

to avoidance behavior in a single-unit T-maze. The two species were *L. terrestris*, an earthworm, and *Eisenia foetida*, a manure worm. Fourteen *Lumbricus* and eight *Eisenia* were run to a criterion of 10 consecutive errorless trials (trials without shock).

The apparatus in the experiment was five simple T-mazes, the stems of which were 8 in. long and each arm was 4 in. long. The alleys were $\frac{1}{2}$ in. wide and $\frac{1}{2}$ in. deep. There was a grid consisting of two No. 18 (B&S) copper bell wires placed $\frac{1}{4}$ in. from each other. The nearest wire was 2 in. from the choice point in the right arm. The magnitude of shock was 15 ma at 7.4 v. A piece of 0/0 sandpaper 1 cm by $\frac{1}{2}$ in. was placed 1 cm from the grid on the choice-point side. There was an exit tube at the end of the left arm of the maze. The exit tube was 6 in. long and had a groove $\frac{1}{2}$ in. by $\frac{1}{2}$ in. milled into it. The exit tube was covered with a piece of wood. The floor of the exit tube and the maze were covered with strips of paper towel that were kept moist with biological water.

The experiment was conducted in a dimly illuminated room. With the exception of a white strip 3 ft wide at the bottom of the wall, the walls and ceiling of the room were painted black. Illumination was provided by four 60-w incandescent bulbs placed in a line parallel to the maze stems. The lights were placed so that the nearest maze was 3 ft from them and the farthest was 9 ft.

The procedure was to remove a worm from its glass dish and place it in a maze. If the worm did not start to crawl readily, it was stroked with a camel's-hair brush. A trial ended when the worm had crawled into the exit tube either with or without shock. The worm was allowed to remain in the exit tube for approximately 20 min before the next trial began. Ten trials were given every other day. When a day's trials had ended, the worm was removed from the maze and placed in a refrigerator maintained at 7°C.

The mean number of trials to the criterion of 10 consecutive errorless trials was 60.5 for *L. terrestris* and 69 for *E. foetida*. A test of the significance of the difference between means indicated that there was no reason to reject the hypothesis that there is no difference.

An important difference was noted in the avoidance behavior of the two species of worms. *L. terrestris* gave avoidance responses to the whole maze, including the stem, as evidenced by backing out of the maze and increased response latency. These first signs of avoidance began between trials 30 and 50. On the other hand, *E. foetida* gave avoidance responses to only a limited circumstance, that is, by moving more slowly only when it was in contact with the sandpaper. In both cases it is clear that the avoidance behavior exhibited is learned, since it appears only after considerable experience in the maze.

It would be misleading to point only to the differences in avoidance behavior without noting one important similarity between the species. Animals of both species would occasionally make contact with the

sandpaper and turn around with the pivotal point on the sandpaper rather than make contact and retreat. This turning response is not tropistic and is extremely unstable. The evidence against the response being tropistic is that the worms would cross the sandpaper readily prior to being shocked a few times. It appears to be unstable because it would appear around the 15th trial and might not appear in any other contacts with the sandpaper.

The finding that *L. terrestris* avoids maze cues remote from the noxious stimulation is the same as the result Robinson obtained with the same species. Similarly, the finding of specific avoidance of stimuli in close spatial contiguity to the source of noxious stimulation by *E. foetida* is in accord with what Yerkes said about that species. Consequently, Robinson's experiment with *Lumbricus* is not a proper basis for his criticism of Yerkes' study in which *Eisenia* was used as the experimental animal.

The results of this study suggest that the problem at issue is not whether a two-factor theory is required to account for learning in worms but rather the proper use of species as an experimental condition. The most satisfactory rule that can be stated at present is: The behavior of different species must be regarded as different until it is proved to be the same. Thus, if that rule is accepted, species would constitute a relevant condition in the comparison, control, and evaluation of behavioral data.

References and Notes

1. R. M. Yerkes, *J. Animal Behav.* **2**, 332 (1912).
2. *Eisenia foetida* is also known as *Allolobophora foetida* and *Heliodrilus foetidus*. *Eisenia* is currently used in preference to other generic names.
3. J. S. Robinson, *J. Comp. Physiol. Psychol.* **46**, 262 (1953).

22 November 1954.

Succinic Dehydrogenase Activity in the Goldfish Gill

Alan W. Sexton and Robert L. Russell

Department of Physiology and Pharmacology,
University of Missouri, Columbia

Succinic dehydrogenase has been demonstrated chemically in the rat kidney by Handley and Lavik (1). Mustakallio and Telkkä (2) have localized this enzyme histochemically in the kidney tubule. The former authors have shown that mercurial diuretics significantly depress total succinic dehydrogenase activity and have suggested that this enzyme system may be involved in active reabsorption of sodium chloride and water from the kidney tubule. In the light of this suggestion, it seemed desirable to check for the presence of succinic dehydrogenase in another tissue where active uptake of sodium occurs. Such a tissue is readily available in the gills of goldfish, which have been shown by Krogh (3), Meyer (4), and others to transport the sodium ion against a diffusion gradient.

Table 1. Oxygen uptake by excised goldfish gills in succinate, sucrose, and succinate plus mercuric chloride substrates (microliter of O₂ per milligram of dry weight of gills).

Intervals	Succinate substrate (avg. of 35 fish ± S.E. _m)	Series I	Series II	Series III
		Sucrose substrate (avg. of 11 fish ± S.E. _m)	Succinate plus 10 ⁻⁵ M HgCl ₂ substrate (avg. of 12 fish ± S.E. _m)	Succinate plus 10 ⁻³ M HgCl ₂ substrate (avg. of 12 fish ± S.E. _m)
30 min	1.99 ± 0.21	0.23 ± 0.14	2.20 ± 0.32	1.18 ± 0.14
60 min	3.86 ± 0.22	.41 ± 0.14	3.71 ± 0.42	1.87 ± 0.21
90 min	5.53 ± 0.26	.71 ± 0.26	5.09 ± 0.50	2.24 ± 0.22
"P" for 90-min interval		< .001	0.297	< 0.001

Thirty-five goldfish obtained from a commercial hatchery were used in these experiments. Filaments stripped from excised gill arches were used for experimental tissues. Each fish served as its own control. The Warburg apparatus was used to determine succinic dehydrogenase activity by the method suggested by Umbreit, Burris, and Stauffer (5). Three milliliters of substrate were used in each flask.

In series I, sucrose was substituted for sodium succinate as a substrate. In series II and III, sodium succinate was used as a substrate. In series II, mercuric chloride was made up in succinate substrate and placed in the side arm of the flask. Its concentration was such that when tipped into the flask it produced a concentration of 10⁻⁵M mercuric chloride for the entire substrate. The same procedure was followed for series III except that the final concentration of mercuric chloride in the substrate was 10⁻³M. In series II and III, unaltered succinate substrate was tipped into the control flasks. After 20 min for equilibration, readings were taken at 0-, 30-, 60- and 90-min intervals. All tip-ins were made at the 30-min interval. Calculations are based on the percentage dry weight of the gill tissue.

The results of these experiments are presented in Table 1. The first column gives the average oxygen uptake for all gills run in the pure succinate substrate. The "P" values presented are for the 90-min interval and compare the oxygen uptake in the succinate substrate with oxygen uptake in the sucrose substrate, succinate plus 10⁻⁵M mercuric chloride substrate, and succinate plus 10⁻³M mercuric chloride substrate, respectively.

The significantly greater uptake of oxygen by gill filaments in a succinate substrate (5.53 µlit of O₂ per milligram of dry weight) as compared with the uptake in sucrose substrate (0.71 µlit of O₂ per milligram of dry weight) at the 90-min interval is considered proof of the presence of succinic dehydrogenase in the gills of goldfish. According to Barron and Kalnitzky (6), mercuric chloride inhibits the activity of this system by combining with essential sulfhydryl groups. Such an inhibition was found to occur when 10⁻³M mercuric chloride was added to the substrate (series III). The uptake of oxygen was reduced

59.5 percent. Since Meyer (7) has inhibited active uptake of sodium in the goldfish gill with mercuric chloride, it is suggested that this work offers additional evidence in favor of the theory that succinic dehydrogenase is involved in active sodium transportation.

References

1. C. A. Handley and P. S. Lavik, *J. Pharmacol. Exptl. Therap.* **100**, 115 (1950).
2. K. K. Mustakallio and A. Telkkä, *Science* **113**, 320 (1953).
3. A. Krogh, *Osmotic Regulation in Aquatic Animals* (Cambridge University Press, Cambridge, Eng., 1939).
4. D. K. Meyer, *Am. J. Physiol.* **165**, 580 (1951).
5. W. W. Umbreit, R. H. Burris, and J. F. Stauffer, *Manometric Techniques and Tissue Metabolism* (Burgess, Minneapolis, 1949).
6. E. S. G. Barron and G. Kalnitzky, *Biochem. J.* **41**, 346 (1947).
7. D. K. Meyer, *Federation Proc.* **11**, 107 (1952).

29 November 1954.

Determination and Inheritance of Nicotine to Nornicotine Conversion in Tobacco

R. B. Griffith, W. D. Valleau, G. W. Stokes
*Agricultural Experiment Station,
University of Kentucky, Lexington*

The presence of a genetic factor in certain low-nicotine strains of tobacco controlling conversion of nicotine to nornicotine during air curing has been reported by Valleau (1). The change of a portion of the nicotine to nornicotine reduces the alkaloid content of the smoke, since the transfer of nornicotine into the smoke is less than one-fourth of the transfer for nicotine (2). Utilization of this genetic factor in commercial varieties could be advantageous, since, with acreage control, overfertilization of burley tobacco, in an effort to increase yields, has tended to raise the nicotine content of some crops to an undesirable level.

The development of the following paper chromatography method, based in part on the work of others (3), has permitted extensive investigations (4). In this method, 1 g of a finely ground tobacco sample was placed in a 15-ml centrifuge tube. Five milliliters