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The Role of Cerebral Ganglia in the Regulation of Locomotor Activity of Ascarids

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Abstract. The paper contains the results obtained in experiments on the role of cerebral ganglia of ascarids in the regulation of their locomotor activity. The experiments, which were performed by various methods (kymographic tracing, visual observation and electrical excitability) showed that cerebral ganglia of ascarids effect the regulation of locomotor activity, promoting and stimulating a constant origin of serpentine movements by means of which the forward progression of the helminth is accomplished.

The studies on locomotor activity of ascarids are of great importance as these nematodes are capable of active movement along the host's intestine (EROPHEEV 1936, KOTLIARCHUK 1936, GESELEVICH 1951, SAVCHUK 1955, KRAVETS 1957, SHISHOV 1961a) and sometimes penetrate to unspecific places of localization, inducing grave afflictions. It is well known that the anthelminthics (santonin and piperazine) used in ascaridiasis are effective only because they upset the coordination of movements of helminths (KROTOV 1957a, b). Nevertheless, only two papers (SHISHOV 1961b and KROTOV 1965a) have been devoted to the study of this problem. Both authors obtained different results and arrived at contrary conclusions.

While studying the regulation of locomotor activity of ascarids SHISHOV observed that by removing the cerebral ganglia incessant long-lasting regular contractions occurred in most experiments. However, the author does not mention the cases when no regular rhythm occurred after the anterior end had been removed and assumes that cerebral ganglia of ascarids form the economical periodic character of movements by force of inhibiting regulatory processes through which these movements are accomplished. On the other hand, KROTOV's data showed that by tying off and removing the anterior extremities of 100 ascarid females the changes described by SHISHOV occurred only in 14 specimens, while in 86 specimens the rhythm slackened and nearly ceased completely. In view of the above-said it was expedient to repeat the experiments by using methods of both authors as well as some additional ones.

MATERIAL AND METHODS

The ascarids of swine *(Ascaris suum)*, collected at a meat-packing plant, were used as the objects of our studies. The experiments were conducted during the first 24 hours following the collection of nematodes. The investigation on the locomotor activity was carried out in a horizontal as well as vertical bath of a helminthograph (Krotov, Olenin 1961) in Krotov's solution (1953). The tracing of locomotor activity was taken:

a) in a vertical bath after Krotov's method, when serpentine movements were recorded from the anterior end of the body and tonic contractions from the posterior end of the helminth (Fig. 1A);

b) in a horizontal bath after Shishov's method, when the helminth was placed in a narrowf horizontal chamber and pinned down about 7–8 mm behind the lips. From the posterior end of its body a general contraction was recorded, while the serpentine movements were traced by a recorder connected with the anterior end of the body (Fig. 1B):

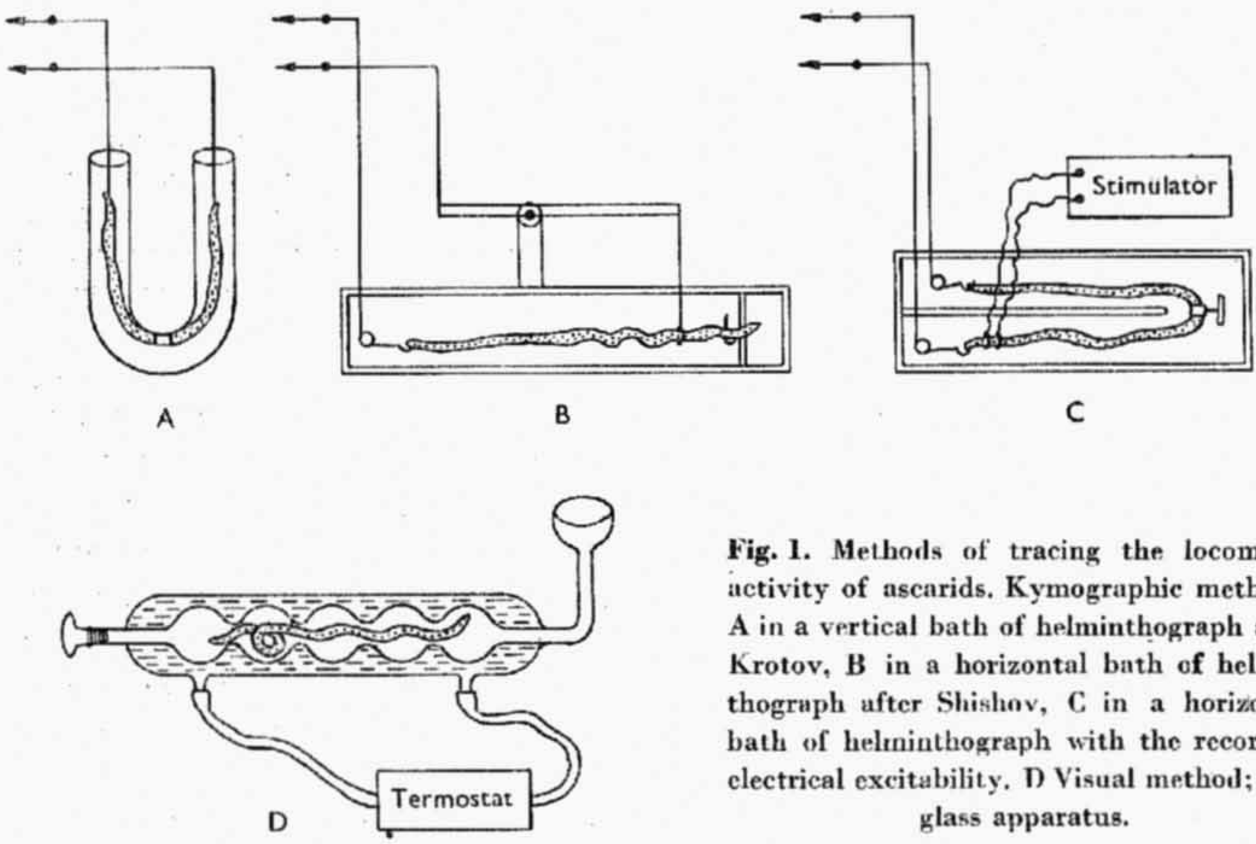

Fig. 1. Methods of tracing the locomotor activity of ascarids. Kymographic methods: A in a vertical bath of helminthograph after Krotov, B in a horizontal bath of helminthograph after Shishov, C in a horizontal bath of helminthograph with the record of electrical excitability. D Visual method; in a glass apparatus.

c) in a horizontal bath while determining the changes of the excitability of ascarids. A loose ligature was tied around the centre of the body, while the anterior and posterior parts of the body were connected with two myographs (Fig. 1C) by means of threads provided with hooks caught in the cuticula. Tracings were taken of contractions by which the ascarids responded to the stimulation induced by silver chloride electrodes connected to a box of organic glass which was affixed to the anterior extremities of the nematodes immediately next to the oesophagus. The interpolar distance was 5 mm. A stimulator ICE-01 was used for stimulation. The period of stimulation was 5 seconds;

d) by visual observations of the movements of ascarids.

The helminths were placed in a special apparatus consisting of two-polar non-communicating glass tubes 60 cm long, inserted into each other. The outer tube was connected with an ultrathermostat by a rubber pipe through which water heated to 38 °C was advancing to it. The inner tube was filled with saline solution in which the ascarids were suspended (Fig. 1D).

While using all above-mentioned methods the relative standard of locomotor activity of ascarids was recorded for 20—30 minutes and then in a series of experiments the anterior end was severed either by applying a ligature 5 mm behind the lips and by cutting it off before the ligature, or by injecting into the anterior end 0.001 mg of nanofin or 0.005 mg gamma-aminobutyric acid diluted in 0.01 ml of Krotov's saline solution. Afterwards a kymographic record was taken or visual observations were made for 1—2 hours. It was established that the mentioned amounts of nanofin and gamma-aminobutyric acid produce the cessation of movements of the anterior end, but do not influence the locomotor activity of nematodes, if injected into the body cavity. In the control experiment the same volume of liquid devoid of preparations was injected into the anterior end or into the body cavity of the ascarids. No changes were observed

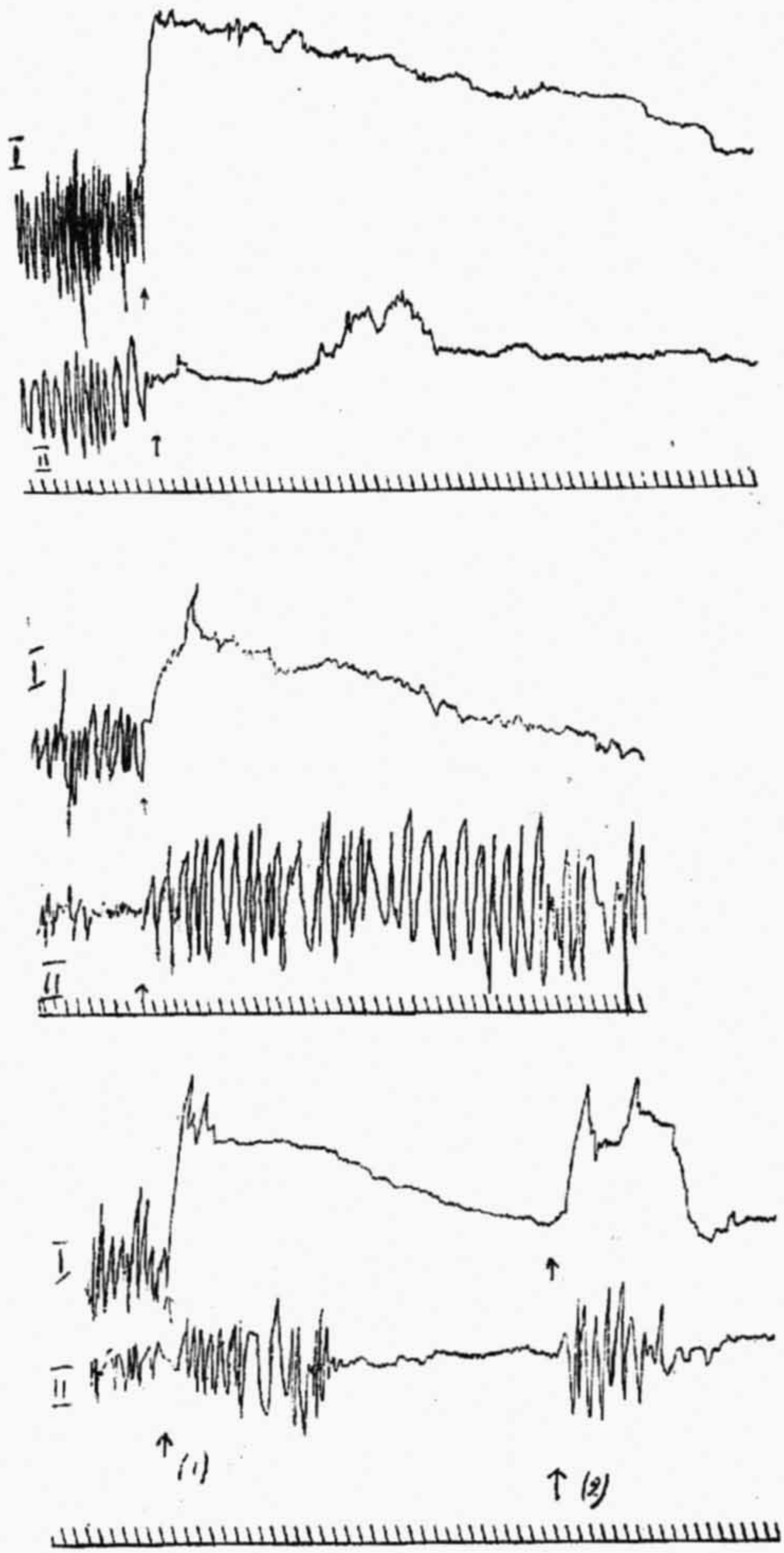

Fig. 2. Changes of the locomotor activity of ascarids after the removal of anterior end (Shishov's method), a) cessation of rhythmic contractions, b) emergence of constant serpentine movements of the body, c) effect of repeated load of ligature (2) after the removal of anterior end (1) upon the tonus and serpentine movements of the body; I — recording of total length of the body, II — recording of serpentine movements of anterior third of the body; arrows indicate the moment of application of ligature and removal of anterior end. Time unit — 1 minute.

in the moving reactions of the helminths. Each series of experiments was carried out with 50 ascarids. A total of 400 experiments were conducted and the results obtained on the excitability of the ascarids were assessed statistically.

RESULTS

The data obtained in experiments carried out by method of horizontal tracing (Shishov 1961b) have shown that in 45 out of 50 experiments after the ligature had been applied and the anterior end removed a considerable decrease of amplitude and less frequent serpentine movements of the body occurred in the helminths, along with a sharp spasmodic increase of tonus (Fig. 2a). In five cases only regular serpentine movements instead of irregular moving activity as observed in standard, occurred after the anterior end had been removed (Fig. 2b). Of the 50 experiments carried out by method of vertical tracing (Krotov 1965b) the changes in locomotor activity described by Shishov occurred in 11 cases only. In individual cases the incessant rhythm induced after the application of ligature gradually subsided. It should be noted that a sharp increase of tonus was observed after the application of ligature even in ascarids with already cut-off anterior extremities (Fig. 2c).

After injecting 0.001 mg of nanofin into the anterior end a marked change of moving activity occurred in the form of slow irregular contractions interrupted by longer and longer intervals, while the body tonus was somewhat decreasing. The ligature applied thereafter evoked a sharp increase of tonus similar to that observed in ascarids with cut-off anterior extremities (Fig. 3a). After injecting 0.005 mg of

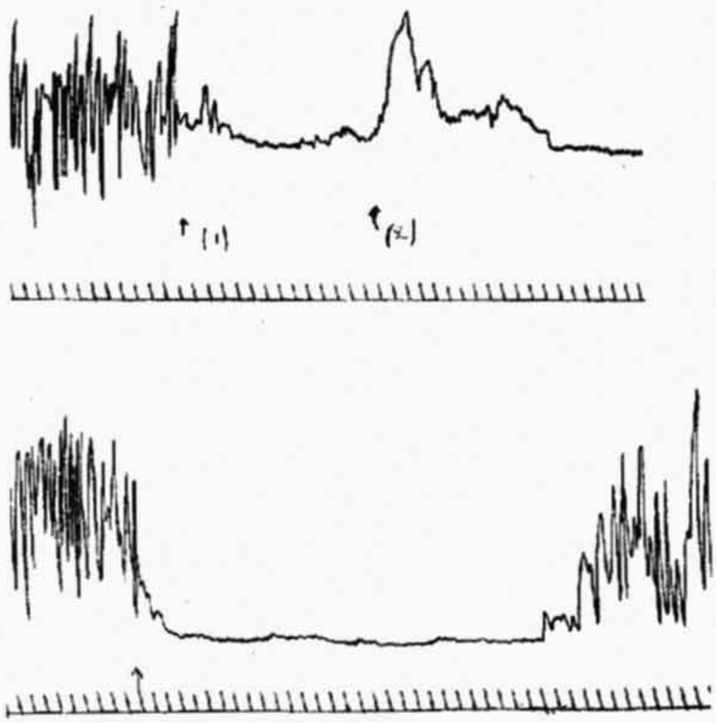

Fig. 3. a) Suppression of locomotor activity of ascarids following the injection of 0.001 mg of nanofin into anterior end (1). Increase of tonus after subsequent application of ligature (2) (Krotov's method). b) Suppression and subsequent restoration of locomotor activity of ascarids after injecting 0.005 mg gamma aminobutyric acid into anterior end (Krotov's method); time unit—1 minute.

gamma-aminobutyric acid into the anterior end all types of moving reactions ceased immediately and were restored in 15—20 minutes (Fig. 3b).

By measuring the excitability of intact ascarids it was established that in 25 experiments the average threshold for response of the anterior third of the body was 0.55 + 0.034 V and of the whole body it was 1.86 ± 0.17 V. The excitability of the anterior end measured immediately after the application of ligature was unstable, but in most experiments it was somewhat decreased and on average equal to 0.7 V. Despite the prevalent values testifying to the decreased excitability, the mathematical evaluation indicated that the results obtained were unauthentic. The excitability of the anterior end of body measured after the application of ligature and removal of the anterior end showed that this value decreased 2—2.5 times only 30 minutes after the anterior end was cut off ($P < 0.01$), while the excitability of the whole body did not differ from the standard ($P < 0.5$). The excitability of the anterior end measured 5 minutes after nanofin and gamma-aminobutyric acid had been injected, decreased to 1—1.6 V ($P < 0.01$), while the excitability of the whole body remained unchanged ($P < 0.05$) (Tab. 1).

Table 1. Changes of the electrical excitability of anterior third and of the whole body of ascarids after the removal of anterior end (average of 25 experiments)

Time of determination	Threshold of stimulation in Volts The average of 25 experiments	
	Anterior end of the body	The whole body of ascarids
Before the removal of anterior end	0.55 ± 0.034	1.86 ± 0.17
Five minutes after the removal of anterior end	0.7 ± 0.05	1.96 ± 0.15
Thirty minutes after the removal of anterior end	1.1 ± 0.1	2.3 ± 0.18

As is generally known, the intact ascarids in vitro accomplish intricate and diverse movements (Krotov 1953). The periodic serpentine movements or waves which are basically characteristic for the anterior end of the body are observed most frequently. The posterior end of the body is often stretched and its curves are weakly marked. Periodically the body of ascarids exhibits light contractions. The described movements result in the forward progression of the helminth accomplished at a speed dependent both on the physiological condition of the specimen and on the factors of outer environment. Ascarids can change the direction of their movements. If the anterior end is less mobile and performs no active serpentine movements, the nematodes are at rest. The ascarids deprived of the anterior end (by application of ligature), mostly rolled themselves up into a ball and remained still for hours. The ascarids which had 0.001 mg of nanofin or 0.005 mg of gamma-aminobutyric acid injected into their anterior extremities immediately slackened and stretched their bodies and their serpentine movements ceased.

DISCUSSION

As mentioned above there exist two contrary views (Shishov 1961b, Krotov 1965a) on the role of cerebral ganglia in the regulation of locomotor activity of ascarids.

It should be noted that in all our experiments and in those conducted by Krotov, after the application of ligature and following the removal of cerebral ganglia, a sharp contraction of the helminth's body was observed in the form of spasmodic increase of tonus. Shishov also observed this fact and associated it solely with the moment of the cutting off of the cerebral ganglia. However, when the ligature was applied to different body parts of the ascarids with previously removed anterior end, the characteristic spasmodic increase of tonus was observed again. It has been established that in cutting off the cerebral ganglia by administration of nanofin or gamma-aminobutyric acid the tonus decreases sharply and the helminth's body becomes limp. The data obtained in determining the threshold of excitability of the anterior end of the body immediately after the application of ligature proved to be unauthentic and when the same values were measured in 30 minutes, a sharp decrease of excitability of the anterior end of the body was observed. After nanofin or gamma-aminobutyric acid had been injected into the anterior end of the body, the excitability decreased sharply as early as in 5 minutes. These facts clearly show that the sharp increase of tonus does not depend on the removal of cerebral ganglia as stated by Shishov, but rather on the stimulation of the exteroreceptors and possibly of the stem part of the nervous system.

The papers by many authors (Rico 1926, Baldwin 1943, Baldwin and Moyle 1947, Krotov 1965b etc.) state that the rhythmic contractions of ascarids have been observed in kymographic records of movements of ascarid fragments. The question arises as to whether the emergence of rhythmics is associated with the fixation method of the helminths, the influence of load and other stimulators. Thus Krotov has shown that the rhythmics of skin-muscular zones of ascarids may disappear or appear depending on load. It follows from the afore-said that the cerebral ganglia promote and stimulate constant appearance of serpentine movements and enable the active forward progression of ascarids. The experiments on the excitability of the anterior extremities of the body and intact bodies of helminths and of same helminths with removed anterior extremities are a clear evidence to this proposition.

While studying the role of cerebral ganglia in the locomotor activity of earthworms Vereshchagin and Sytinsky (1960) proved that the administration of gamma-aminobutyric acid and beta-alanin to the anterior end of animal causes an inhibition of the moving reactions. Apart from this, Kovaleva (1961) demonstrated that after the removal of a cerebral ganglion the tension of the skin-muscular sac relaxed and the moving activity of the earthworm was sharply reduced. Bharucha-Reid Rodabe (1961) revealed that the removal of the cerebral ganglion causes a disturbance in the coordination of movements. The dependence of the emergence of rhythmic contractions of the decapitated earthworms upon the stimulation induced by application of load (0.5—1 g) was demonstrated by GRAY, LISSMAN (1938), COLLIER (1939). The mentioned data indicate that the role of the cerebral ganglion in round worms of the species *Ascaris suum* is similar to that of annelids.

CONCLUSIONS

1. While using four methods of investigation it has been established that the removal of the anterior end of ascarids or the administration of nanofin or gamma-aminobutyric acid into it, as a rule, evokes a decrease of body tonus and relaxation or disappearance of serpentine movements as well as a decrease of electrical excitability.
2. The increase of body tonus or the coiling of body observed after the application of ligature and the removal of anterior end is not dependent on the cutting-off of the cerebral ganglia, but is apparently due to the stimulation of exteroreceptors and the nervous stems of helminth.
3. The cerebral ganglia of ascarids are responsible for the regulation of the moving activity, promoting and stimulating the origin of constant body movements and thus securing the forward progression of the helminth.

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