Glucose Oxidase: A Food Protective Mechanism in Social Hymenoptera[1]

D. MICHAEL BURGETT[2]
Department of Entomology, Cornell University, Ithaca, NY 14850

ABSTRACT

The previously suggested protection from microbial attack of ripening nectar by the addition of glucose oxidase by the common honey bee, *Apis mellifera* L., has been found to occur in 9 other eusocial Hymenoptera from 3 superfamilies. The antibacterial effect results from the accumulation of hydrogen peroxide produced by the enzyme during ripening of the stores.

Honey-storing social insects are confronted with the problem of protecting colony stores against microbial contamination. Ripened honey produced by the honey bee, *Apis mellifera* L., is protected by its hyperosmotic state. Only honey with less than about 18.6% moisture shows such bacteriostatic activity. Dilute or unripened honey was shown by Sackett (1919) to display antibiotic activity.

The antibacterial character in dilute *Apis mellifera* honey, termed inhibine (Dold et al. 1937), was identified as the enzymic production and accumulation of hydrogen peroxide (White et al. 1962, 1963). The enzyme responsible is a glucose oxidase. The presence of a glucose oxidase in the hypopharyngeal glands of the honey bee was reported by Gauhe (1941), who did not connect its presence in these glands with the antibacterial nature of dilute honey. This was the first discovery of a glucose oxidase from an animal source.

The following is a report on the taxonomic breadth of glucose oxidase in the stored carbohydrates of social insects.

MATERIALS AND METHODS

An assay involving the colorimetric determination of evolved hydrogen peroxide in dilute honey was developed for the analysis of glucose oxidase in *Apis mellifera* honey (White and Subers 1963). Color formation by an oxidized chromogen (o-dianisidine) in the presence of hydrogen peroxide and peroxidase is the basis of this assay. With modifications for small sample sizes (0.2–1.0 g), this peroxidase-dye assay was employed for the determination of glucose oxidase activity in honeys of eusocial Hymenoptera. Ten-ml breakers were substituted for White and Subers 8-oz (225 ml) bottles in the incubation of the diluted honeys and/or honey protein extracts. Incubation was done in an agitating (100 shakes/min) water bath.

Enzyme activities were determined for dilute whole honeys and crude honey protein extracts prepared by dialyzing honey with running tap-water (20°C) for 24 h. Enzyme activity was expressed as the μg H_2O_2/mg honey protein/h. Protein content was determined by the method of Lowry (1951). The results of the protein determination are seen in Table 1.

Honeys for investigation came from the following species, Formicoidea: *Myrmecocystus mexicanus* Wesmael (honey ant); Vespoidea: *Protonectarina sylveirae* (Saussuer) (honey wasp); and Apoidea: *Bombus perplexus* Cresson, *B. fervidus* (F.) (bumble bees), *Trigona (Trigona) fuscipennis* Friese, *T. (Scaptotrigona)* sp. (stingless bees), *Apis mellifera* L., *A. indica* F., *A. florea* F. (honey bees).

The diluted whole honeys and protein extracts (1 ml of extract was added to 4 ml of a 0.4 M glucose substrate) were incubated in a 35°C agitating water bath. Peroxide accumulation was determined at the end of 1 h.

RESULTS AND DISCUSSION

Glucose oxidase was a component of the honeys from all the species examined. No hydrogen peroxide was found in any of the honeys prior to dilution with a sodium-potassium phosphate buffer, *p*H 6.4. The buffer served as both a diluent and to adjust the *p*H of the honey solutions to eliminate any photo-oxidation of the enzyme (White and Subers 1964). Peroxide was liberated only upon dilution and controlled incubation of the honeys. The quantitative results for peroxide accumulation from diluted whole honeys, protein extracts, and lyophilized protein extracts are summarized in Table 2. Peroxide accumulation values are valuable qualitatively but do not represent strict quantitative values comparable between species.

All the honey-producing species examined in this

Table 1.—Protein content of eusocial Hymenoptera honey.

Species	mg protein/g honey[a]
Formicoidea	
Myrmecocystus mexicanus	3.72
Vespoidea	
Protonectarina sylveirae	2.18
Apoidea	
Apis mellifera	2.02
Apis indica	2.72
Apis florea	2.98
Bombus perplexus	0.88
Bombus fervidus	1.40
Trigona (Trigona) fuscipennis	3.99
Trigona (Scaptotrigona) sp.	4.54

[a] Mean value of actual honey examined for enzyme activity. Protein content of a given honey sample will depend largely upon the floral source of the honey.

[1] Received for publication Jan. 8, 1974.
[2] Present address: Dept. of Entomology, Oregon State Univ., Corvallis 97331.

Table 2.—Glucose oxidase activity in eusocial Hymenoptera honey.

Sample	Activity—μg H_2O_2/mg honey protein/h		
	Whole honey diluted	Dialyzed Protein Extract—Substrate, 0.4 M glucose	
		Aqueous solution	Lyophilized
Formicoidea			
Myrmecocystus mexicanus	[a]	271.8	265.9
Vespoidea			
Protonectarina sylveirae	493.1	459.4	320.8
Apoidea			
Apis mellifera	140.6	55.6	45.9
Apis indica	20.4	22.3	15.6
Apis florea	172.3	25.6	18.3
Bombus perplexus	[a]	943.3	386.4
Bombus fervidus	[a]	124.6	63.5
Trigona (Trigona) fuscipennis	103.1	209.7	201.9
Trigona (Scaptotrigona) sp.	248.6	400.0	315.3

[a] Whole honey not tested for enzyme activity.

study are eusocial. Sociality has developed independently many times in the bees, wasps, and ants. The ability of these insects to utilize plant carbohydrates as reserve colony stores developed to a great extent because of the large number of workers per colony and the advanced state of polyethism in eusocial Hymenoptera. Selective pressure has sustained a glucose oxidase system for the protection of these energy stores against microbial contamination.

A honey glucose oxidase represents a defense system at the colony level. This enzyme mechanism is now seen to be present in honeys from representative members of 3 superfamilies of the Hymenoptera: Formicoidea, Vespoidea, and Apoidea. The probability is high that this enzyme occurs among most honey storing social insects.

REFERENCES CITED

Dold, H., D. H. Du, and S. T. Dziao. 1937. Nachweis antibakterieller, hitze und lichtempfindlicher Hemmungsstoffe (Inhibine) in Naturhonig (Blutenhonig). Z. Hyg. Infektkrankh. 120: 155–67.

Gauhe, A. 1941. Uber ein glukoseoxydierendes Enzym in der Pharynxdruse der Honigbiene. Z. Vergl. Physiol. 28: 211–53.

Lowry, O. H., N. J. Rosebough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with folin phenol reagent. J. Biol. Chem. 193: 265–75.

Sackett, W. G. 1919. Honey as a carrier of intestinal diseases. Bull. Agric. Exp. Stn., Colorado Agric. College 252: 1–18.

White, J. W., M. H. Subers, and A. I. Schepartz. 1962. The identification of inhibine. Am. Bee J. 102: 430–31.

1963. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide, and its origins in a honey glucose oxidase system. Biochem. Biophys. Acta 73: 57–70.

White, J. W., and M. H. Subers. 1963. Studies on honey inhibine. 2. A chemical assay. J. Apic. Res. 2: 93–100.

1964. Studies on honey inhibine. 4. Destruction of the peroxide accumulation system by light. J. Food Sci. 29: 819–28.

Reprinted from the
ANNALS OF THE ENTOMOLOGICAL SOCIETY OF AMERICA