use of the Mann-Whitney U-test, a nonparametric alternative to the t-test (*5*), confirms the significance of the difference in learning in the two groups. Analysis of brush and flashlight applications reveals that the evening group required significantly ($p < 0.05$) less stimulation per trial than did the morning group. Stimulation-per-trial measures were used because of the significant differences in the total number of trials that the two groups were run.

Since the morning group was run by one experimenter (J.P.M., *2*) and the evening group by me, an additional four animals were run in the evening by J.P.M. to insure that the procedure for running and stimulating animals was the same for both experimenters. No differences were noted between these four worms and the six others run in the evening group on any of the measures.

No specific control over temperature cues was exercised in this study; a record was kept, however, of the temperature of the room at the time of the first trial on each day for each worm. On the basis of the mean temperature for each worm, the 12 subjects were divided into a high-temperature group of six and a low-temperature group of six. No significant difference in the number of trials necessary to achieve the learning criterion was found between these two groups. Although this does not completely preclude the possibility that temperature may be an important variable (since there may have been temperature fluctuations during the five trials on any one day)—in particular, a temperature effect interacting with the time parameter—the experimental conditions of the present study did not lend themselves to greater control.

It may be concluded that the diurnal cycle plays an important role in maze learning in earthworms. This cycle appears to carry over to a learning situation where light-dark cues are eliminated from the environment. Wells (*6*) has noted the existence of diurnal activity cycles in the lugworm which continue for several days after the animal is placed in an environment free of diurnal cues, while other investigators report this persistence of cyclical activity in other species (*7*). It is possible that, like the feeding and irrigation cycles of the lugworm, the earthworm diurnal cycle is controlled by an "internal pacemaker."

At present there is relatively little evidence concerning the neurological structures which may act as pacemakers in regulating the biological activity of the worm. Scharrer and Scharrer (*8*) have noted the existence of neurosecretory cells in the brain of the earthworm; the function of these cells is as yet undetermined. Ziller-Perez (*9*) has indicated that the chromaffin cells in certain species of worms secrete an adrenalinlike substance. A tentative hypothesis might attribute the cyclical activity of the earthworm to the products of these cells. This hypothesis, however, sheds no light on the basic problem of the manner in which the cyclical pattern is imposed on the functioning of these cells.

Just as Schmidt (*1*) has shown that one cannot generalize from the avoidance behavior of one species of worm (*L. terrestris*) to the avoidance behavior of another species (*Eisenia foetida,* a manure worm), this study shows that time of learning relative to the worms' activity cycle is an important variable in research with Annelida.

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Extension of a Tracheole into Cytoplasm

In the course of an electron microscope study of spermatogenesis in the grasshopper (*Melanoplus differentialis*), a cell of the testicular theca was observed which is penetrated by a tracheole (*1*). The electron micrograph (Fig. 1) is presented because it illuminates two points: the structural details of the terminal portion of the tracheole and its position in relation to the cell.

The tracheole, which seemingly penetrates the cytoplasm of the thecal cell, clearly has taenidia (*TN*). This confirms the findings of Keister (*2*), Richards and Korda (*3*), and Beams and Anderson (*4*) that all respiratory tubes of the insect species examined possess taenidia. Furthermore, the taenidia are accompanied by cytoplasm. Buck (*5*), Keister (*2*), and Beams and Anderson (*4*) have observed tracheoles that emerge from their originating cells accompanied by protoplasmic processes. At the junction of the cytoplasm of the thecal cell and that of the tracheole is a double membrane, of which one part (*CL*) appears to be a limit of the cell cytoplasm and the other (*TCL*), the limit of the cytoplasm associated with the tracheole. At the region *D* each membrane appears to be double.

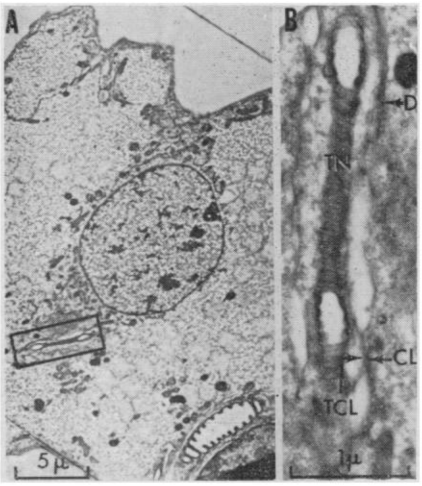

Fig. 1. (*A*) Thecal cell from grasshopper testis, penetrated by a tracheole (× 1850). (*B*) Enlargement of the tracheole-entered area revealing taenidia (*TN*), thecal cell membrane or limit (*CL*), and tracheolar cytoplasmic limit (*TCL*). At various regions (for example, *D*) each limit appears to be composed of a double membrane (× 15,000).

In this species, the terminal portion of the tracheole has an intima with recognizable taenidia, whose lumen measures 0.30 μ in diameter. In addition, beyond the limits of the taenidial border, this tracheole is surrounded by cytoplasm and has its own limiting membrane. The over-all diameter of the tracheole is about 0.67 μ. There is also evidence here that the tracheole does not end on the peripheral surface of the thecal cell but extends into the interior. However, the presence of the double membranes suggests that the tracheole has assumed its position, not by actual intracellular penetration, but by causing or following an invagination of the thecal cell membrane to the inner portion of the cell.

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