Ants, a source of ant-igenic activity? A case history

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Abstract

The suggestion that ants may produce ABH antigen-like substances was tested, using Argentine ants (*Iridomyrmex humilis*). Prolonged tracking by these ants did not leave ABH antigen-like substances on filter paper, and did not act to produce an ABH antigen-like reaction on non-secretor saliva. An extract of the head and thoracic regions showed activity that mimicked that of an A secretor. Lesser amounts of A antigen-like material were found in other circumstances. *Key words*: Blood grouping; Ants; Secretor status.

Journal of the Forensic Science Society 1987; 27: 241–245
Received 2 September 1986

Introduction

The experiment presented in this paper may seem of trifling importance to the criminal justice system, or to biological science. Not even entomologists have been interested enough prior to this time to learn whether or not ants exhibit ABH antigenic activity. Unfortunately, this matter became of critical importance in two murder cases.

In 1981, a laboratory examined a knife which was believed to be stained with blood of a person accused of murder. The laboratory reported that "the knife stains are human blood and exhibited antigenic activity indicative of type A and type B or type AB". The two accused were both type A.

The prosecutor sought a way to explain the type B in the stain. He engaged a criminalist from a different laboratory who re-examined the evidence and also noted from a police report that ants had been observed on the bloodstained knife. It was estimated that the knife had lain unattended for about 12 hours before it had been found.

At trial, the criminalist testified in regard to experiments he had done to test his theory that ants had been the cause of the type B antigen activity in the blood on the knife. He permitted ants to walk for approximately 30 minutes on a cloth stained with wet type O blood and on an unstained control cloth. He also collected some red ants and black ants. Ten to fifteen live and dead ants of each color were crushed, extracted and centrifuged, and the supernatants were subjected to antigen testing. He testified that black ants gave group B reactions, but that red ants gave negative results. ABO grouping tests on the samples from the two cloths were negative for A or B antigenic activity.

A year or so later, two men went on trial in the same jurisdiction charged with the rape and murder of twelve young women. The nude body of one of the victims had been found on a grassy hillside. Analysts from the crime laboratory determined that the victim's blood type was ABO group A. They tested a swab that had been taken from the area of the victim's right nipple, and reported that a chemical test "gave a positive result indicating the possible presence of saliva" and that "the material exhibited antigenic activity indicative of type B (ABO system)". Both accused were type AB non-secretors. Police photographs at the scene showed ants trailing across her face and body.

The defence undertook to prepare a challenge to any prosecution theory that ants could have produced the B antigenic activity. At the request of the defence, an experiment was set up at the University of California at Berkeley.

Experimental design

The experiment was designed to answer three questions: whether the pheromone or chemical trail left by ants would produce an ABH antigen-like reaction; whether the pheromone of ants would act upon the saliva of a non-secretor to produce an ABH-like reaction; and whether the extracts from macerated ants would produce an ABH antigen-like reaction. Samples and appropriate controls were tested in parallel by absorption inhibition and absorption elution.

Preparation of samples

Ants were collected from a nest at the site where the body had been found and were subsequently identified as Argentine ants, *Iridomyrmex humilis*.

Clean, new disposable gloves (unpowdered) were worn during the collection. New gloves were worn subsequently when handling anything within the containers in which the ants were kept and any materials with which the ants might come in contact or which were to be used in the ABO antigen grouping tests. The soil which contained the ants was placed in two 23 cm × 23 cm × 15 cm plastic containers with tight sealing lids. The con-

tainers were newly purchased, and had been rinsed with 95% ethanol and distilled water before use. In the laboratory, the containers were kept on portable shelving over jars of water (a water barrier to prevent escape). The ants were maintained in the laboratory for several weeks prior to preparation of the experimental samples. They were fed Empress brand clover honey (pure US fancy white). By means of a sterile, disposable syringe, the honey was placed in a small petri dish lid which was placed directly on the soil within the ant containers. Lids and honey were changed every three or four days. All materials (petri dishes, gloves, scalpel blades, microscope slides, etc) were taken from sealed packages to minimize the possibility of contamination.

In preparation of the first experimental samples, a petri dish lined with a filter paper was installed in one of the containers. Honey in a small petri dish lid was placed in the center of the filter paper and exposed to the ants for a period of 72 hours. The honey and the filter paper were used in the subsequent antigen tests. The number of ants on the filter paper was monitored. It was noted that there were 50 ants on the paper 5 minutes after installation, 200 ants after 24 and 48 hours of installation, and 100 ants at the end of the 72-hour limit.

In preparation of another experimental sample, ants in the same container were permitted to walk for 24 hours on glass slides and filter paper smeared with a sputum sample of the defendant, a type AB non-secretor. Again, the number of ants was monitored at regular intervals. The saliva from a slide was used in the subsequent antigen tests. At the conclusion of this 24-hour period, one hundred ants were cut into a head and thorax section and abdomen or gaster section. All ants were from the original nest site and had been fed on honey, and most had been exposed to the sputum samples on the slides and filter paper. The separated ant sections were macerated in 0·8 ml sterile saline and refrigerated prior to the antigen testing.

A second group of ants was collected from the original site for the purpose of demonstrating trail-following by ants. Since a larger amount of concentrated material was needed for the behavioral assay than for the antigen tests, a different collecting technique was employed. For 72 hours, the ants were forced to pass over a bridge consisting of a narrow solid glass rod covered with Whatman No 1 filter paper (4·25 cm) in order to reach a honey source. This paper was then extracted in 1 ml methylene chloride and the resulting solution was used in the behavioral assays. A control solution was prepared by extracting a clean filter paper in methylene chloride.

To demonstrate a response to the filter paper extract, 20 behavioral assays were performed with the treatment extract and 20 with the control extract. In each assay, a single ant was removed from the ant container and placed for 20 minutes in a large glass petri dish (150 × 20 mm) containing a piece of

TABLE 1. Investigation of the effect of ant pheromone on the saliva of an ABO group AB non-secretor

		Absorption-inhibition			*Absorption-elution*		
Sample	*Dilution*	*A*	*B*	*H*	*A*	*B*	*H*
saliva	Neat	—	4	—	4	—	3
A_2 secretor	1/2	—	4	—	3	—	2
	1/5	—	4	—	3	—	—
saliva	Neat	3	4	4	2	—	—
A_1 non-secretor	1/2	4	4	4	2	—	—
	1/5	4	4	4	3	—	—
saliva	Neat	4	—	2	3	3	—
B secretor	1/2	4	—	3	—	4	—
	1/5	4	—	3	—	3	—
saliva	Neat	4	4	—	—	—	4
O secretor	1/2	4	4	—	—	—	4
	1/5	4	4	—	—	—	4
saliva	Neat	4	4	4	—	—	—
O non-secretor	1/2	4	4	4	—	—	—
	1/5	4	4	4	—	—	—
saline	Neat	4	4	3	—	—	—
blank	1/2	4	4	3	—	—	—
	1/5	4	4	3	—	—	—
saliva of defendant	Neat	4	4	4	—	—	—
AB non-secretor	1/2	4	4	4	—	—	—
(on slide)	1/5	4	4	4	—	—	—
honey exposed	Neat	4	4	4	3	—	—
72 hours to ants	1/2	4	4	3-4	3	—	—
	1/5	4	4	4	2	—	—
honey control	Neat	4	4	4	2-3	—	—
Empress Clover	1/2	4	4	4	3	—	—
	1/5	4	4	4	3	—	—
filter paper	Neat	4	4	4	—	—	—
control for honey,	1/2	4	4	4	—	—	—
saliva	1/5	4	4	4	—	—	—
filter paper on which	Neat	4	4	4	—	—	—
ants moved for 24 hours	1/2	4	4	4	—	—	—
	1/5	4	4	4	—	—	—
filter paper on	Neat	4	4	4	—	—	—
which ants moved	1/2	4	4	4	—	—	—
for 72 hours	1/5	4	4	4	—	—	—
control filter	Neat	4	4	4	—	—	—
paper for tracking	1/2	4	4	4	—	—	—
ants	1/5	4	4	4	—	—	—
soil from collection	Neat	4	4	4	—	—	—
site	1/2	4	4	4	—	—	—
	1/5	4	4	4	—	—	—
gasters-ants	Neat	4	4	4	2	—	—
(N = 100)	1/2	4	4	4	—	—	—
	1/5	4	4	4	—	—	—
heads and thoraxes	Neat	—	3	4	2	—	—
(N = 100)	1/2	—	3	4	3	—	—
	1/5	—	4	4	2–3	—	—
defendant's saliva	Neat (1)				—	—	—
on slide over which	Neat (2)				—	—	—
ants tracked for 24 hours							

tracing paper upon which a straight 10 cm line had been drawn with 10 μl test solution. A positive response was recorded if, during the 20 minute period, the ant followed at least half of the 10 cm line, and paused upon reaching a terminus, indicating that it was actually responding to the line rather than coincidentally moving in that general direction.

Eighty-five percent of the ants responded positively to the extract from the filter paper over which ants from the same nest had tracked for 72 hours. None responded to the control extract. This strongly suggests deposition by the ants of relatively persistent chemical compounds (pheromones) on substrates over which they walked.

Results

Table 1 shows the results of the absorption inhibition and absorption elution tests. The absorption elution tests did not produce any spurious type B activity. However, control saliva from an ABO type A_1 non-secretor showed type A antigenic activity by absorption elution only. The uncontaminated honey control and the honey that had been exposed to ants both showed type A antigenic activity by absorption elution only. The head and thorax extract showed a relatively strong type A antigenic activity by both absorption inhibition and absorption elution, while the gaster extract showed a weak type A activity by absorption elution. Saliva from the defendant, (an AB non-secretor), exposed to ants for 24 hours, showed no ABH activity.

Conclusions

Since there are 15,000 species of ants in the world, and 100 species of ants in southern California, it is unwise to generalize about ants on the basis of these experiments. However, prolonged trailing by these Argentine ants did not leave ABH antigen-like substances on filter paper and did not act to produce an ABH antigen-like reaction in non-secretor saliva. The experiment does not show whether the antigenic activity in macerated ant extract was of physiological origin, or whether it resulted from a diet of honey.

This experiment does not support the theory ants could have produced type B antigenic activity in the saliva from a type AB non-secretor.