length of fast, and the delayed feeding effect, found in the higher vertebrates. Because the planarians are very far removed phylogenetically from the vertebrates and are among the most primitive animals that possess a central nervous system, bilateral symmetry, and encephalization, these, as well as other (4), behavior patterns may predate, and be more universal than, the vertebrate brain.

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Relationship between Locomotory Habits and Enzyme Concentration in Insects

Abstract. The activity of alpha-glycerol phosphate dehydrogenase has been measured in muscles from four insect species. In flying insects the enzyme activity is much greater per unit weight for flight muscles than for leg muscles. The results indicate a direct relationship between muscle levels of the enzyme and habitual mode of locomotion.

In contrast to most vertebrate smooth muscles, insect muscles possess low concentrations of lactate dehydrogenase and relatively large amounts of alpha-glycerol phosphate dehydrogenase (1). These enzyme differences are known to reflect differences in glycolytic pathways that are thought to be related to the very different efficiencies of vertebrate and insect muscles. As there are very large differences between insect species in habitual modes of locomotion, we have conducted experiments to determine whether these differences can be related to enzyme activities in insect muscles.

Four insect species were examined. The bumble bee *(Bombus terrestris)* is a strong flier and uses this means for its collection of food. The praying mantis *(Orthodera ministralis)* does fly

but also often walks. The katydid *(Caedicia simplex)*, although winged, rarely flies and depends on walking as its almost sole means of locomotion. The tree weta *(Hemideina thoracica)* is wingless.

Adult specimens of these four species were captured locally. Flight muscles were dissected from the winged insects, and thoracic muscles, corresponding to flight muscles, from the wetas. Leg muscles were removed from all four species. All muscles were dissected into ice-cold, 0.9-percent potassium chloride solution and their weight was derived by difference.

The muscles were ground in a Potter-Elvehjem, all-glass, tissue homogenizer with more of the cold KCl solution. The resulting homogenate was centrifuged at 13,000 rev/min for 20 minutes at 2°C. The supernatant was used as the source of alpha-glycerol phosphate dehydrogenase.

Activity of the enzyme was determined by the following method, which is adapted from that of Chefurka (2). A mixture was prepared containing 0.37 μmole of dihydroxyacetone phosphate (DHAP), 3.0 μmole of reduced nicotinamide-adenine-dinucleotide (NADH$_2$), enzyme extract, and 0.1M phosphate buffer, pH 7.4. The final volume was 3.0 ml.

The reaction was initiated by the addition of DHAP, and the enzyme activity was determined by following the oxidation of NADH$_2$ spectrophotometrically at 340 mμ at 25°C. A control was used containing no DHAP. A unit of alpha-glycerol phosphate dehydrogenase activity is defined as the amount producing an initial rate of oxidation of 0.01 μmole of NADH$_2$ per minute under the above conditions of assay. Results are given in Table 1.

It is apparent that flight muscles contain much more of the enzyme than leg muscles. Moreover, the activity of the enzyme in the flight muscles of the

Table 1. α-Glycerol phosphate dehydrogenase in insect muscles.

Insect	Muscle	Enzyme (units per gram fresh wt.)
Bumble bee	Flight	4500
Bumble bee	Leg	2200
Praying mantis	Flight	1090
Praying mantis	Leg	175
Katydid	Flight	855
Katydid	Leg	85
Weta	Thoracic	250
Weta	Leg	475

winged species seems directly proportional to the flying habits. There is a similar decrease in the alpha-glycerol phosphate dehydrogenase activity in the leg muscles. The flightless weta, however, has a moderate alpha-glycerol phosphate dehydrogenase activity in its leg muscles.

These experiments appear to demonstrate a close relationship between the habitual locomotory habits of an insect and the alpha-glycerol phosphate dehydrogenase activity of its muscles (3).

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Imprinting: Its Effect on the Response to Stress in Chicks

Abstract. Young chicks imprinted to surrogate mothers were compared with nonimprinted controls on two tests designed to measure resistance to stress. Half of each group was run with and half without a surrogate present during the stress. One test involving survival time under starvation showed no effects. However, in the other test, imprinted chicks showed fewer distress calls in response to auditory stimulation than nonimprinted controls.

Several writers have suggested a close relation between emotion and imprinting. Thus, maturation of fear may determine the critical period during which imprinting occurs (1). Conversely, imprinting may have as a main function the reduction of fear in the young animal (2). Gray (3) has reviewed evidence at the human level and suggests that, here also, lack of attachment to a parent or parent-surrogate has deleterious effects on emotional development.

The experiments reported here were aimed at providing further evidence on the relation between imprinting and emotion. Specifically, our aim was to examine both the effects of the im-

printing experience and the presence of a surrogate mother on the responsiveness of chicks to stress. First an extreme and then a mild stress were used.

In the first experiment, 28 Vantress chicks from a local hatchery were used. To eliminate visual contact with each other or with the environment, the chicks were transferred to the lab in closed boxes at 3 hours of age. In the lab, they were kept in individual cages (9 by 8 by 5½ inches) covered with fine mesh nylon screening that allowed light and heat to enter but presumably prevented any effective perception of the environment outside the cages. Lighting was supplied by two 200-watt ceiling bulbs; cage temperature was maintained at approximately 88°F. The chicks were weighed individually at 3, 9, 12, and 16 hours. So they would have no visual contact with the environment, the chicks were placed in metal cans during weighing.

Experimental procedure was divided into two parts, imprinting and stress. Imprinting was as follows. Fourteen of the chicks, randomly assigned to the experimental group, were imprinted to surrogate mothers made of wadded linen wrapped in white cheesecloth and shaped so as to resemble an adult chicken. First, a surrogate was placed in the cage of each experimental chick from 4 hours of age until the introduction of stress at 60 hours. Second, each chick was given a 10-minute imprinting session with a moving surrogate at 8, 12, 16, and 20 hours of age. This was carried out in an unpainted wooden alley, 6 by 2 feet, with walls 3 feet high. Each chick was transferred to the alley in darkness. The room lights were then turned on, and the chick was allowed to follow a surrogate suspended in the alley and moved in front of the chick by the experimenter from behind a screen. Control chicks were kept in their home cages during this whole training session.

Stress involved total food and water deprivation at 60 hours of age. At this time, surrogates were removed from seven of the experimental cages and placed with seven control subjects. This procedure yielded four groups: imprinted with surrogate (IS), imprinted without surrogate (INS), nonimprinted with surrogate (NIS), and nonimprinted without surrogate (NINS). All chicks were

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Table 1. Mean distress calls per minute elicited in imprinted and nonimprinted chicks during and following sound stress. See text for explanation of abbreviations.

Group	Distress calls per minute (mean)	
	During stress	After stress
IS	32.6	37.9
INS	28.7	31.1
NIS	47.1	89.6
NINS	21.3	41.9

weighed again at 60 and 94 hours, and at death.

In analyzing the data, two main variables were of interest in comparing the resistance of the four groups to stress: survival time and weight loss.

No significant differences between groups were found either in respect to survival time or weight loss. A significant negative correlation was found between age at death and percent weight loss ($r = 0.62, p < .01$), indicating, paradoxically, that chicks which showed the largest weight loss by 94 hours (all were still alive at this time) tended to live longer.

In view of these negative results, we designed a new experiment using a milder stress condition and a more sensitive index of responsiveness to stress.

In the second experiment, the subjects were a new group of 28 Vantress chicks, approximately 3 hours old at the time of pickup. Mode of transfer to the laboratory, housing conditions, and surrogates were as in the first experiment. However, a circular imprinting apparatus, like that of Hess (1), was used. This was made of white cardboard painted with irregular black lines. The outside circumference of the alley was 13 feet, the inside was 6 feet. The surrogate was suspended in the alley and moved manually by the experimenter from behind a screen. A view of the alley farthest from the experimenter was supplied by an appropriately located mirror.

Imprinting procedure was similar to that used previously. The 14 experimental chicks lived with a surrogate in their home cages and, in addition, were given three 10-minute imprinting sessions in the circular alley at 9, 12, and 15 hours of age. Each chick was removed from its cage to the alley in darkness and allowed to follow the moving surrogate. Mean following distances for all experimental chicks for

the sessions at 9, 12, and 15 hours were, respectively, 164.5, 333.1, and 339.7 feet. Control chicks lived in their home cages during this time.

Stress was introduced at 28 hours of age. Each chick was carried in a metal can, in darkness, to a test box (2 by 2 by 1 foot) containing a doorbell. Half the experimentals and half the controls had a surrogate with them during stress sessions, thus giving four groups (IS, INS, NIS, NINS) as in the first experiment. Stress consisted of a 3-minute session during which the doorbell was rung for 10-second periods interspersed with 10-second periods of silence. After this 3-minute session, chicks were left in the test box for 5 minutes without the bell ringing. Two measures of the response to stress were used: activity-level and vocalization. Since the first of these yielded such low scores from all chicks, it is omitted in presentation of results. Vocalization was scored by a hand-counter in terms of number of "distress" peeps per minute during both the 3-minute sound trial and the subsequent 5-minute trial.

Mean peeps per minute in the two trials are shown in Table 1. A mixed model variance analysis indicated two significant effects: between stress and nonstress condition ($F = 20.26$, $p < .001$) and the interaction between imprinted-nonimprinted and stress-non-stress ($F = 12.48$, $p < .01$). All chicks tended to vocalize more following stress than during stress; this difference was larger for nonimprinted than for imprinted chicks.

Thus, we may conclude that although imprinting does not appear to affect responsiveness to severe stress, it does influence responsiveness to a mild stress inasmuch as imprinted chicks showed a reduced amount of "distress" vocalization following auditory stimulation (4).

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