *Gallus gallus* L., and Western Fence swifts, *Sceloporus occidentalis* Baird and Girard, without inducing gross behavioral modification; (2) the rate of loss of rubidium from the peripheral blood of these hosts; and (3) how rubidium levels in mosquitoes that have parasitized marked Japanese quail, *Coturnix coturnix* L., change with the period host quail have been marked and the length of the interval following the mosquito blood meal.

To measure the highest dose of rubidium which could be administered to hosts with no acute effect, they injected mice, chicks, and lizards with single i.p. isotonic doses of rubidium chloride (RbCl) at rates of 0, 500, 750, 1000, 1250, or 1500 mg/kg. Following injection, changes in behavior and deaths among these animals were recorded hourly for 12-h and subsequently at 24-h intervals. No behavioral depression or acute effect occurred in animals injected with 500 mg/kg RbCl. Depressed activity occurred immediately in all the 750 mg/kg groups, lasting less than 3 h in chicks and mice and as long as 5 h in lizards. Acute signs developed in animals injected with $\geq$ 750 mg/kg; longer periods of depression and deaths occurred in 1000 mg/kg groups. Thus, the 500 mg/kg dose was used in all subsequent studies.

To discover how long rubidium would circulate in the blood of host animals, Kimsey and Kimsey (1984) monitored the concentration of rubidium for 7 days in injected lizards, mice, and chicks, by assaying 3 μl blood samples taken daily, a volume close to that imbibed by small mosquitoes (2.74 μl) (Gooding 1972). Sample preparation followed standard digestion procedures (Smith 1953); analysis was by standard flame emission spectrophotometry. They detected no rubidium in the blood of these animals before these experiments were begun. Following injection, the most rapid loss occurred in mice; lizards and chicks lost rubidium at relatively slower rates, although chicks had the highest average blood concentrations on the 7th day. Reconstituted from log transformations, these mean 7th-day concentrations were: mice 33.9, lizards 109.7, and chicks 117.5 ppm. Thus, rubidium circulated in the blood of diverse animals for at least a week following i.p. injection of 500 mg/kg.

By exposing marked and unmarked Japanese quail to the bites of *Culex tarsalis* Say mosquitoes, Kimsey and Kimsey (1984) then sought to determine if rubidium contaminated mosquitoes. Each day following injection, a marked and a nonmarked

bird were introduced unrestrained into separate cages in the evening, along with 20 female *Cu. tarsalis*. Beginning the following morning, one blood-fed mosquito was killed each day and later subjected to rubidium analysis. They found rubidium in all 31 blood-fed mosquitoes (Table 1). Only one blood-fed mosquito died, 7 days after feeding on a quail injected 5 days earlier. Therefore, rubidium did not seem to be acutely toxic to mosquitoes. Additionally, there was little indication of daily decreasing rubidium concentration in marked mosquitoes. Rubidium was not detected in any mosquito that parasitized nonmarked quail, nor was it found in any nonfed mosquitoes from the feeding experiments. Thus, mosquitoes fed on marked hosts may easily be distinguished from those which have obtained blood from nonmarked hosts.

TABLE 1[a]. Rb^+ (ppm) in Mosquitoes (Blood Meal and Hemolymph) as a Function of Days After Feeding on Marked Quail (Rows) and Days After Quail Were Marked (Columns). Quail Were Injected on Column Day 0.

	Day Mosquito Fed										
Day Mosq. Killed	1	2	3	4	5	6	7	8	9	10	11
1	82	21	105	125	[b]	47	36	110		36	54
2			37	52	61	201		32		48	49
3	66		34	26	16[c]	93					
4			52	26	98						
5	67		27		27						
6[c]				103,64							
7[c]				35,16[d]							

[a] Taken from Kimsey and Kimsey 1984.
[b] Lost sample.
[c] Two mosquitoes killed per day.
[d] Concentration close to maximum resolution of atomic emission procedure.

To determine if marker circulating in a host altered the ability of mosquitoes to locate blood, the daily numbers of mosquitoes that parasitized marked and nonmarked quail were compared. Of 440 mosquitoes, 37 fed on control and 31 fed on rubidium-marked quail. There was no significant difference in the frequency of mosquitoes feeding on rubidium-marked quail and nonmarked quail ($P=0.62$). Taken as a whole, this preliminary study confirmed the idea that alkali metals could be used as experimental tracers to determine the relationship between insect parasites and their vertebrate hosts.

MARKING TO IDENTIFY INSECT PARASITES OF VERTEBRATE HOSTS

The first application of alkali metal marking of vertebrate hosts and their insect parasites arose in studies of the insect vectors of parasites that cause malaria in lizards in central Panama (Kimsey 1991). Entomological inoculation rates, a

critical parameter in such studies, derive in part from a list of insects which feed on the vertebrate host, in this case a lizard, *Anolis limifrons* Cope . Such a list usually develops by identifying antigens in blood meals taken from field-collected insects. But low temperature preservation of blood meal samples taken from such insects proved impossible, and blood antigens often degraded within the insect before collection. Additionally, creating antibodies that could distinguish *A. limifrons* blood antigen from those of a complex of closely related species seemed an expensive and unfeasible alternative. Elemental marking seemed a more reasonable approach.

To determine what insects fed on the lizard host, Kimsey (1991) systematically captured, marked with rubidium, and released lizards weekly in an established study site in the Zona de Canal. A 3 μl blood sample was taken from each lizard followed by an intraperitoneal injection of isotonic RbCl at a rate of 500 mg/kg. Blood-fed mosquitoes and sand flies were collected from tree buttress cavities in the plot every second day. Prior to the first marking, blood samples collected from 20 lizards and 150 field-derived sand flies contained no measurable levels of rubidium. Insects and blood samples were prepared and analyzed in the standard way (Smith 1953, Kimsey and Kimsey 1984). None of 48 blood-fed sand flies taken over the 6-wk duration of the study contained rubidium mark. Of 11 blood-fed *Culex* L. mosquitoes, 2, including 1 *Culex* (*Anoedioporpa*) spp. and 1 *Culex* (*Melanoconian*) spp. contained levels of rubidium marker (67 and 91 ppm, respectively). Later studies on related parasites and lizards in Florida confirmed a *Culex* (*Melanoconian*) *erraticus* Meigen vector (Kline et al. 1988).

IDENTIFYING MULTIPLE BLOOD MEALS

Particular evidence suggests that many mosquitoes may take a number of small blood meals during each reproductive cycle and are likely to take them in rapid succession from clusters of the same host species, such as colonial nesting birds (Washino and Tempelis 1983, Romoser et al. 1989). For example, the mosquito *Culiseta melanura* (Coquillett) seems to transmit Eastern Equine Encephalitis virus (EEE) among flocking birds, the reservoir hosts of EEE, at a greater rate than accounted for by a single host contact per reproductive cycle. Here, employing serology to distinguish these hosts becomes completely intractable; antibodies cannot distinguish how many such birds contribute to the blood meal of an engorged mosquito. Because the rate of spread of EEE virus depends on how often infected mosquitoes contact susceptible birds, multiple feeding would directly increase the vectorial capacity of a mosquito (De Foliart et al. 1987, Spielman and Rossingol 1984).

To discover if *Cs. melanura* takes multiple meals, Anderson et al. (1990) designed semicontrolled field experiments where paired hosts, each circulating a different alkali metal in the blood, were exposed to the bites of these mosquitoes. In parallel with Kimsey and Kimsey (1984), they first sought to ascertain (1) if rubidium and cesium markers could be distinguished separately and combined in *Aedes aegypti* L. mosquitoes and chickens, and (2) whether these elements affected the ability of *Culex quinquefasciatus* Say mosquitoes to obtain blood from a host. They then analyzed field derived *Cs. melanura* taken from locations where chickens marked with cesium or rubidium were caged.

Anderson et al.(1990) determined that rubidium and cesium would remain circulating in the blood of chickens for 3 days at levels sufficient to mark feeding *Ae. aegypti* mosquitoes if injected with 500 mg/kg of rubidium, or 780 mg/kg cesium.

Analysis of *Ae. aegypti* and *Cs. melanura*, that sequentially parasitized rubidium or cesium marked hosts, revealed both elements. When caged overnight with pairs of chickens, one marked with rubidium and the other with cesium, no difference could be measured in the number of blood-fed *Cu. quinquefasciatus* marked with either rubidium or cesium the following day. Interestingly, 19% of these mosquitoes contained both elements. Thus, laboratory evidence strongly suggested that a multiple marker element experiment could be used to identify multiply-fed mosquitoes.

The field study site near the Hockamock Swamp at Raynham, Massachusetts, was characterized by hardwood trees and sparse understory. A total of 12 chickens, 6 injected with 500 mg/kg rubidium and 6 with 780 mg/kg cesium were caged in pairs, one marked with rubidium and the other with cesium. The cages were placed in a small clearing and surrounded by resting boxes that faced the cages. The close proximity of the chickens in this experiment simulated the colonial nesting of many natural bird hosts of *Cs. melanura* (Scott 1988). The chickens were left overnight for 2 nights and resting mosquitoes were collected from the boxes and surrounding vegetation each morning. Of 101 mosquitoes collected in their 1987 experiment, Anderson et al. (1990) determined that 34 contained rubidium, 16 cesium, and 5 both elements. Another experiment in 1988 yielded 620 engorged mosquitoes, 95 with rubidium, 31 with cesium and 10 with both, thus revealing that *Cs. melanura* may take blood from different hosts in rapid succession.

MARKING WITH ELEMENTS OF THE LANTHANIDE SERIES

In attempting to identify released tsetse, *Glossina morsitans morsitans* Westwood, in dispersal studies, Curtis et al. (1973) proposed "activable elements" of the lanthanide series, specifically lanthanum, europium and dysprosium as markers. In this case, assaying marked insects requires that these be exposed to a thermal neutron source such as a nuclear reactor, creating radioisotopes of such elements, which then may be identified with a suitable detector. This constitutes an interesting departure from alkali metal marking and flame photometric analysis, and others later applied activable elements successfully in the marking of bark beetles (Naumann-Etienne et al. 1977). Curtis et al. (1973) found that 10^{-3} mol/l europium or dysprosium (chlorides) administered to adult tsetse in the blood meal could be easily detected upon analysis of the flies.

To further develop activable elements as markers for tsetse, in laboratory experiments Hamann and Iwannek (1979) marked *Glossina palpalis palpalis* Robineau-Desvoidy with europium, dysprosium or lanthanum by injecting flies, adding lanthanum to blood presented artificially to flies as food, dipping pupae in solutions of lanthanum with and without dimethylsulfoxide (DMSO) and spraying such solutions on adult tsetse. These experiments were terminated after 6 days. At least 70% of elements remained in injected flies for this period; however, 3 days following oral uptake of the same compounds, only 16% remained. Although it seemed that more adult flies contained marker when dipped as pupae in solutions with higher concentrations of DMSO, the proportion marked was not consistent enough for use in field studies. Lanthanum significantly contaminated sprayed flies for 6 days and, coincidentally, 10% of this element on the surface of male flies transferred to female flies during mating contact. In their experiments with these elements, Hamann and Iwannek (1979) ruled out the use of dysprosium because the isotopes created during analysis decay too rapidly for easy analysis, a problem not

encountered with europium or lanthanum. Although lanthanide marking presents an intriguing alternative to alkali metals, activation with neutrons from a nuclear reactor is not feasible for most studies; perhaps flame spectrophotometric assays for lanthanide elements present a more available form of analysis (also see Stimmann, Akey and Burns, this supplement).

CONCLUSIONS

It is clearly unfortunate that elemental marking has not found wider application in studies of medically important arthropods. Numerous technical advantages and very low cost characterize alkali metal marking, but among the most important advantages include low toxicity and little or no apparent behavioral modification to either host or parasite (Anderson et al. 1990, Kimsey and Kimsey 1984, Stimmann et al. 1973). Nor does there seem to be low gustatory rejection thresholds to these elements in diverse insect groups (Frings 1946, Pappas 1978). Hamann and Iwannek (1979) found neither problem in their use of the lanthanides.

How this technology may be applied seems to be restricted only by the mind of the researcher. For example, in field surveys such markers could be used to determine how far and where insects disperse after feeding on a marked host, or in the case of ticks and mites, where these arthropods detach from the host. By marking such arthropods and analyzing artificial media from which they have imbibed food, the amount they salivate or regurgitate may be quantified in the laboratory. Thus, in addition to the studies reviewed here, elemental marking may critically test ideas about the epidemiology of particular arthropod-borne diseases and the mechanisms by which pathogens that cause such disease may be transmitted.

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