8. The metathoracic gland was dissected from each bug and extracted in *n*-pentane. The composition of the secretion, excluding acetic acid and 2-*n*-butyloct-2-enal, was determined with a gas-liquid chromatograph (GLC) (Tracor 222) with a flame ionization detector coupled to a chromatography data system (Varian CDS-111) peak area integrator on a 3 percent OV-1 column programmed from 50° to 215°C at 10°C per minute. Four adult bugs were analyzed separately for each age class. The mean percentage is reported for each compound, followed by the 95 percent confidence interval. Mass spectra were obtained for each compound with a GLC and mass spectrometer (LKB-9000 GLC-MS) and a 1 percent SP-1000 column programmed from 50° to 225°C at 10°C per minute.
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11. High-resolution mass spectra for both of these GLC peaks gave diagnostic ions at *m/e* 229.1797 ($C_{13}H_{25}O_3$), 201.1841 ($C_{12}H_{25}O_2$), 101.0972 ($C_6H_{13}O$), and 83.1857 (C_6H_{11}), plus metastable ions at *m/e* 50.75, 68.20, and 36.45.
12. The bugs were chilled, their metathoracic glands were removed intact, and the secretion was mixed with 100 μl of cold 0.1*M* tris-HCl buffer (*p*H 8.2). Each sample (30 glands) was divided into three equal portions and placed on the gels. Electrophoresis was performed on 7.5 percent polyacrylamide gels with spacer gels, at 1.5 mA per gel for 4 hours; the gels were then stained differentially for esterases, proteins, and alcohol dehydrogenase. When assayed for 1-hexanol or hexanal dehydrogenases, the gels were washed in 0.1*M* tris-HCl buffer (*p*H 7.4) for 5 minutes and then incubated at 37°C in 50 ml of 0.1*M* tris-HCl buffer containing 1 ml of either 1-hexanol or hexanal (20 percent in acetone), 25 μmole of tetrazolium, 30 μmole of nicotinamide adenine dinucleotide phosphate, and 15 μmole of phenazine methosulfate for 10 minutes.
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14. Few nonvenomous secretions have been analyzed for proteins. We electrophoretically analyzed the defensive exudates of some opilionids (*Leiobunum* spp.) and found an array of low-molecular-weight proteins.
15. The secondary accessory glands of whole excised metathoracic glands from *L. phyllopus* stained positively for esterases using α-naphthyl acetate as a substrate.

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Biosynthesis and Accumulation of Formic Acid in the Poison Gland of the Carpenter Ant *Camponotus pennsylvanicus*

Abstract. *Formic acid synthesis in the poison gland of* Camponotus pennsylvanicus *is closely related to the C-1 metabolism of the glandular cells. Serine, glycine, and histidine are potential C-1 donors to formic acid by several tetrahydrofolate intermediates. Formic acid is accumulated by its transfer to an insulated reservoir, so that the ant avoids the acid's cytotoxicity. This combination of biochemical and morphological features provides an autodefensive mechanism. Possible factors that regulate the biosynthesis of formic acid in the poison gland apparatus are discussed.*

Formic acid, identified from a distillate of formicine ants (Formicidae: Hymenoptera) more than 300 years ago, was the first natural product isolated from insects (*1*). Later it was identified in the defensive secretions of moth larvae (Notodontidae:Lepidoptera) (*2*), and as a pygidial gland product of ground beetles (Carabidae:Coleoptera) (*3*). In the notodontids and carabids, formic acid is utilized as a defensive compound, but in some species of formicine ants it also functions as an alarm pheromone (*4*). In view of the high reactivity of formic acid, we were interested in determining how insects biosynthesize and accumulate large amounts of this cytotoxic acid. In order to understand this mechanism, we have studied its in vitro synthesis and storage of formic acid in the poison glands of the carpenter ant, *Camponotus pennsylvanicus*.

We assumed that (i) formic acid was derived from one or more of the free amino acids in the glandular tissue, and (ii) that its biosynthesis was closely related to the C-1 metabolism in the gland and probably involved tetrahydrofolic acid (H_4folate). All incubations were conducted with the 20 intact poison glands dissected from worker ants belonging to the same colony (*5*). Time-course studies, in which poison glands were incubated with uniformly labeled serine, in vitro, indicated that this amino acid serves as a precursor for formic acid production. Formic acid was synthesized at a rate of 40 nmole per hour for the first 3 hours of incubation. Incubation of poison glands with [U-^{14}C]serine, [3-^{14}C]serine, or [1-^{14}C]serine, suggests that serine contributes both its α and β carbons to formic acid but not its carboxylic carbon, since the incorporation of [U-^{14}C]serine was twice that of [3-^{14}C]serine (Table 1). The α carbon of glycine was also converted to formic acid by the poison glands, suggesting that both serine and glycine serve as precursors of formic acid. Histidine also contributes a carbon fragment to the C-1 pool in cells (*6*), and in fact contributes its C-2′ (in the imidazole ring) to formic acid (Table 1). These results suggest that the production of formic acid by the poison gland is linked to the C-1 metabolism in its glandular cells.

To test our second hypothesis, that H_4folate is involved in the transfer of the β carbon of serine to formic acid, we studied the incorporation of labeled H_4folate derivatives, that could be precursors. An H_4folate-free extract of poison glands (desalted on a Sephadex G-25 column) synthesized formic acid when incubated with 5,10-[^{14}C]methylene H_4folate—which is formed spontaneously when [^{14}C]formaldehyde and H_4folate are mixed in solution (*7*)—whereas a similar extract supplemented only with [^{14}C]formaldehyde failed to do so (Table 2). But when [^{14}C]formaldehyde was incubated with intact poison glands, it was readily converted to formic acid. This was probably due to its intracellular combination with H_4folate, forming 5,10[^{14}C]methylene H_4folate. A second H_4folate derivative, 5,10[^{14}C]methenyl H_4folate, had an even higher degree of incorporation when incubated with intact poison glands, suggesting that both H_4folate derivatives are intermediates in formic acid biosynthesis.

Table 1. Incorporation of labeled amino acids into formic acid in the poison glands of *C. pennsylvanicus*.

Precursor	Formic acid produced (dpm/nmole)	Total formic acid (nmole)	Percent incorporation
L [U-^{14}C]serine	983	56 ± 5	1.67
DL [3-^{14}C]serine	574	56 ± 5	0.98
L [1-^{14}C]serine	21.4	56 ± 5	0.036
[2-^{14}C]glycine	605	47 ± 3	0.87
L-[2′-^{14}C]-histidine	347	42.5 ± 3	0.45

Table 2. Incorporation of labeled formaldehyde and H_4folate derivatives into formic acid by intact poison glands, or poison glands extracts, of *C. pennsylvanicus*.

Precursor	Tissue	Formic acid produced (dpm/nmole)	Percent incorporation
5,10[^{14}C]Methylene H_4folate	Gland extracts	924	1.41
[^{14}C]Formaldehyde	Gland extracts	12	None
	Intact glands	832	1.27
5,10[^{14}C]Methenyl H_4folate	Intact glands	1180	1.80

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Finally, we attempted to isolate the possible H_4folate intermediates formed when [3-^{14}C]serine was incubated with poison glands. 5,10-Methenyl-H_4folate, a rather stable intermediate, was isolated (i) by descending paper chromatography, which revealed a fluorescent, radioactive spot at R_F 0.5, corresponding to the R_F of standard 5,10-methenyl-H_4folate, and (ii) by column chromatography on Whatman cellulose CF-11 (*8*). The eluted fractions from the column whose absorbance ratios at 350 nm and 305 nm were higher than 1.6, characteristic of 5,10-methenyl-H_4folate (*8*), showed ^{14}C labeling (that is, originated from the [3-^{14}C]serine in the incubation medium). These fractions were pooled, lyophilized, and then reacted with adenosine diphosphate, inorganic phosphate, and a pure preparation of 10-formyl-H_4folate synthetase (E.C. 6.3.4.3.) from *Clostridium thermoaceticum* (*9*). The labeled compound isolated from the column was completely converted to [^{14}C]formic acid, demonstrating unequivocally that it was indeed 5,10-methenyl-H_4folate.

In view of these results, we suggest the following pathway of the biosynthesis of formic acid in the poison gland of *C. pennsylvanicus*. Serine, serving as the main precursor, contributes its C_2 and C_3 carbons to 5,10-methylene-H_4folate; this is subsequently converted to 5,10-methenyl-H_4folate and 10-formyl-H_4folate which is hydrolyzed to the final product, formic acid. In the last step H_4folate is regenerated and adenosine triphosphate (ATP) is produced (*6*); in turn, the ATP may be utilized for an energy dependent transport of formic acid through the glandular membrane into the gland lumen. Since the equilibrium between formic acid and 10-formyl-H_4folate is 1:20 (*6*), only small amounts of formic acid are produced in the glandular cells. For the acid to accumulate, it is therefore necessary to transfer it to a second compartment (the poison gland reservoir), which is insulated by a cuticular intima (*10*). Thus the biochemical equilibrium inhibits the accumulation of the acid in the glandular cells, and the insulated reservoir prevents it from diffusing out into the body cavity. When the reservoir fills up, the proposed formic acid carrier will be saturated, resulting in the accumulation of 10-formyl-H_4folate as well as ATP in the glandular cells, and inhibiting the biosynthetic pathway. This system would be readily reactivated as soon as the ants eject their reservoir contents in a defensive encounter.

The biosynthesis of formic acid in the ant poison gland deduced from our experiments is not different from that reported for bacterial and mammalian cells (*6*). The novelty of this system, however, is the adaptation of the biochemical characteristics of this pathway and the morphological compartmentalization of the gland for accumulating large amounts of this toxic compound.

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Adherence of Group A Streptococci to Pharyngeal Cells: A Role in the Pathogenesis of Rheumatic Fever

Abstract. *We used an assay in vitro to investigate the possible role of streptococcal adherence to human pharyngeal cells in the pathogenesis of acute rheumatic fever. There was no difference in adherence of rheumatic fever–associated and non-associated strains of group A streptococci to pooled pharyngeal cells of normal people. Likewise, streptococci not associated with rheumatic fever adhered equally well to cells taken from normal people and from patients with rheumatic heart disease. However, the pharyngeal cells of all nine rheumatic heart disease patients tested had increased avidity for adherence for a rheumatic fever–associated strain of streptococcus compared to the pharyngeal cells obtained from age- and sex-matched controls. Increased streptococcal adherence to pharyngeal cells of rheumatic fever–prone patients may play a role in the pathogenesis of rheumatic fever.*

There seems to be a special relation between pharyngeal infection with group A streptococci and the subsequent development of acute rheumatic fever. It is known that streptococci must be present in the pharynx for a prolonged period of time and must evoke an antibody response, and that treatment for less than 10 days may not eradicate the infection or prevent the occurrence of rheumatic fever (*1*). Streptococcal infections of other sites such as the skin do not lead to rheumatic fever (*2*). It is unclear why pharyngeal infection is necessary. Various postulates have been forwarded, including an antigenic change in the organism induced by the oral environment or the neutralization of some toxic or antigenic streptococcal products by the skin (*3*). There are also no currently recognized host or bacterial factors that fully account for the observation that less than 3 percent of individuals with untreated group A streptococcal infection of the pharynx develop acute rheumatic fever (*4*). Although it has been thought in the past that any strain of group A streptococcus is potentially rheumatogenic, epidemiologic studies have indicated that impetiginous strains may not have the potential to produce acute rheumatic fever even when they infect the pharynx (*5*). It is therefore unclear whether the most important factor in the pathogenesis of acute rheumatic fever is the strain of streptococcus or the site of infection or both.

Studies with streptococci, as well as

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