Reports

Enzymatic Basis of Mannose Toxicity in Honey Bees

Abstract. Honey bees have a negligible amount of phosphomannoseisomerase, together with a high content of a hexokinase which phosphorylates mannose more efficiently than fructose or glucose. Competition at the phosphorylation level plus accumulation of mannose-6-phosphate can fully account for the toxicity of mannose in honey bees.

In the course of a thorough investigation on the sense of taste in honey bees, von Frisch (*1*) incidentally found, 30 years ago, that mannose was strongly toxic for honey bees.

Mannose is a common and widely utilizable hexose, which is known to be metabolized through the reactions:

$$\text{Mannose} + \text{ATP} \rightarrow \text{mannose-6-P} + \text{ADP} \quad (1)$$

and

$$\text{Mannose-6-P} \rightleftarrows \text{fructose-6-P} \quad (2)$$

Reaction 1 is catalyzed by hexokinase, an enzyme common to glucose, mannose, and fructose (*2*), which is widely distributed (*3*). Reaction 2 is catalyzed by phosphomannoseisomerase (*4*), which also is of wide occurrence (*5*). The work presented in this report shows that honey bees have a high content of hexokinase, while they have no more than a trace of phosphomannoseisomerase.

Worker honey bees (*Apis mellifera*) were obtained from local sources (*6*) and maintained on honey until used. A number of trials with different batches consistently confirmed the toxicity of mannose. In parallel experiments, lots of about 20 bees each were offered 1*M* mannose, water, and 1*M* glucose, respectively. Of those offered mannose, 50 percent died within 1.5 hours, and over 90 percent, within 3 hours, at which time more than 90 percent of the controls (those given water) still survived. Within 12 hours over 90 percent of the controls had died, as against less than 10 percent of the honey bees given glucose.

For the exploration of enzymes, bees fasted for 1 hour were killed by freezing at −20°C. The frozen bees were homogenized in a Waring blender for 2 minutes with 4 volumes of cold 0.005*M* ethylenediaminetetraacetic acid neutralized to *p*H 7. The suspension was freed of residual pieces of hard tissue by squeezing through two layers of gauze. The clarified homogenates were used without delay or were stored frozen until assay. The results in Table 1 show a high phosphorylation activity toward glucose, mannose, and fructose, as well as strong inhibition of the activity toward fructose by both aldoses, a high phosphoglucoseisomerase activity, and a barely detectable trace of phosphomannoseisomerase activity.

These results indicate a hexokinase common for glucose, mannose, and fructose. This conclusion is supported by the following complementary observations: competitive inhibition of the three hexoses by N-acetylglucosamine and phosphorylation of 2-deoxyglucose and glucosamine. Inhibition by glucose-6-P tends to lower the apparent phosphorylation rate of glucose with respect to that of mannose. Nevertheless, even under conditions of minimal product accumulation, the maximal phosphorylation rate of mannose appears to be as high as 1.7 that of glucose, while the apparent affinities are similar (Michaelis constants about 1×10^{-4} *M*) (*8*).

Phosphoglucoseisomerase is in excess over hexokinase. This fact has been repeatedly observed even in tissues where glycolysis proceeds from free hexose rather than from glycogen. Nevertheless this must not be interpreted as a superfluous excess. Owing to the free reversibilty of the reaction catalyzed by the phosphoglucoseisomerase, the enzyme must be "in excess" to create sufficient net activity within the glycolytic chain without requiring levels of glucose-6-P which could seriously disturb normal metabolism.

This excess makes more obvious the insufficiency of the phosphomannoseisomerase in bees. The highest value found has been less than one-tenth of the mannose phosphorylating capacity. At these low levels of activity it is difficult to ascertain to what extent there is any true phosphomannoseisomerase at all in honey bees. Among other considerations there is the possible contribution of enzymes from microorganisms when whole, normally raised bees are used.

These results indicate that the mannose toxicity in honey bees is a metabolic disease due to lack of balance between hexokinase and phosphomannoseisomerase, presumably due to mutational loss of the ability to make the latter. Mannose would both competitively inhibit glucose and fructose phosphorylation and give rise to an accumulation of mannose-6-P which could interfere with glycolysis in a number of ways; it is a competitive inhibitor of phosphoglucoseisomerase (*9*). Both the high hexokinase content and the speed with which intoxication by mannose occurs must be related to the fact that honey bees are markedly dependent for activity on a high blood-sugar level, which in turn depends on recent food intake (*10*). This metabolic disease bears analogies to the galactosemia syndrome (*11*) and to the experimental interference with glucose metabolism by 2-deoxyglucose (see *12*).

An exploration of mannose toxicity and phosphomannoseisomerase, or of phosphomannoseisomerase alone, in other bees and eventually in other Hymenoptera could give interesting clues to the genetic relationships among closely related species.

Table 1. Hexokinase and phosphohexoseisomerases in honey bees.

Substrate	Activity (μmole/gm/15 min at 30°C)		
	Phosphorylation*		Isomerization†
	Sugar	Ketose	
Glucose	105		
Mannose	190		
Fructose		100	
Fructose + glucose		35	
Fructose + mannose		20	
Glucose-6-phosphate			700
Mannose-6-phosphate			About 1‡

* Estimated by substrate disappearance essentially as described by Crane and Sols (*3*), 0.1 ml of homogenate and 3 μmole of substrate (plus 6 μmole of the second substrate in the fourth and fifth experiments) being used, in a total volume of 0.5 ml. † Studied by ketose formation with the borate method of Alvarado and Sols (7), 0.01 to 0.1 ml of homogenate in a total volume of 0.4 ml and incubation times of from 10 minutes to 2 hours being used. ‡ Values from about 0.3 to 10 were obtained in different experiments.

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

Note added in proof. After the preparation of this report, our attention has been called to a paper from von Frisch's department, in which the results of an exploration of mannose toxicity in a number of insects are reported [T. Staudenmayer, *Z. vergleich. Physiol.* **26**, 644 (1939)]. Mannose was to be toxic to several apidae examined and to *Vespa vulgaris*, but not to other Hymenoptera.

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Identity of a Rust on Ephedra

Abstract. The aecial stage of a rust on *Ephedra* has been called a *Peridermium*. Clues as to its telial stage have been lacking. It is here described as resembling more a form of *Roestelia*, and the reasons for its possible relation to *Gymnosporangium multiporum* Kern are presented: the two sets of hosts have similar distributions: the two rust stages have the same geographic range; they have been found in close proximity in the field.

Ephedra is a genus of the joint-fir family (Ephedraceae) distributed over the arid regions of the northern hemisphere. The drug ephedrin, administered as an astringent, is obtained from a Chinese species. The small scale-like leaves and jointed stems of these plants make them resemble somewhat the horsetails. Botanically the joint-firs are classed with the gymnosperms, which bear naked seeds in contrast to the angiosperms, with seeds enclosed in an ovary. The joint-fir family has points of agreement with both Gymnospermae and Angiospermae, making the group in some ways an intermediate one between the two classes.

A rust fungus (order Uredinales) has been known as a parasite on species of *Ephedra* since 1877. This rust has been reported as, "A common and conspicuous species along the southwestern border of the United States and southward into Mexico" (*1*). It is usually listed as *Peridermium ephedrae* Cooke (*2*). In the Natürlichen Pflanzenfamilien, Dietel referred to it as *Aec.* [*Aecidium*] *Ephedrae* Cke. (*3*).

This rust on *Ephedra* is an aecial (or aecidial) stage only, and without a known perfect (telial) stage can be referred only to a form genus. *Peridermium* is a form genus name for various aecia on gymnospermous hosts, whose telial connections are unknown. Many of the forms temporarily placed here have been connected to telial stages. All of these aecial forms have been on coniferous hosts (order Coniferales), and their telial stages belong to the rust family Melampsoraceae.

The *Ephedra* rust doubtless has been called a *Peridermium* chiefly because the host is classed as a gymnosperm. *Ephedra,* however, is not a conifer and the aecial stage on it is in certain respects unlike the forms of *Peridermium* on these hosts.

It more closely resembles some of the highly differentiated species of the form genus *Roestelia,* in which the peridium (outer coat or investment of the sorus) is cylindrical, elongated (up to 5 mm), and dehiscent at the apex. For many years the forms of *Roestelia* were believed to have a restricted host range: species of the tribe Pomeae or family Malaceae (depending on the classification used). We now know that a few species with *Roestelia*-like aecia in habit not only Malaceae but other families—Rosaceae, Hydrangiaceae, Myricaceae—belonging to the angiosperms. These rusts are heteroecious and those for which a telial stage is known belong to the genus *Gymnosporangium.* Without exception species of *Gymnosporangium* have their telial stages on the family Juniperaceae which is a part of the Gymnospermae. Here we have heteroecious rusts alternating between angiosperms and gymnosperms.

We are now strongly suggesting that the rust on *Ephedra* is a *Roestelia* and that it is the aecial stage of a *Gymnosporangium* on a *Juniperus* species. We are aware that this will create an anomalous situation. It will be the first case where aecial and telial stages of a heteroecious rust inhabit two families both belonging to the Gymnospermae. Perhaps this may be regarded as supporting the view that the *Ephedra* family is not truly gymnospermous, as has been pointed out by some taxonomists.

The species of *Gymnosporangium* believed to be the telial stage of the *Ephedra* aecial stage is *G. multiporum* Kern (*4*). The geographical range of these two stages is essentially the same. The similar distribution of the two sets of hosts—the various species of *Ephedra* and *Juniperus deppeana* Steud. (*J. pachyphlaea* Torr.), *J. monosperma* (Engelm.) Sarg., and *J. osteosperma* (Torr.) Little [*J. utahensis* (Engelm.) Lemmon]—and their association on desert lands make possible the harboring of a fungus which must pass from one set to the other. This lends credence to the suggestion that *Gymnosporangium multiporum* is the telial stage of the *Ephedra* rust. Not only do the ranges and distribution of these rust stages and of their hosts favor this conjecture, but there is additional evidence from field observations. *Gymnosporangium multiporum* and the aecia on *Ephedra* have been found in proximity in an area of the South Rim of the Grand Canyon, Coconino County, Arizona. Our prediction is made with much confidence. Proof can be had only through cultures, involving inoculations under controlled conditions.

It is pertinent to note that there are numerous collections of the rust on *Ephedra* but that there has been no suggestion till now of a possible telial stage. *Gymnosporangium multiporum* is so inconspicuous as to elude easy detection even when present. Relatively few collections of *G. multiporum* have been made. In any event its collectors would doubtless have sought *Roestelia* on Malaceae (the usual alternate stages for *Gymnosporangium*) and naturally would have disregarded the *Ephedra* as being of no concern. These facts may help explain why the present hypothesis has not been suggested sooner (*5*).

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