sibility has some empirical support. As Winans pointed out, judgments on the basis of luminous flux can be made by animals with visual-cortex damage (*5*). Reducing the size of the figure as in the Winans study is not an unequivocal test, since the subjects may have learned to attend selectively to smaller black-white gradients. A simple control test such as the one described here gives an indication of whether the discrimination is based on form or on relative distribution of light within the figure.

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Buchtel's study with normal rats does not bear directly upon the major conclusion from my report—that, contrary to indications from previous studies, striate decorticate cats are capable of discriminating stimuli equated for total luminous flux (*1*). However, Buchtel's data are particularly relevant to a second consideration which I raised in that report concerning the possible cues for discrimination of the stimuli. The erect and inverted triangles which have been used in classical and recent studies of visual form discrimination (*2*) display localized flux cues on corresponding restricted regions of the stimuli, as well as flux gradients, either of which could facilitate the discrimination of these stimuli by animals capable of discriminating regional flux differences within areas equated for the total flux.

As I indicated in my reply to Dodwell and Freedman (*3*), after the striate decorticate cats had mastered the series of triangle discriminations, the cats were presented with a number of transfer tests. Among those tests was a black-white figure-ground reversal similar to that described by Buchtel. When three of the striate decorticate cats were presented with black triangles (7.62 cm high and 4 cm at the base) centered on lighted panels (21.6 cm by 11.4 cm) for 40 differentially reinforced trials (erect triangle rewarded, as in discrimination training), the correct responses averaged 53.3 percent. Similarly, unoperated cats which had mastered the same series of triangle discriminations responded to this test with an average of 50.6 percent correct responses over the 40 test trials. Unlike Buchtel's rats, the cats did not prefer the inverted triangle. However, in Buchtel's experiment, the rats were rewarded regardless of whether they chose the erect or inverted triangle, whereas in my study, the cats were rewarded only for selecting the erect triangle. This differential reinforcement may have counteracted a preference for the inverted triangle and resulted in the apparent absence of differential responses.

The results of this black-white figure-ground reversal test with the cats support the conclusion that these animals were dependent upon flux gradients or localized flux cues for discrimination of the triangles. But, in other transfer tests (in preparation for publication) these same lesioned and unoperated cats chose the erect triangle on critical trials (food behind both doors) when the triangles were presented in outline form and in outlines with the bases deleted. In these figures the flux gradients were greatly reduced and the localized flux cues were altered. Thus, if the striate decorticate cats relied on flux gradients or localized flux cues to discriminate the triangles, they were capable of utilizing surprisingly subtle cues of these types to guide their discrimination behavior.

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Are Honeybees Deficient in Phosphomannose Isomerase?

Sols, Cadenas, and Alvarado (*1*) have interpreted mannose toxicity in honeybees as a metabolic disease characterized by a deficiency in phosphomannose isomerase, presumably caused by a mutational loss of the bee's ability to synthesize this enzyme. Their conclusions were based on experiments with homogenates of honeybees prepared in 5 m*M* ethylenediaminetetraacetic acid (EDTA), *p*H 7.0. Since phosphomannose isomerase, isolated from brewers' yeast (*2*), is a zinc-metalloenzyme (*3*), the question arose of whether this apparent lack of phosphomannose isomerase (*1*) is in fact a deficiency, or whether it was perhaps caused by the chelating agent in the extraction medium. We have, therefore, made measurements of phosphomannose isomerase activities in honeybee extracts prepared and stored under a variety of conditions in the absence or presence of chelating agent.

Conditions for extraction of the enzyme were chosen to resemble as closely as possible those described (*1*). Normal worker honeybees (*4*) were fasted for 1 hour and killed by freezing at −18°C; they were stored frozen until use. Portions (5 to 10 g) of the frozen bees were ground for 5 minutes, with twice the amount of buffer, in a chilled mortar; the suspension was centrifuged at 1°C for 20 minutes at 29,000*g*. The supernatant was poured through glass wool to remove the pollen and was assayed immediately to obtain the initial phosphomannose isomerase (PMI) activity. The extracts were then incubated at 30°C or kept frozen at −15°C. Extractions were made either in 0.1*M* piperazine bis-ethanesulfonate (Na^+) buffer, *p*H 7.15, selected for its nonchelating property (*5*), or in 5 m*M* EDTA, *p*H 7.0, as used by Sols *et al.* (*1*). Phosphomannose isomerase was assayed at 30°C through coupling to phosphoglucose isomerase and glucose-6-phosphate dehydrogenase, by a continuous spectrophotometric method (*2*). Phosphoglucose isomerase and hexokinase (with glucose as substrate) were also measured by coupling to the glucose-6-phosphate dehydrogenase system. For all enzyme measurements, emphasis was placed on determining true initial velocities.

When the phosphomannose isomerase activities of various bee preparations were compared, extraction with the piperazine-bis-ethanesulfonate buffer always yielded higher PMI activities (5.9 ± 0.6 μmole of substrate converted per 15 minutes per gram of bee; seven preparations) than extraction with EDTA (4.1 ± 0.7 μmole units, as above; three preparations). The values for the piperazine-bis-ethanesulfonate extracts are about six times higher than the average PMI ac-

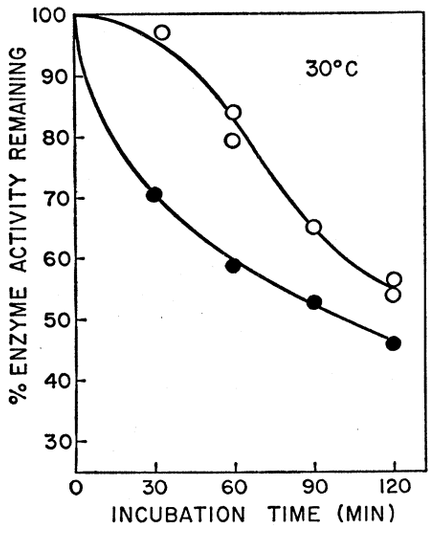

Fig. 1. Rapid loss of activity of phosphomannose isomerase at 30°C. Extracts of honeybees were prepared as described and incubated at 30°C. At the indicated times, samples were removed and immediately assayed for residual PMI activity. (Open circles) 0.1*M* piperazine-bis-ethanesulfonate (Na^+) buffer, *p*H 7.15; (closed circles) 5 m*M* EDTA, *p*H 7.0.

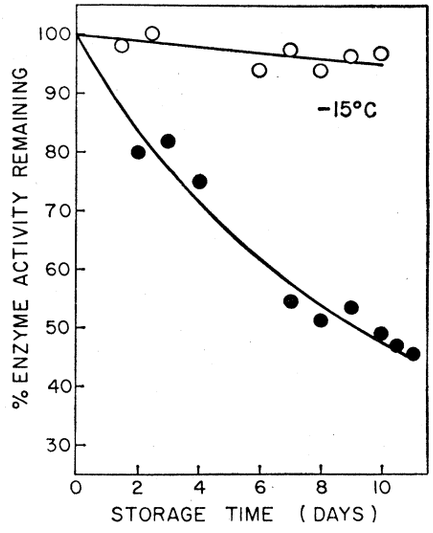

Fig. 2. Ethylenediaminetetraacetate-induced loss of activity when phosphomannose isomerase was stored at −15°C. Extracts of honeybees prepared in either piperazine-bis-ethanesulfonate buffer (open circles) or EDTA (solid circles) were kept frozen at −15°C and were thawed at times indicated, when samples were removed for measurement of enzyme activity.

tivities reported by Sols *et al.* (*1*). But it was surprising, at first, that our EDTA extracts contained substantially more PMI than those of Sols *et al.* especially since both phosphoglucose isomerase (690 μmole units, as above) and hexokinase activities (106 μmole units, as above, with glucose as substrate) under all our extraction conditions were essentially the same as theirs. However, the inhibition of yeast PMI by EDTA is a time-dependent process (*3*). If honeybee PMI were also a metalloenzyme, loss of enzyme activity caused by EDTA would likewise be expected to occur as a function of time. Such behavior was indeed observed, but it was complicated by the fact that, at 30°C, the enzyme is apparently not very stable, not even in the inert buffer (Fig. 1). Nevertheless, the activity loss is considerably more rapid with the chelating agent than without it. The difference becomes even more pronounced when the activities in the two extraction media are compared after storage for various lengths of time at −15°C (Fig. 2). Here, the piperazine-bis-ethanesulfonate extract shows virtually no loss of PMI activity whereas, in the EDTA extract, PMI decreased to less than half of its original value within 10 days.

The data shown in Figs. 1 and 2 may also provide an explanation of why Sols *et al.* observed much lower average PMI concentrations in their extracts, and why the range of their values was scattered over two orders of magnitude. Their measurements were made on homogenates in EDTA that "were used without delay *or* (our italics) stored frozen until assay" (*1*). As demonstrated in Fig. 2, any variation in the length of time, during which the samples were kept frozen in EDTA, would have caused substantial variations in the measured PMI activities. Furthermore, their assays were performed by a discontinuous chemical stop method (*6*) with incubation times ranging from 10 minutes to 2 hours at 30°C (*1*). This type of assay, in contrast to the recording spectrophotometric determination, does not allow continuous monitoring of initial velocity conditions. Since, at 30°C, as much as 50 percent of the initial PMI activity is lost within 2 hours (Fig. 1), any calculation of substrate turnover rates, based upon the assumption that no loss of activity has occurred during the incubation period, would have resulted in values that are too low.

It may, therefore, be concluded that the extremely low activity of phosphomannose isomerase found by Sols *et al.* (*1*) may be attributable to the choice of experimental conditions. Our data suggest that significant and reproducible amounts of this enzyme can be shown to be present in honeybees when possible chelation and instability effects are excluded. The measured PMI activities correspond to about 1/100 that of phosphoglucose isomerase. Similar or even lower ratios have been observed for a number of animal tissues (*2*, *7*) as well as for various yeasts (*2*, *8*), in cases where both phosphohexose isomerases have been determined under comparable conditions. Thus, compared with other systems performing glycolysis, the PMI activity in honeybees cannot be considered to be abnormally low. It is, on the other hand, still substantially lower than that of hexokinase.

Whether or not this *normal* imbalance between the two enzymes is still large enough to create a bottleneck for the catabolism of large quantities of mannose, and to result in accumulation of mannose-6-phosphate sufficient to cause a lethal breakdown of glycolysis, must at present remain a subject for speculation. However, it does not seem to be warranted any longer to classify mannose toxicity in honeybees as a genetically caused metabolic disorder.

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