THE REGULATION OF THE ACTIVITY RHYTHM OF THE CRAB (TRICHODACTYLUS PETROPOLITANUS)

by

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INTRODUCTION

A considerable amount of work has been accomplished in the last twenty years in respect to the coordinating systems of crustaceans, principally in reference to the molting process. Thus the eyestalk has been the object of a number of experiments designed to study the true functions of the sinus gland and X-organ, since their discovery by HANSTROM (1931; 1933; 1939). ABRAMOWITZ and ABRAMOWITZ (1939) showed that extirpation of the eyestalks provoked precocious molting, and were led to conclude (1940)that removal of the eyestalks of *Uca pugilator* accelerates molting and shortens the interval between the first and second molt but does not influence copula or the shedding of the eggs. Bilateral eyestalk extirpation in *Cambarus* results in gastrolith formation whereas sinus gland implantation in an eyestalkless animal retards the formation of gastroliths (SCUDAMORE, 1947) Eyestalkless animals show a diurnal rhythm in the deposition of the calcium of the gastrolith (SCUDAMORE, 1947). Eyestalk removal also influences water metabolism (GUYSELMAN, 1953) and the metabolism of inorganics, as shown by several authors (SCUDAMORE, 1942; 1947; DRACH, 1944; and BAUCHAU, 1948, 1949)

The eyestalk hormone has been shown to exert a considerable influence in metabolism, principally respiratory metabolism (SCUDAMORE, 1942; 1947; BAUCHAU, 1948; 1949; EDWARDS, 1950; BLISS, 1951; 1953). Normally in *Uca* the *oxygen* consumption is greater during the night than during the day (EDWARDS, 1950; BROWN, 1954) In *Uca* there appear to be two rhythms of respiratory exchange, one of 12.4 hours and the other of 24 hours duration. The former corresponds to the tidal rhythm; the oxygen consumption and tidal rhythms coming into phase each 15 days. The 15 day rhythm is independent of temperature but may be changed by illumination shifts. The daily rhythm can be modified by extreme changes in temperature or changes in illumination at "sensitive times" of the cycle (BROWN, 1954). Removal of the eyestalks causes an increase in rate of oxygen consumption (SCUDAMORE, 1942; 1947; EDWARDS, 1950), and injection of eyestalk hormone or sinus gland implantation causes a decrease towards normal in eyestalkless animals (SCUDAMORE, 1942; 1947; EDWARDS, 1950)

The eyestalk hormone has also ben shown to be concerned with the control of activity KALMUS (1938) demonstrated a decrease in motor activity and loss of diurnal rhythm following extirpation of the eyestalks of *Potamobius* and *Cambarus*. On the other hand, Roberts (1941) showed a decrease in activity in *Cambarus* following the injection of eyestalk extract, and SCHALLEK (1942) found that removal of the eyestalks of *Cambarus* augmented activity In three species of the normally nocturnal *Uca*, EDWARDS (1950) showed that eyestalk removal causes a decrease in coordinated motor activity, elimination of the normal rhythm, and that injection of eyestalk hormone or sinus gland implantation in the eyestalkless animals evokes an increase in activity once again.

According to PASSANO (1953) the X-organ is a neuro-secretory gland, i. e. it consists of neurons which have a secretory activity and that these axons are connected directly with the sinus gland. Thus there exists a similarity between the endocrine systems of the insects and crustaceans, a fact which lends support indirectly to the concept of BARGMANN and SCHARRER (1951) of the neuro-secretory nature of the hypothalamus-hypophysis complex of vertebrates.

In view of the above, we have initiated a study of the control of coordinated motor activity and related processes in the fresh water crab, *Trichodactylus petropolitanus,* in order to compare the responses of this species with those previously studied animals, and with the hope of contributing to the knowledge of the site and function of the eyestalk hormone. To the present we have studied the influence of eyestalk removal upon coordinated motor activity

MATERIAL AND METHODS

A. ANIMALS:

The animal used in the present study was *Trichodactylus petropolitanus* GOELDI, Crustacea, Decapoda. These crabs are common to the waterways of the south of Brazil and occur in large numbers in the Tieté river and its tributaries in the vicinity of São Paulo. In the laboratory the animals were maintained in glass aquaria in tap water which was constantly renewed and aerated by means of a compressor. The temperature within the aquarium was kept equal to that of the natural environment of the crab, i. e. approximately 22 degrees C. During the course of the registration of activity the animals were unfed.

B. MEASUREMENT OF COORDINATED MOTOR ACTIVITY:

One of the simplest criteria of activity is that of the coordinated walking movements. Such activity can be measured easily over a long period of time by means of a simple, plastic wheel (EDWARDS. 1950), containing a sanded inner surface to provide traction, a number of orifices for ventilation and a small door for the entrance of the animal. The wheel turns on an axle supported by two metal arms. The entire mechanism is so evenly balanced that a small crab can easily turn it in normal walking, even with the addition of the small amount of water necessary for aquatic or semi-aquatic animals. The movement of the wheel is registered graphically by means of a bristle glued to the outer surface of the runway normal to the long axis of the wheel and writing tangentially on a kymograph made from a converted 8 day barograph. Knowing the circumference of the wheel, the distance walked by the animal is easily calculated. In our experiments the wheels had a circumference of 50 cms. With such instruments we measured the activity of normal, one-eyed, and eyestalkless crabs.

C. ABLATION OF EYESTALKS:

Before operation the crabs were placed at 10°C for some minutes in order to avoid excessive bleeding. This method has the advantage of increasing the viscosity of the blood and decreasing the coagulation time. Six to 12 hours were permitted to elapse between the cutting of the first and second eyestalks, and several hours elapsed between the second operation and the measurement of activity No animals died from operations performed in this manner.

The eyestalks were collected in a desiccator in the refrigerator at 3°C. Extraction of the active principle of the eyestalk tissue was as follows. When the eyestalks were thoroughly dried, they were triturated and dissolved in hot ethanol and then filtered, the resulting filtrate being allowed to crystallize by evaporation. Extraction and crystallization were repeated three times. The final crystals were dissolved in saline and conserved in the refrigerator until the moment of use. For the replacement experiments, o.1cc. of a solution equivalent to 20 eyestalks per cc. were injected, giving a concentration of the equivalent of 2 eyestalks per animal.

TABLE ONE

COORDINATED MOTOR ACTIVITY OF TRICHODACTYLUS PETROPOLITANUS

(WALKING TIMES)

Animal	Start	End	Conditions
I	1830	0815	normal
II	2045	0645	normal
III	1800 1806 1807 1749	0530 0531 0407 0619	normal
IV	2057 1854	0809 0627	normal
V	2026 2038 1945 1912 2036	0356 0814 0642 0712 0624	normal
average	1916	0704	
VII VIII IX X XI	continuous and arrhythmic		one-eyed " " " " " " eyestalkless

RESULTS

Trichodactylus is normally nocturnal. Registering graphically, as described, the coordinated walking activity of 5 crabs it was found that, on the average, the crabs began the evening activity at 1916 hours and stopped walking at 0704 hours in the morning. Generally there was no recordable activity during the day (Graph 1, Table 1) If, however, some disturbance occurred during the day, e. g. opening the door of the experimental room, or a sharp noise, etc., the crabs walked several turns of the wheel before becoming once again completely inactive. The activity remained the same in constant light or constant darkness. The activity at night appeared to continuous, i. e. few breaks in the curves are observable. The distance covered by the crabs averaged 210 meters per night.

Upon removal of one eyestalk the animals became rigid, were inclined to stand high upon outstretched legs and demonstrate quivering and other non-coordinated movements. Inmmediately after operation walking activity was considerably less than normal. Several hours after the operation the animals began a continuous, but very slow and arrhythmical, walking activity, i. e. were active both day and night with no particular rhythm but the activity was considerably diminished (Graph 2). This type of activity persisted for the seven days of the experiments.

Removal of the second eyestalk did not change the activity evidenced by the one-eyed animal, i. e. the activity continued arrhythmic and continuous for the seven days following operation. It is interesting that one animal so treated showed an increase in activity on the 4th day and an almost normal amount of activity on the 5th day, but still of the continuous, non-rhythmic type (Graph 3).

Experiments have been initiated on the influence of injection of eyestalk hormone upon the activity of the various animals. Injection of ESH at 5 pm into an eyestalkless animal that had been walking continuously caused an immediate cessation of activity, the effect lasting 24 hours. Three days later, when the initial type of activity had returned, a second injection was given at 6 pm, once more causing a cessation of activity for 16 hours (Graph 4). As a control on this experiment, ESH was also injected into a normal animal at 8 pm. As above, the animal ceased walking for 24 hours (i. e. the normal nocturnal activity was eliminated) and the activity thereafter remained erratic for 4 days, at the end of which time the animal once again began to show the normal nocturnal rhythm (Graph 5)

DISCUSSION

The graphs show that *Trichodactylus* is normally nocturnal in its habits and that the rhythm of coordinated motor activity continues under a variety of conditions in the laboratory, such as normal dark and light, constant illumination and constant darkness. Thus, *Trichodactylus* is similar in its activity to the North American *Cambarus* of fresh waters, and to the marine and estuarine species of *Uca*. The laboratory records coincide with the observations in nature, where it is found that the animals remain quiet and hidden during the day and are active in hunting and feeding at night. For example, trapping the animals with nets or traps is practically useless during the day, never more than one or two being caught; whereas in the same locale one can catch 50 to 100 animals by the same means in the evening, i. e. 7 to 11 pm. We have not seen in our animals any evidences of crepuscular activity rhythms, or bimodal rhythms of activity such as found by BROWN (1954) in the rhythms of color change and oxygen consumption in *Uca*, or by EDWARDS (1950) in the walking activity of certain individuals of various species of *Uca*. This may well be related to the fact that *Uca* is marine and hence would more likely have its activities related to the tidal rhythms and cycles, whereas *Trichodactlyus* is strictly a freshwater crab. It would certainly be most interesting to determine with more exact means if comparable rhythms may exist in both the freshwater and marine forms in related species or groups.

The control of activity in *Trichodactylus* seems to be by means of the same system as that in other crustaceans so far investigated. i. e. by means of a substance produced in the eyestalk. Removal of either one, or both, eyestalks causes a loss of rhythm and a decrease in amount of activity thus causing the animal to walk continuously at a very slow rate. Injection of eyestalk extract causes a return toward the normal in the eyestalkless animals, and in normal animals causes a diminution of normal nocturnal activity. Our experiments have not yet shown the locale of active production of hormone, but it is assumed that the substance is a product of the sinus gland — X organ complex of the eyestalk. Further experimentation is underway to determine the site of secretion and to study the regulation of other processes, e.g. oxygen consumption, that appear to be under the control of the eyestalk hormone.

SUMMARY

An apparatus has been constructed, consisting of a plastic running wheel and barograph converted into 8 day kymograph, for the registration of the walking activity of the fresh water crab, *Trichodactylus petropolitanus*.

Trichodactylus is normally nocturnal, and its rhythm of activity is unchanged in constant darkness or constant illumination.

Removal of one or both eyestalks causes a decrease in total activity and interrupts the normal 24 hour rhythm.

Injection of eyestalk extract into eyestalkless animals causes a return toward normal in amount, but not rhythm, of activity.

Injection of eyestalk extract into normal animals during the period of evening activity causes a cessation of the activity

Trichodactylus resembles *Cambarus* and *Uca* in its activity and in the hormonal regulation of this activity

SUMÁRIO

Para registro da atividade locomotora do caranguejo de água doce, *Trichodactylus petropolitanus* foi construido um aparelho compreendendo uma roda plástica e um barógrafo adaptado para registro em 8 dias.

Trichodactylus normalmente mostra atividade noturna e seu ritmo de atividade é invarável no escuro constante e na iluminação constante.

A remoção de um ou ambos os pedúnculos oculares causa uma diminuição na atividade total e interrompe o ritmo normal de 24 horas.

Injeção de extrato de pedúnculo em animais sem os dois pedunculos causa a volta, em quantidade, ao normal, mas não restabelece o ritmo de atividade.

Injeção de extrato de pedúnculo em animais normais, durante o período de atividade locomotora, provoca a parada da atividade.

Trichodactylus, assim, assemelha-se na sua atividade e na regulação hormonal a *Cambarus* e *Uca*.

ACKNOWLEDGEMENTS

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GRAPH ONE

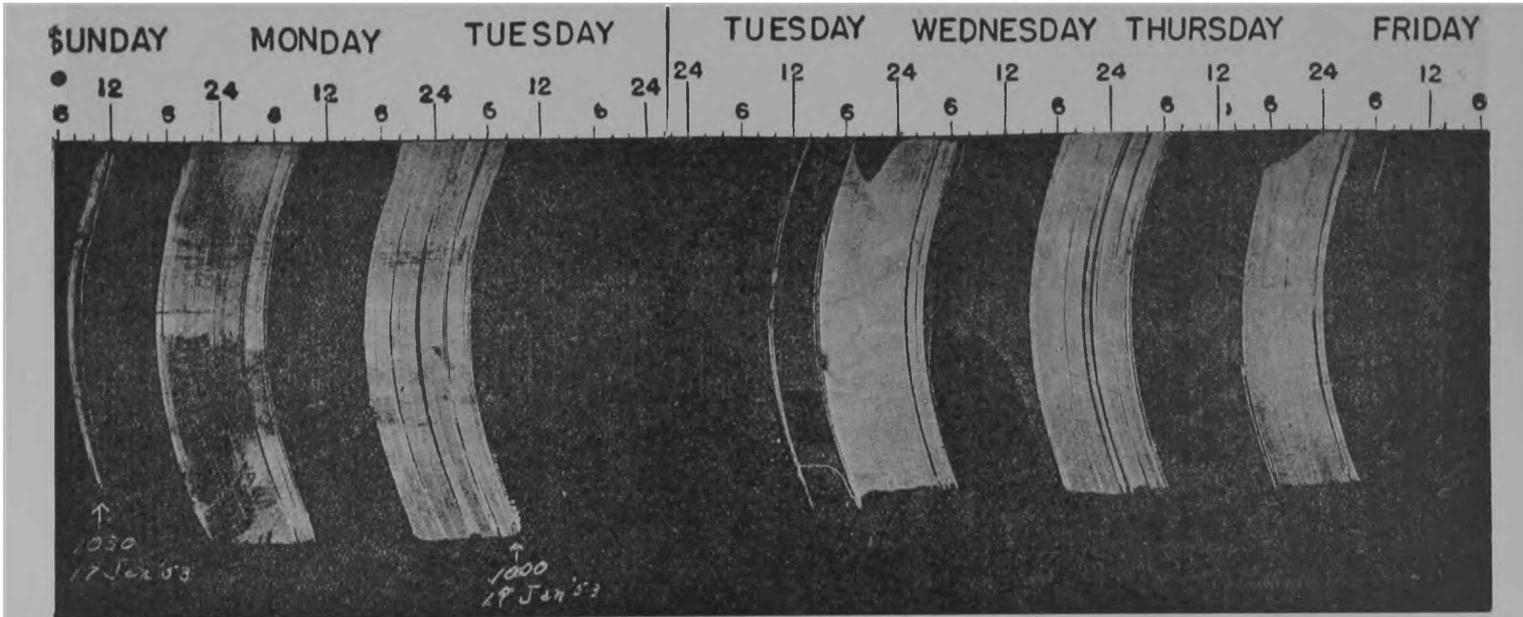

Fig. 1. — Graphs of normal walking activity of 2 *Trichodactylus*.

GRAPH TWO

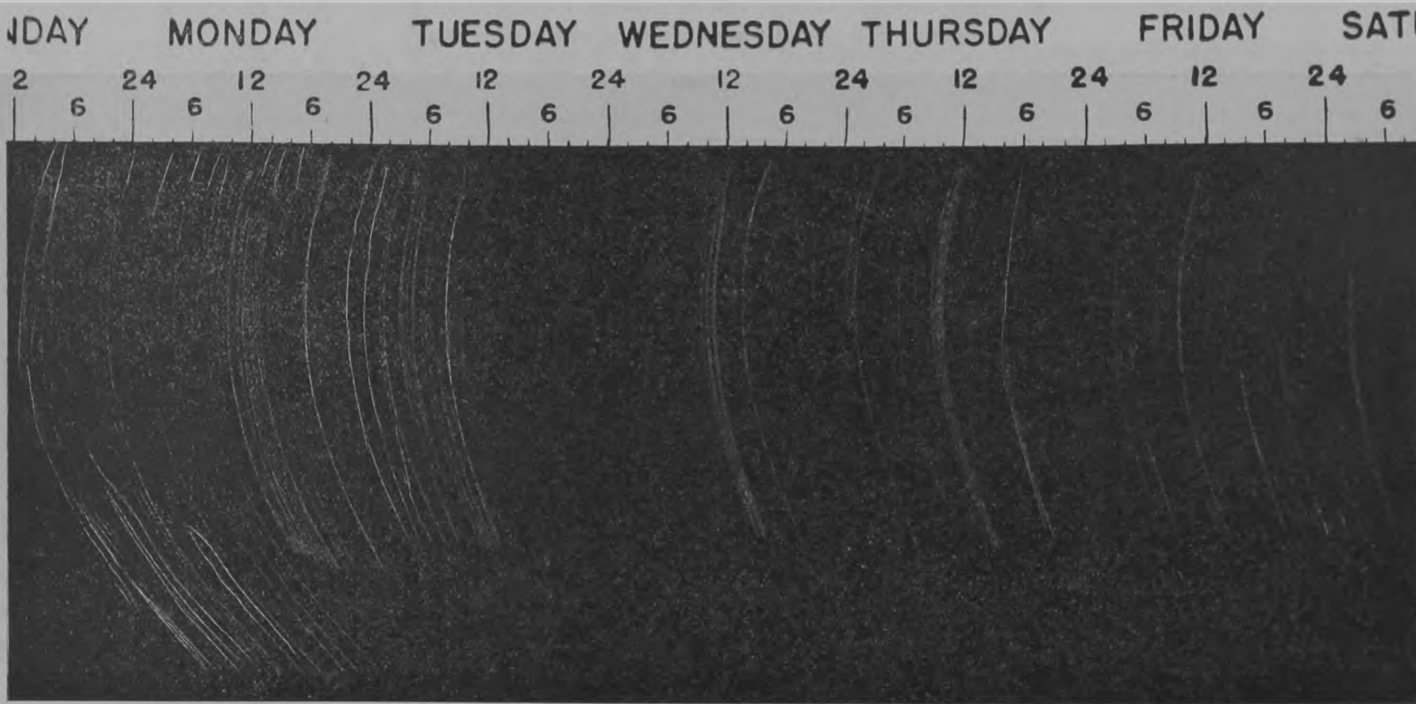

Fig. 2. — Graph of walking activity of *Trichodactylus* with one eyestalk removed.

GRAPH THREE

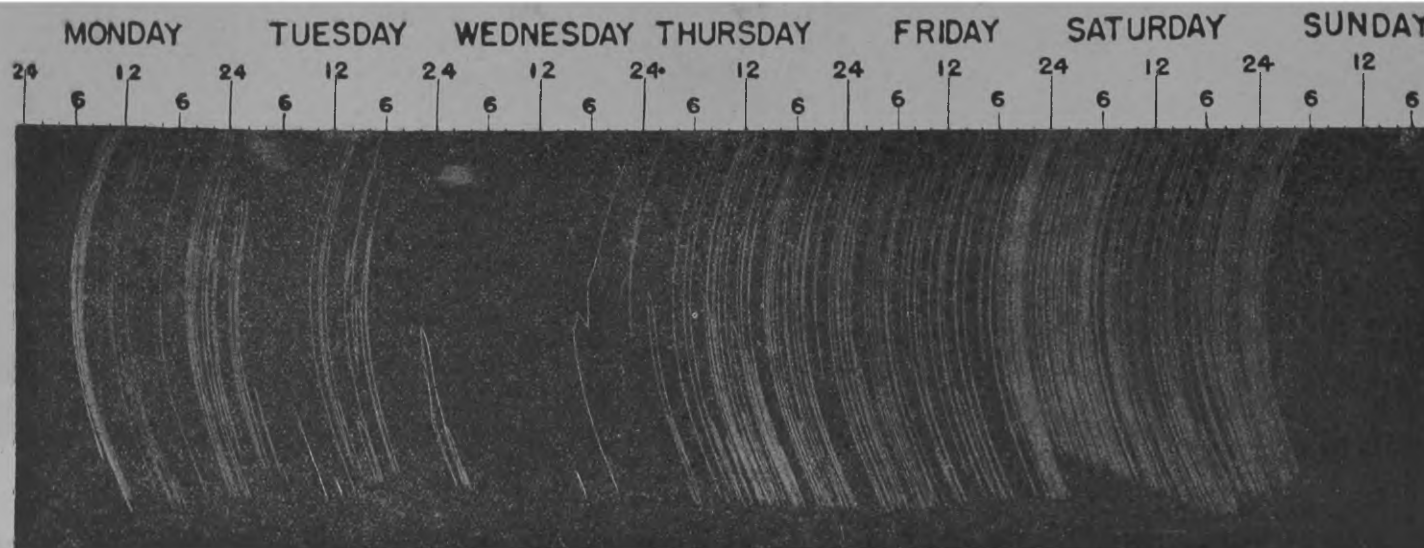

Fig. 3. — Graph of walking activity of *Trichodactylus* with both eyestalks removed.

GRAPH FOUR

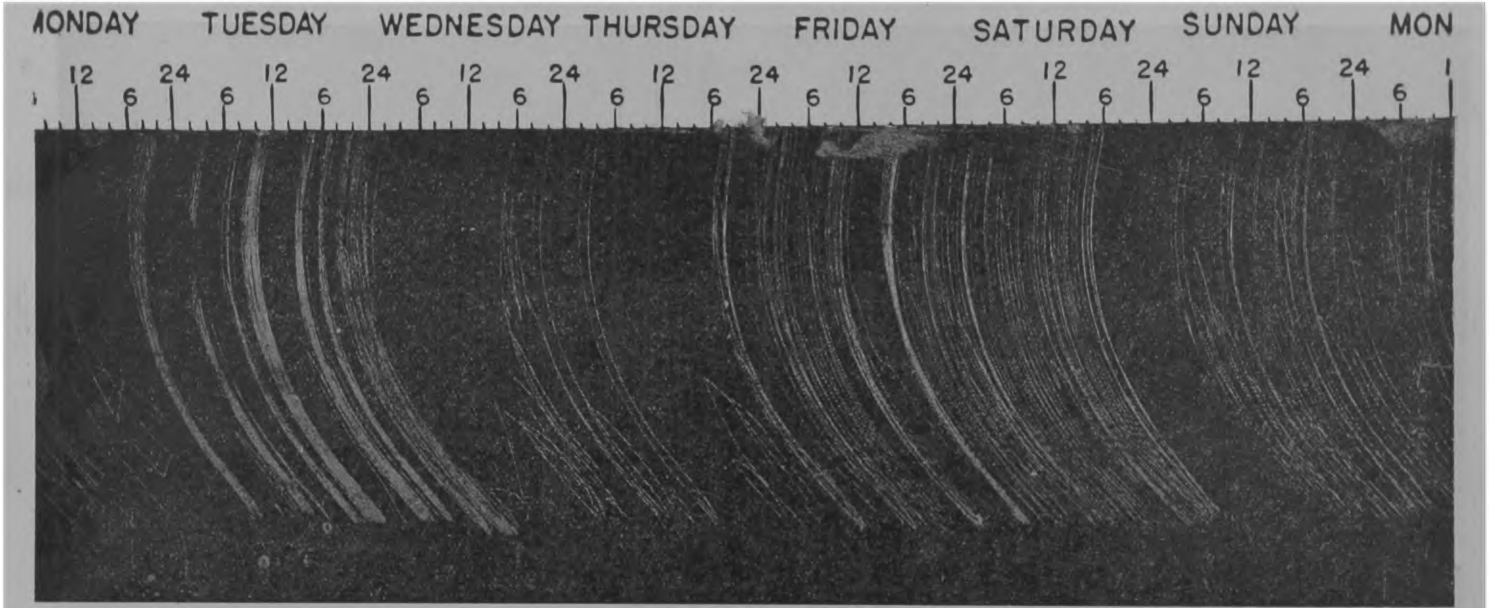

Fig. 4. — Graph of walking activity of *Trichodactylus* with both eyestalks removed and with ESH injected twice during the week, causing a cessation of activity.

GRAPH FIVE

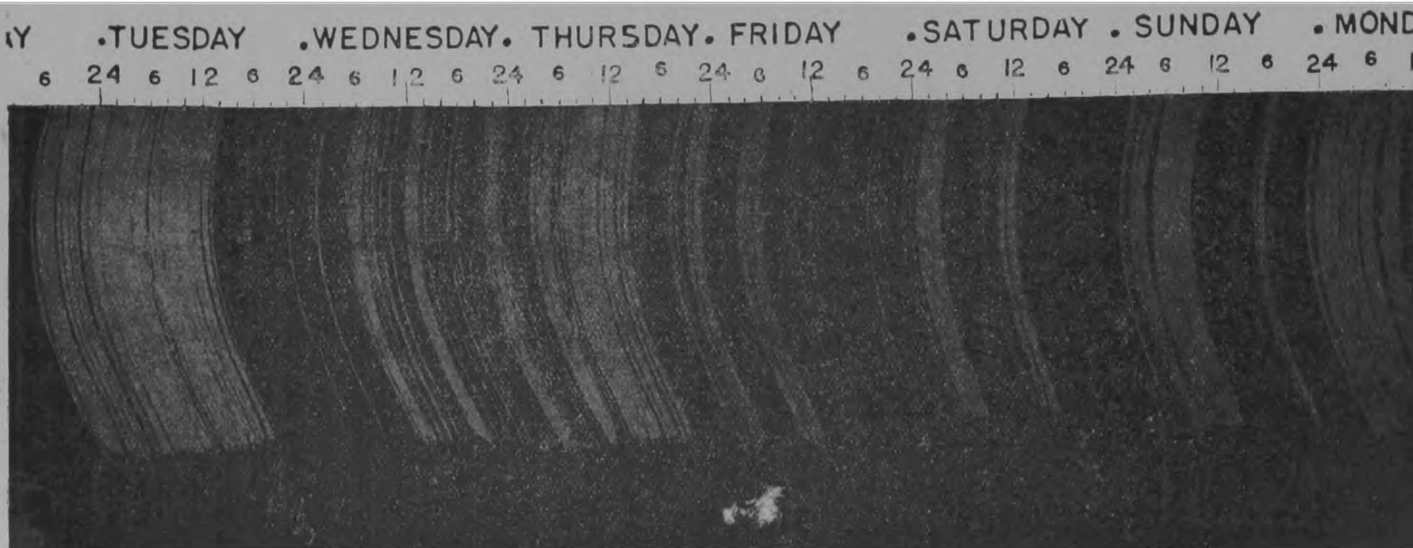

Fig. 5. — Graph of walking activity of normal *Trichodactylus* showing influence of injection of ESH during period of normal activity.