Physiological Studies on the Neuromuscular Systems of Lower Worms

I. *Caridinicola indica*

By

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With 3 Text-figures and Plate XIII

I. Introduction

Since LOEB (1894) published a work on the brain-physiology of worms, many studies dealing with the neuromuscular mechanisms of lower worms have been made exclusively on a class of Plathelminthes, viz. Turbellaria. Surveying the results of these studies which were summarized by TEN CATE (1931), it may be said that the behavior of the Turbellaria is now pretty well analyzed from the standpoint of neuromuscular physiology. On the contrary, the other groups of lower worms being rarely adopted as materials for such a study, it is properly supposed that the neuromuscular analysis of their behavior is interesting and will contribute more or less to the knowledge of the neuromuscular physiology of the lower animals.

I began with *Caridinicola indica*, a tiny worm belonging to the Temnocephaloidea, commonly regarded as a rather rare and curious class among the Plathelminthes; and tried not only to observe its behavior in nature, but also to analyze its neuromuscular mechanism by means of the following series of operations. This worm was first reported by ANNANDALE (1912), who found it in India as a parasite on two species of prawn of the Genus *Caridina*. The appearance and movements of the worm had such a striking resemblance to those of Hirudinea that it was naturally mistaken by an

assistant of ANNANDALE for a small leech. Thus my study was also directed to the comparison of the nervous mechanisms of these two forms whose nervous structures were remarkably different from each other.

This study has been carried on in 1933 at the Otsu Hydrobiological Station (on Lake Biwa) of the Kyoto Imperial University, under the kind supervision of Professor T. KAWAMURA, to whom I am deeply indebted. Before entering my principal descriptions, a short review of the habitats and the morphological characters of the animal will be given.

Habitats.—In Japan the common host of *Caridinicola* is *Xiphocaridina compressa* DE HAAN, a small prawn which is so abundant in Lake Biwa that it is utilized by the people for food. The season when the prawn is found in abundance among the waterweeds thickly growing in shallow zones of the lake is only from the end of March to the beginning of July (water temperature 10°–26°C). During this season the eggs of the prawn attached to the mother's swimmerets become gradually mature till, the end of the season, we can find some prawns bearing only the split egg-cases after the young individuals have been thrown out. When the youngs begin to hatch, the prawns are rarely found in the shallow zones because they migrate far into the deep regions of the lake and stay there until the next spring.

Caridinicola is generally found in the branchial cavity, less often being attached to the appendages, the carapace and the pleurae of the abdominal somites of the prawn. There are often as many as twenty or more in a single host, or there may be none in some cases, while five or six is the average number. The size of the worm is variable, the fullgrown being as long as 2.8 mm., while the immature just after hatching is only 0.7 mm. Besides the active worms the gill-chamber of the prawn is provided with many solitary eggs of the worms, the maximum number counted being 28 in one side of the gill-chamber. The egg has a somewhat spheroidal form and measures on an average 0.24×0.19 mm. from the side view (quite similar to the measurements of ANNANDALE). Each egg is attached to the gill-filaments with one end of its long axis, while the other free end is endowed with a short stalk. Some of the eggs contain in their chitinous egg-cases a young worm, already hatched, bent in a U-form towards the ventral side, and showing sluggish movements. If we break an egg-case and let the young worm free, its movements

are scarcely different from those of an adult. Thus it is evident that *Caridinicola* undergoes no metamorphosis but develops directly from the egg layed on the gill-filaments of the prawn, as is the case with other forms of Temnocephaloidea.

It is farther noticeable that the hatching season of the worm is nearly the same as that of the prawn, though the former precedes the latter more or less. When the young prawn is perfected in the egg-case, many young worms are already hovering about the inner side of the carapace in the abdominal pleurae and among the eggs of the host. Therefore we can presume that they have ample opportunity to infect the young host as PLATE (1914) has suggested.

Morphological characters.—Since a very good anatomical account of *Caridinicola* is given in the descriptions by PLATE, I will give here only an outline supplemented with some new observations. The animal has a cylindrical body, elongated in the forward direction. (cf. Fig. 1). At the anterior end is a pair of tentacles, each on one side of the mouth. Each tentacle has a cylidrical basal portion and a nipple-shaped distal portion, both of which are separated by a constriction. The mouth communicates with a big muscular pharynx, which often protrudes as a proboscis surrounded by a ring of eight papillae at the distal end. Paired eyes are situated on the dorsal surface of the middle portion of the pharynx. The pharynx opens into a simple cylidrical intestine ending blindly near the posterior end of the body. The intestine of the adult worm is coloured green, yellow or brown according to its food, while that of the young worm just after hatching is light yellowish gray in colour due to the yolk-mass being not yet absorbed. On both lateral sides of the intestine there are rather conspicuous vitelline glands and a pair of testes, while the principal parts of the genital organs, i. e. ovary, oviduct, vas deferens, vesicula seminalis, penis etc. lie on the ventral side of the intestine, and a genital opening also opens ventrally near the middle portion of the body. The excretory system is arranged symmetrically on either side of the body and is composed of branching canals and gland-like terminal organs. A pair of excretory pores are located on the dorsal surface a little posterior to the eyes. The posterior end of the body is occupied by a large sucker, not round but horseshoe-shaped, and notched on the ventral margin.

Histological study has made clear the following facts. The body surface is covered by chitinous cuticle. The inner epidermis consists

of syncytia with scattered nuclei but without rhabdites. Inside the
epidermis there are three layers of dermal muscles, viz. circular,
diagonal and longitudinal muscles, arranged in order from the outside.
The diagonal muscles are composed of two sets of muscle-fibres,
which run diagonally to the body axis and cross each other at right
angles. A thin layer of circular and diagonal muscle extends uni-
formly all over the body, while the longitudinal muscles are highly
developed at the posterior region, especially on the ventral side.
Besides these, muscle-fibres run here and there in the dorso-ventral
direction and, at the posterior end, develop into the axial muscles
of the sucker. The ventral side of the constricted part of each
tentacle is also provided with axial muscles derived from the longi-
tudinal muscles, and furthermore the epidermis of that part becomes
hollow, thus becoming the anterior sucker. The pharynx is provided
with one pair of retractor muscles on the ventral side. The
wall of the pharynx consists of an outer layer of circular and longi-
tudinal muscles and an inner one of circular muscles, epidermis and
cuticle, both layers being connected by the radial muscle-fibres
mingled with the reticular connective tissue. Stripes of gland-cells
are found in the parenchym along the lateral sides of the body,
among the musculatures of the anterior and the posterior suckers,
and around the genital opening.

With regard to its nervous system, the most obvious structure
is a band-like cerebral ganglion or brain attached to the dorsal sur-
face of the pharynx (cf. Fig. 2). From each anterior corner of the
brain there passes forwards a tentacular nerve, which soon ramifies
into nerve-fibres anastomosing with each other and innervating to
the tip of the tentacle. From each posterior corner of the brain
branches off in the forward direction a lateral nerve and backwards
a posterior nerve which divides into a ventral and a lateral nerve
at a point between the pharynx and the intestine. Both the lateral
nerves run just under the layer of dermal muscles till they disap-
pear into the muscular tissue; the anterior one is traced to the basal
portion of the tentacle, while the posterior extends to the neighbour-
hood of the testis. The ventral nerve of each side running along
the ventral side of the body, thickens towards the posterior end,
ramifies into nerve-fibres and thus forms the nerve-plexus of the
posterior sucker. Though I am able to detect only a transverse
commissure connecting two ventral nerves just anterior to the genital
opening, it is naturally supposed that all the nerve-cords are indirectly

connected by the nerve-nets embeded in the musculature and connective tissue. In the brain the ganglion-cells are mainly located in the dorso-lateral parts while the middle portion is occupied by transversal nerve-fibres. Some ganglion-cells are also found in the nerve-plexus of the tentacles, the posterior sucker and the pharynx. The eye, being situated close to the postero-dorsal margin of the brain beneath the layer of dermal muscles, is composed of a cup-shaped mass of black pigment-granules and a refractive body led to the brain by a short optic nerve. Generally speaking, the nervous system of the *Caridinicola* is far simpler than that of the *Temnocephala rouxii* (studied by MERTON, 1914), while it considerably resembles to that of *T. chilensis* (studied by WACKE, 1905).

II. The Behavior of Normal *Caridinicola*

Keeping the prawn in a glass dish of 10 cm. diameter it is easy to observe with the naked eye the *Caridinicola* living in the branchial cavity. The worm usually assumes a more or less shrunk form and attaches itself to the surface of the gill or the inner wall of the carapace by means of its posterior sucker. When we disturb the worm by slightly pressing the carapace with a needle, it soon begins to creep forwards slowly but continuously till it comes out of the gill-chamber and leaves the body of the host through the antennae, the antennules, the eye-stalks or the abdominal pleurae. Such an isolated worm exhibits a series of movements as follows.

a) *Progressive movement.*—The locomotion of the worm is only effected by means of a leech-like progression. First of all the body expands, adheres to the substratum with the tentacles (or exactly speaking, the anterior suckers of tentacles), then the posterior sucker detaches from the substratum and at the same time the body contracts, becoming slightly concave on the ventral side. Thus the posterior sucker is drawn forward and adheres near the position of the tentacles. Then the tentacles get free, and again the body is stretched, the tentacles stick to the substratum and so on. Why the ventral side becomes concave during the general contraction of the body is explained by the above-stated histological fact that the longitudinal muscles on the ventral side are more powerful than those on the dorsal side. The progressive movement is composed of a functional antagonism between the anterior and the posterior suckers, and between the longitudinal and the circular muscles. The walking rhythm is usually 1.5–3 sec. for each step, being faster in

the smaller animals and when in a state of excitement. If the
tentacles touch the point of a needle or any such object during the
progression, they avoid it. If the object is the smooth bottom of a
dish or any other favourable subject, they adhere to it. Thus the
tentacles appear to have a selective ability in their adhesions, while
the posterior sucker is entirely indiscriminative and readily adheres
to whatever it touches. Since the posterior sucker is able to attach
itself to even the point of a needle, we must presume either that
any portion of the posterior sucker can work by itself as an inde-
pendent effector or that the adhesion is by means of secreted mucus.
The former method is more probable because during its progression
it is noticed that, when we precisely observe an animal walking on
the slender antenna of its host, the portion of the posterior sucker
attached to the antenna is curved so as to fit to the curved surface
of the latter. This view is also supported by the fact that the animal
progresses with much difficulty on such a coarse surface as one
covered with paraffin, owing to the imperfect suction of the posterior
sucker.

b) *Resting posture.*—With the decline of its activity the worm
assumes a resting posture. Then the posterior sucker attaches itself
to the bottom of the dish, the body muscles strain to a moderate
degrees, the body axis is maintained at various angles with the
substratum, and the tentacles are directed obliquely upwards in the
water. This posture is continued over a period of many days unless
the worm is disturbed. Even when the posterior sucker is attached
to the surface of the water, the worm is able to hang from and
beneath the surface film in the same manner as on the bottom of
the dish. That the posterior sucker adheres to the surface film of
water indicates that the adhesion of the sucker in the resting state
is also due to suction rather than to the mucous secretion. When
the worm rests for a long time, however, the adhesion of its posterior
sucker seems to be assisted by a secreted mucus which collects
about the sucker. Hanging from the surface film the worm has
difficulty in locomotion. It seems vainly to move by repeating the
expansion-contraction movements with both the tentacles and the
posterior sucker attached to the surface film. Its inability to move
is probably due to the fact that the tentacles can not adhere suf-
ficiently to liberate the posterior sucker. Frequently the animal
takes a modified posture in which the body is contracted and curved
a little bit to the dorsal side with the tentacles perked up. Since

such a posture seems to be particularly convenient for catching the host when it passes by, it may be called the "ambushing" or "waiting posture."

c) *Seeking movement.*—The worm often shows remarkable motions that I call "seeking movements" after HERTER (1928), who gave the name "Suchbewegungen" to similar movements of the leech, *Hemiclepsis marginata*. In the resting position or at every step of progression the worm oscillates the body slowly from side to side and rarely violently, by alternate contractions of both the lateral longitudinal muscles. The worm often swings in such a wide range that the tentacles now touch a point of substratum and then touch another point of substratum ; thus the body moves as on the surface of a cone. When this lateral oscillation is accompanied by a flexion and extension of the body, the worm swings at random in all directions. During the oscillatory movement we often notice another complicated movement in which the body is expanded asymmetrically, the anterior region, particularly the tentacles, being twisted around the body axis. This twisting motion seems to be mainly effected by an antagonistic action between two sets of muscle-fibres in the diagonal muscular system, even though there be some cooperation of the longitudinal and dorso-ventral muscles. We can presume that the contraction of the circular muscles is a necessary condition for the twisting motion because the motion always occurs when the body is in an expanding state. It is clear that these motions are for the purpose of seeking an object to which it may attach its tentacles, for, if the tentacles come in contact with any suitable material during these movements, they attach to it instantly which results in changing the position of the worm.

d) *Righting movements.*—If the worm is made to lie on its side or upside down, both the tentacles and the posterior sucker being liberated, it reflexively makes a few contraction-expansion movements followed by violently twisting the whole body. When the tentacles happen to touch and adhere to any object or the ground, the worm at once shortens itself, draws the posterior sucker forward and fixes it on the substratum, liberates the tentacles and then takes a natural resting posture. If the tentacles do not succeed in finding the ground, the animal begins all the righting motions again. Sometimes we notice that the worm catches the ground first by the posterior sucker and easily takes the upright posture. If the prostrate worm is gently touched by a needle just when its body is fully expanded, the next

twisting movement is checked, the body contracts and curves to the dorsal side. Thus the animal maintains the prostate position until the next stimulus comes to begin the twisting motion. In short, the twisting motion of the body is the most important element in the righting process. Now the worm hanging under the water surface does not make any righting motions, which leads us to believe that the stimulus for the righting reflex is not based on a sense of gravity but on the tactile sense due to the liberation of the posterior sucker.

f) *Feeding movements.*—As the feeding motions are never detected in the isolated worm, we must observe the worm in the gill-chamber (through the carapace) of the host. The worm in the gill-chamber usually undergoes lateral oscillations and twisting movements which are thought to be motions in which it seeks its prey. Meanwhile the animal protrudes the whole pharynx with astonishing rapidity, captures the prey by the unfolded papillae of the proboscis and then withdraws it rapidly. It seems that the extrusion of the pharynx is effected by the longitudinal contraction of the dermal muscles, while the retraction depends on the retractor muscles of the pharynx. Of course the musculature consisting of the pharynx itself has an important rôle in the extrusion-retraction movements. These motions of the pharynx seem to be readily induced when the body is in a compressed form in the branchial cavity of the host. When the worm is compressed under the cover-glass during the microscopicobservations, we often notice some peristaltic and anti-peristaltic waves, though local and non-periodic, pass along the intestinal wall. By means of these peristaltic motions the food may be transferred from the buccal region to the intestine and *vice versa*. The animal seems to prey upon the planktons which are brought into the branchial cavity together with water current induced by the vibrating appendages of the host. But the digestive power of the animal is so thorough that we can scarcely find any waste, save for some desmids and diatoms.

g) *Reactions to mechanical stimuli, Avoiding movements.*—When the worm is exposed to a weak mechanical stimulus the general reaction is a seeking movement; i. e. whenever the body is touched at any point with a needle, the whole body reacts to the stimulus with the tentacles twisted forward towards the needle. If this stimulus is increased, an effort to avoid the stimulus is observed, which effort varies somewhat according to the portion of the body stimulated. When one of the tentacles is repeatedly stimulated the tip of

that tentacle retracts into the basal portion, while the stimulus being further increased, the body bends in the opposite direction or wholly contracts including even another tentacle. When the head region is strongly stimulated on the dorsal side the worm first contracts and becomes short and then extends again in another direction. When any part of the posterior sucker is stimulated even to a moderate degree, the worm expands its body at once and starts locomotion; the stronger the stimulus, the more rapid and continued the progress. Thus the tentacles, the posterior sucker and the parts near the brain, being richly provided with nerves, delicately react to the mechanical stimulus, while the middle portion of the body appears to be highly insensible. For instance, a strong stimulation to the intestine usually produces no effect, but sometimes leads to lateral oscillations or progressions. With regard to the ventral side of the body, it is difficult to stimulate it so freely and so intensely as the dorsal side; but it might be said that, when it is lightly stimulated, the body bends and catches the needle by the tentacles and, being more intensely stimulated, by a lateral oscillation seeks to avoid the stimulus. In general, the worm gives a positive reaction (produces a seeking motion) to a weak mechanical stimulus and a negative reaction (or avoiding motion) to a strong stimulus.

h) *Reactions to stimuli of water current.*—If we agitate the water in which the worms are resting by means of a needle or water-drops, they soon vigorously exhibit seeking motions in reaction to the agitation of the water. Thus the question is raised; how do they react to the directive current of water? Being exposed to a current of water ejected by a small pipette, the animal adjusts its body to the stimulus by twisting the tentacles as in the case of the mechanical stimulus. This response is given regardless of the direction of the current, but it is especially strong when the stream comes from the rear because of the strong bending of the body to meet the stimulus. Soon after the response the animal resumes its previous position and does not again react while the current is continued or when it ceases, so the response may be regarded as being invoked only by the initial impulse of the current. This is further ascertained by the following experiment: A round dish of 4.5 cm. diameter is placed in a larger one of 10 cm., several worms are scattered in the ring-shaped space between two dishes and a steady current is led into that space from a tap. In the moment required to produce the current the worm reacts as described above and soon returns to

a state of repose. When subjected to a second local current from the pipette, they instantly exhibit the typical reactions. Therefore we can say that the worm reacts not to a constant stimulus but to a sudden increase of the intensity of stimulus. This positive reaction evidently enables the worm to catch the host passing by it. When the strong current is against the animal it is passively pushed away with the posterior sucker anchored to the substratum. If the current becomes still stronger the animal begins a longitudinal contraction of the body which increases the adhesive force of the posterior sucker and prevents the animal's being torn from the substratum. The fact that the animal assumes a contracted shape in the branchial cavity of the host may be partially due to the strong current in the branchial cavity. The animal attached to the anterior margin of the carapace is in this manner pushed out of the branchial cavity towards the head of the host.

    i) *Reactions to photic stimuli.*—Observing the actions of worms emerging from the host, it was noticed that they were apt to crowd into the lighter side of the dish. Thus the following experiments were performed : after scattering 20 individuals uniformly in a dish of 10 cm. diameter, the container was completely covered with black paper save for a small lateral window of 5 mm. square. After being thus exposed to diffused sun-light for two hours the dish was uncovered so as to examine the distribution of the worms. 16 individuals were crowded under the small window or in its vicinity, and 4 individuals were scattered in the centre of the dish. From this it may be said that the worm exhibits a positive phototaxis to diffused sun-light. Next in the dark room, a dish was placed at a distance of 20 cm. from a 40 watt lamp so that the direct light might be projected obliquely on the dish. Along the lighter side of the dish was set a screen throwing its shadow on one-fourth of the dish. Several animals were put into the dish on the side opposite the screen. All the animals at once began to go towards the light source. Vigorously making the seeking motions they advanced straight towards the shadow zone. As soon as they reached the shadow line they ceased locomotion, continued the seeking motions for a while and then returned to the illuminated zone. Thus after various efforts of orientations the animals finally came to rest in the lightest zone of the dish. Such behavior indicates that the positive phototaxis of the animal is of a phobotactic nature. It is further noticeable that the inactive worm, several hours after leaving the host, is still indifferent to the photic stimuli.

Against other sorts of stimuli the animal generally seems to be indifferent. Being brought near a capillary tube filled with the body-fluid of prawn, it does not show any chemotactic reaction. Neither does it seem to have a positive thigmotaxis, since it does never creep in underneath a cover-glass plunged into the dish. Accordingly we can not understand why the worm is fond of getting into the dark gill-chamber of the host. We cannot but suppose that it is a reaction to the stimuli of water current arising from the gill-chamber or the contact stimuli characteristic of the host-body.

III. Behavior of *Caridinicola* on which Operations have been performed

To analyze, if possible, the neuromuscular mechanism in the behavior of *Caridinicola* more closely, and particularly the function of the central nervous system, a series of experiments were made which are of a common extirpation method, i. e. the changes which occur in the above-stated normal movements after the removal or the destruction of various regions of the worm were observed. A small round tray, 4.5 cm. in diameter lined with a mixture of paraffin, wax and animal charcoal was used as an operation table. Worms contained in a small quantity of water were cut with small knives and sharp needles under a binocular microscope. When an animal was too vigorous to permit operation, a concentrated solution of carmotin was dropped into the medium-water until the animal no

Fig. 1

longer reacted against the touch of the operative instruments. The animal was so slightly narcotized by the carmotin, however, that the strong stimulus of cutting naturally caused violent reactions. Other narcotics, such as chloreton, cocain, etc., were not usable for this purpose since they produce exciting behavior in the worm. The animal satisfactorily operated upon was returned to the common glass dish and carefully observed under a high power binocular microscope. The results of the experiments are as follows:

Experiment I. Removal of the tentacle, as for instance the right tentacle (fig. 1.). In this case the shock effect of the operation was very slight. There occurred no remarkable change in the form of the body after operation, but sometimes the left tentacle curved a little to the right as a result of the constriction of the wound. Not only could the left tentacle properly
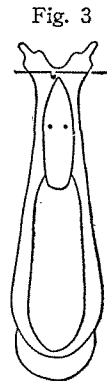
respond to a mechanical stimulus but the cut end of the right ten-
tacle showed also an ordinary response to the stimuli, and the worm
could twist its body to meet a needle or could bend from side to side.
The seeking and the avoiding motions were apparently normal, and
the progressive movement nearly so.   The worm was able to make
a straight progression, but the process of drawing the body forward
was a bit difficult owing to the reduction of one-half of the adhesive
force of the anterior suckers though the left tentacle retained its
normal force of adhesion.   There was also no change in the adhesive
force of the posterior sucker.   It was as difficult as in the case of
the intact individual to detach the sucker from the substratum by
applying a strong current of water.   The righting movement was
also normally performed, the left tentacle or the posterior sucker
being first used for catching the substratum.   In short, the worm
minus one tentacle can behave almost normally.   The single tentacle
disconnected from the body, shrunk, lost its adhesive ability and
showed no more response to a mechanical stimulus.

Experiment II.   Making an incision at the base of the right
tentacle, or cutting a tentacular nerve (fig. 2).   No apparent change
in the body was noticed except that the right tentacle showed a
slight shrinkage due to the operation shock.   Responses
of the left tentacle to stimuli were normal, while respon-
ses in the right tentacle were only local, these local dis-
turbances being unable to induce a twisting and a bend-
ing of the body.   Since the right tentacle was able to
adhere to the substratum, it prevented these various
movements by its local and independent reaction.   If the
right tentacle adhered simultaneously with the left one
during the progressive movement, the animal required a
long time to start the right tentacle after drawing the
posterior sucker forward and could barely detach it by
forced extension of the body; thus the movement was
far more difficult than when one tentacle had been entirely removed.
The seeking movement was also difficult for the uncoordinated ten-
tacle adhered to the bottom whenever it touched.   The righting
could be finished rapidly if the left tentacle or the posterior sucker
happened to touch the substratum first, but when the injured right
tentacle alone adhered first, the worm repeated its reflex-movements
in vain.   If the injured tentacle was shrunk and had lost its adhe-
sive ability due to the heavy shock of the operation, the movements
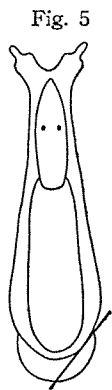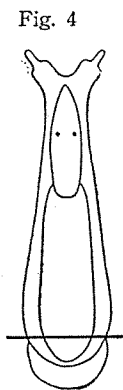

Fig. 2
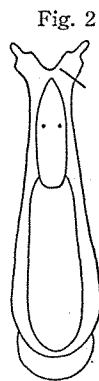
were rather smooth as in the case of Experiment I. In short the tentacle, whose nervous connection with the brain has been destroyed, still has its adhesive ability, while the centripetal and the centrifugal conduction of impulse is of course damaged between that tentacle and the body.

Experiment III. Removal of both right and left tentacles (fig. 3). No change in the body form, the wound being shut by contraction of the circular muscles. Even if the cut end was gently stimulated by a needle, the worm usually gave no positive seeking motion but a violent swing in order to avoid the stimulus, and occasionally repeated the alternate extension-contraction movements which would be effective to locomotion in the intact animal. There was of course no adhesive ability in the cut end of the tentacle, but the adhesive force of the posterior sucker was normal. If such an operated animal was stimulated at the posterior region of the body, it swung the body so violently as to form a ring by the curved body-axis on both sides, but could not progress without the tentacles to adhere. That is, the strong stimulus, which ordinarily induces the animal to progress, is directed to the oscillatory movement in the tentacleless worm. The righting reflex was almost normal, the worm being capable of quickly righting itself by catching the substratum with its posterior sucker. The worm was also able to react rather sensitively to the current stimuli. It is noticeable in the animal lacking both tentacles that the excitability as a whole is strikingly increased together with a predominance of the oscillatory movement. Two tentacles of an anterior piece severed from the body, were cramped for a while and soon became shrunk and senseless. Although the tentacles disconnected from the body were not capable of adhesion, it was ascribed, considering the case of Experiment II, not to nervous obstruction but to the heavy shock of the operation.

Fig. 3

Fig. 4   Fig. 5

Experiment IV. Cutting off the posterior sucker (fig. 4, 5). The wound surface was shut by contraction of the circular muscles after expelling some intestinal contents, and the posterior half of the body became thicker

as a result of the shrinkage of the longitudinal muscles. After two
hours, however, the animal recovered from the shock of the opera-
tion and regained its normal body form. Apparently no change
occurred in the adhesive ability of the tentacles, whose responses to
a mechanical stimulus were also normal, i. e. they vigorously twisted
towards a needle or induced the whole body to contract or to oscillate.
Since the altered worm was not able to assume the resting position
but laid on the substratum, the righting reflex was properly revealed.
In this case the animal first attached its tentacles to the substratum
after some vigorous normal reflex-movements and then drew the
posterior region forward, but it could go no further since the pos-
terior sucker did not stick permanently to the substratum. Thus,
an impulse from the posterior sucker not being started, there was
no opportunity to detach the tentacles and the worm repeated in
vain a bending-expanding motion with the tentacles attached to the
substratum, until the tentacles naturally became detached and then
the worm began the righting motions again. On the other hand, if
the posterior end of the body happened to be in violent contact with
the substratum by a longitudinal contraction of the body, the ten-
tacles could be liberated with the result that the animal remained
on its side, or, in some exceptional cases, it took hold of the sub-
stratum with the posterior end and walked one or two steps. Such
a liberation of the tentacles appears possible because any stimulation
given to the cut-ends of the ventral nerves has the same effect as
in the case of the adhesion of the posterior sucker. From these
results and those of Experiment II and III it may be generally in-
ferred that an impulse which, stimulated by adhesion, originates in
the posterior sucker runs to the tentacles through the central ner-
vous system (ventral nerves—brain—tentacular nerves) ; and at this
point releases the anterior suckers from adhesion ;- and *vice versa*.
The animal lacking the posterior sucker finds it impossible to pro-
gress or to maintain the natural resting posture, but repeats only
the righting motions. The severed posterior sucker adhered rather
firmly to the substratum and further responded to a mechanical
stimulus by local contractions. Thus the adhesive power of the
posterior sucker is able to be retained merely by its own nerve-plexus.

Experiment V. Severing the body at the level of the eyes, or
the removal of the anterior half of the brain (fig. 6). The anterior
piece was strikingly shrunk and did not react to a mechanical
stimulus. The posterior piece also shrank soon after the operation,

but later extended gradually to normal length. Its be-
havior was very sluggish. Accordingly, even though it
could normally give a positive or a negative reaction to
a mechanical stimulus, the threshold of the stimulus had
to be higher than that for the intact individual. A strong
stimulus primarily induced a longitudinal contraction of
the body. Being stimulated at the posterior sucker it
reacted with certain convulsive or impulsive expansion-
contraction movements rather than with lateral oscilla-
tions. If we agitated the medium-water the animal was
also able to react with expansion-contraction movements.
There was no change in the adhesive power of the pos-
terior sucker which was able to adhere even to the surface film of
water. The most interesting point in this operation was the dis-
appearance of the twisting motion and the seeking motion. When
the posterior sucker was detached from the substratum, the animal
repeated only the expansion-contraction movements, no longer twist-
ing the whole body, but made some irregular and inconspicuous
movements of the sucker. Thus it was impossible to right itself at
once, but the sucker was brought into use gradually so as to attach
to the substratum until it finally recovered the natural upright posi-
tion. Sometimes, a part of the sucker only being attached to the
substratum, the animal rested in an inclined position. In short, the
noticeable facts in this experiment are the remarkable decline of
excitability, the absence of the twisting motion, and the predominance
of the expansion-contraction movements over the oscillatory motions.
In these respects we perceive a remarkable contrast between the
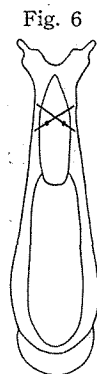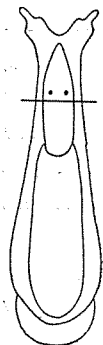worm in this experiment and that in Experiment III.

Fig. 6

Experiment VI. *Cutting the body just behind the eyes
or decapitation* (fig. 7). In the shrunk anterior piece,
the tentacles responded to a strong stimulus by local con-
tractions but did not exhibit adhesive abilities. Consider-
ing this result together with those of the former experi-
ments, the function of the tentacles seems to be checked
as to sensitivity by the shock given to the brain, though
they should have their own adhesive abilities. Concern-
ing the posterior piece, the body was shrunk to two-third
its size after operation, but soon almost recovered its
normal length. The posterior sucker was able to adhere
even to the surface film of water. The worm had no

Fig. 7

spontaneity of motions nor positive reactions to any ordinary stimulus. Against a strong stimulus applied to the cut end, the reaction was a longitudinal contraction of the body, but, when the animal was unilaterally stimulated, it was able to slowly bend the body to the side opposite that stimulated. Strong currents also induced a longitudinal contraction of the body. The posterior sucker being strongly stimulated, the animal reacted with impulsive extension-contraction movements, the rhythm of which was slower than that of the progressive motion in the intact animal. As the twisting motion was never detected, the prostrate specimen was unable to quickly right itself as in the case of Experiment V. Generally speaking, the decapitated animal exhibits to a greater degree only the abnormal behavior seen in Experiment V. It is clear that the brain is indispensable for maintaining the excitability of the whole animal, the twisting movement and the regulation of the extension-contraction movement.

Fig. 8

Experiment VII. Cutting the body through its middle portion (fig. 8.). The shock effect of this operation was so slight that both the anterior and the posterior pieces regained their extending forms soon after the operation. The tentacles retained their adhesive abilities and responded to stimuli by bending or twisting of the body. Thus the anterior piece behaved just as the anterior piece in Experiment IV. On the other hand, the posterior piece was more inactive than that in Experiment VI. Very strong stimulation at the posterior sucker could barely provoke impulsive extension-contraction movements. Any stimulus given to the anterior portion produced only a local contraction of that portion, while the body side close by the sucker, being strongly stimulated, produced an avoiding bending motion of the body to the side opposite from that stimulated. The seeking and the progressive motions were of course impossible, and the righting, being performed solely by the extension-contraction movements, required a very long time. The coordination between the two sides of the animal is retained without the brain and the transversal commissure, probably through the nerve-plexus of the posterior sucker.

Experiment VIII. An incision was made, reaching the median line, on one (in this case the right) side just behind the eyes, or by cutting both lateral and ventral nerves of one side (fig. 9). On

account of the shrinkage of the muscles near the wound, the body was curved a little to the right at the point of incision, but we could not detect any shrinkage of the muscles posterior to the incision of the right side. The reactions of the tentacles to weak mechanical stimuli were almost normal, but not so to strong ones. That is, if we strongly stimulated the right tentacle the animal bent in its normal manner to the left, but, if the left tentacle was stimulated the only avoiding reaction was a longitudinal contraction of the anterior part, a bending motion to the left. Such asymmetrical reactions were evidently due to the nervous connection between the tentacles and the posterior muscles of the injured side that had been unusually damaged by the incision. When either side of the body posterior to the incision was stimulated, the avoiding motion was equally almost normal. Against current stimuli the animal was apt to bend to the left. Although progression was normal and generally along a straight line, the worm was apt to turn to the right especially when its progress was forced by stimulating the posterior sucker. Righting required a somewhat longer time though without unusual abnormality. Thus this operation does not generally obstruct the various reflex motions of the worm, except the asymmetry of its reactions to external stimuli.
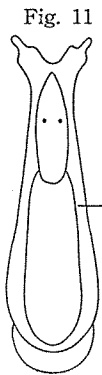
Fig. 9

Experiment IX. Making a deep incision, reaching the intestine, on one (in this case the right) side of the middle portion, or cutting both lateral and ventral nerves of one side (fig. 10). The body usually curved to the right at the operation position, but, when the intestinal contents were expelled on the right side, it crooked somewhat to the left. The tentacles when mechanically stimulated reacted in much the same manner as in the case of Experiment VIII. Though the worm made almost a straight advance in its locomotion, it described a circle when a heavy stimulus was applied to the posterior region. The circle motion of the right-curved individual was directed to the right side, while the left-curved one turned to the left. Accordingly the circle motion seemed not to be caused by unequal tonicity of musculatures between both lateral sides of the body, but was due merely to the body-shape. With regard to the righting reflex, the twisting was far more conspicuous than the

Fig. 10

bending-extending motion, and owing to the curvature of the body, a long time was required to assume a natural position.   Since the results of this experiment are scarcely different from those of Experiment VIII, it may properly be said that the transverse nerve plays no important rôle in the lateral coordination of the body as has already been described in Experiment VII.   Since the decapitated individual can maintain the normal length of its body and can make the circular motion in two directions, we can conclude that the muscle tonus of the body is not under the control of the brain.

Experiment X.   Making a superficial incision, on one (in this case the right) side of the middle portion, without reaching the intestine or cutting a lateral nerve (fig. 11).   The body shape was

Fig. 11

scarcely changed.   Both the function of the tentacles and the adhesive power of the posterior sucker were apparently normal.   The worm showed a straight progression and a normal seeking movement, and when stimulated on the right side just behind the wound, there was only a local contraction of that part without a general bending motion to avoid the stimulus.   The righting movement was also normal.   From these facts the lateral nerve is supposed to be only a sensory nerve, having almost no effect upon important reflex-movements of the worm.

Experiment XI.   Making a deep incision, passing the median line, on one (in this case the right) side of the middle portion or cutting a lateral and both ventral nerves.   The body was considerably crooked to the left at the operated position, owing to excessive expelling of the intestinal contents.   The body-part anterior to the incision twisted vigorously and the tentacles adhered to the substratum.   But the posterior sucker not being liberated, the posterior part merely repeated the impulsive extending-contracting motions.   Both parts behaved respectively just as did the anterior and the posterior pieces in Experiment VII, without there being any coordination between them.   It is clear that the ventral nerves are the most important conductors of the various reflex-motions of the animal.

Experiment XII.   Thrusting the eye-region, or destroying the brain.   A perfect performance of this operation was not expected, because, as the brain is spread over the dorsal surface of the pharynx, it was very difficult to destroy the entire organ only by the thrust of a needle.   When the various parts of the brain were repeatedly

thrust, the operation shock led to a shrinkage of the tentacles. Thus the results were quite similar to those in the case of decapitated individuals. One or two thrusts were sometimes sufficient to secure satisfactory results. In such cases the shock effect was still so considerable that the body shrunk becoming concave on the ventral side. The tentacles also shrunk and were faintly cramped, and when stimulated, contracted locally without transmitting the impulse to other parts of the body. Their adhesive powers were entirely lost or remained so slightly that they were easily detached by the contraction of the body. There was scarcely any change in the adhesive capacity of the posterior sucker. Against the current stimuli the animal reacted at random with impulsive expansion-contraction movements or bending motions. When the body-side was strongly stimulated with a needle, the worm dully made avoiding motion, bending the body towards the side opposite to that stimulated. Though stimulated strongly at the posterior sucker, tentacle adhesions were never induced, hence progression was impossible and there occurred only some ineffective expansion-contraction movements. The righting process was impossible because it could not make the twisting motions. Its excitability as a whole was remarkably decreased as compared with the normal animal. While destruction of the brain deprives the animal of its locomotive ability, we can not hastily suppose that the centre of the progressive motion is localized in the brain. Since the destruction of the brain damages the tracts between the tentacles and the posterior part of the body, the progressive motion would be impossible although there exists the centre. On the other hand, the operated animal is still capable of the impulsive extension-contraction movement when strongly stimulated. But the rhythm of this movement is far slower than that in normal progression, and we can not observe that the liberation of the posterior sucker is coordinated with the contraction of the longitudinal muscles. Therefore, since the brain is indispensable at least for ordinary progression, it may be said that the coordination-centre of the progressive motion is situated in the brain.

Remarks.—(1) Regeneration seems to be very difficult in *Caridinicola*. A worm whose tentacles were cut off, survived 40 days in a glass dish, the water of which was renewed every day. Meanwhile the body length became about one half normal size, but the animal was unable to make progress. Observed under a binocular microscope, there was no sign of regeneration. Thus the tentacles

can not be regenerated, not to speak of the other parts of the body. Worthy of passing attention is the fact that a decapitated worm layed 4 eggs on the bottom of the dish the eleventh day after operation.

(2)　The influence of certain narcotics on the behavior of *Caridinicola* was observed. The animal being plunged into 0.02% cocain hydrochloride solution, the body contracted longitudinally, often so far as to push out the proboscis. As the adhesive powers of the suckers generally became abnormally stronger, progression and righting were rather difficult. Even if the anterior suckers were attached to the substratum, the posterior sucker was not easily liberated (often this required more than 10 sec.). Moreover, the extension-contraction movement being powerful but slackened, the walking rhythm was remarkably slower than normal. Cocain appears to raise the tonicity of the body muscles as a whole. In a 0.001% strychnin sulphate solution, on the other hand, the body was enormously stretched and crooked to the ventral side. Though the anterior suckers seemed to have their weak adhesive abilities, the posterior sucker was not capable of adhesion. The animal lay on its side and faintly repeated the extension-contraction movements. The effects of strychnin appears to be just opposite those of cocain.

IV.　Consideration of Results in Comparison with Annelida

Besides the so-called " leech-like " locomotion, in its general behavior the *Caridinicola* remarkably resembles that of the Hirudinea. Now, the observations of the normal behavior of *Caridinicola* compared with the results obtained by Herter (1929) who observed the behavior of several German leeches, are as follows. With reference to the seeking and the avoiding motions, it resembles *Piscicola*, *Hemiclepsis* and *Protoclepsis* in the respect that the whole body vigorously swings. The resting posture is similar to that of *Piscicola*, since both the anterior and the posterior suckers are never attached at the same time. The progressive movement generally resembles that of Glossosiphonidae, but in *Caridinicola*, there are not 4 types of locomotion, the third type only being effective—namely, in the contraction phase, the body is curved to the ventral side and the posterior sucker adheres close to the anterior ones. The breeding and the rolling-in motions (*Brutpflege- u. Einrollbewegungen*) are never detected as in *Piscicola* and *Herpobdella*. Against external stimuli, the activity is as vigorous as *Piscicola*, *Hemiclepsis* and *Protoclepsis*. Generally speaking, it can be said that the behavior of

Caridinicola is similar to that of *Piscicola* and the parasitic forms of Glossosiphonidae, but is strikingly different from that of the free-living groups, *Glossosiphonia* and *Herpobdella*. As to the differences between *Caridinicola* and the Hirudinea, there are two noticeable points. In the first place, *Caridinicola* can not become flattened in the dorsoventral direction as a natural consequence of which the dorsoventral muscles are not so developed as in the Hirudinea. In the next place, we can not detect in *Caridinicola* any motion due to the transmission of the contraction wave along the body as seen in the swimming motion of *Piscicola* or the respiratory motion of *Hemiclepsis*, etc. The lack of these undulatory motions is obviously ascribed to the non-metameric structure of *Caridinicola*. Referring only to the nervous system, *Caridinicola* has but a cerebral ganglion, while the leech is further provided with a chain of ventral ganglia, each ganglion taking part successively in the undulatory motion.

With regard to the neuromuscular analysis of the movements of the Hirudinea, *Hirudo medicinalis* was exclusively adopted by LOEB (1894), MAXWELL (1897) and VON UEXKÜLL (1904). VON UEXKÜLL asserted that the decapitated individual was not capable of progressive motion because of the inactivity of the circular muscles, and thus it was naturally concluded that the brain was a centre for the chain-reflexes: the adhesion of the anterior sucker, the contraction of the longitudinal muscles, the adhesion of the posterior sucker, the contraction of the circular muscles and so on. This opinion, however, was recently refuted by SCHLÜTER (1933), who removed the subœsophageal ganglion of *Hirudo* and observed that the contractions of the circular and the longitudinal muscles were still coordinated and, in connection with the function of the latter, that the posterior sucker was detached from the substratum. On the other hand, JANZEN (1931) studied the leech-like forms of Oligochaeta, *Branchiobdella parasita* and confirmed that the localized centre for its typical progressive motion was the subœsophageal ganglion which could alone regulate the typical walking rhythm. The nervous process underlying the progressive motion of the *Caridinicola* is evidently not so adaptable as was described by SCHLÜTER, nor so strictly concentrated in the brain as was mentioned by VON UEXKÜLL. On the contrary, the opinion of JANZEN seems to be most applicable in our case; i. e. the brain of *Caridinicola* is the centre of the process of the typical progressive motion as is the subœsophageal ganglion of the *Branchiobdella*. After removal of the subœsophageal

ganglion it was noticed in *Hirudo*, that activity was remarkably decreased, the seeking motion was barely induced by a strong stimulus and the righting was impossible (SCHLÜTER). These facts strikingly resemble the effects of the decapitation of our *Caridinicola*. Hence the brain of *Caridinicola* is a centre of excitation as is the suboesophageal ganglion of *Hirudo*. Further in *Branchiobdella*, the coordination of the progressive motion was intermediated by the ventral cord. The anterior and the posterior suckers had their own independent centre of motion and the avoiding motion became inconspicuous as the anterior ganglions were removed. These facts are remarkably consistent with those of *Caridinicola*.

As regards behavior and mechanism, *Caridinicola* comes rather close to Annelida, especially the parasitic forms of this class, in spite of its primitive organization as a member of Temnocephaloidea. This resemblance can easily be understood from the fact that the modes of life in both groups are very similar, quick movements always being required to live a parasitic life. Of course it can not be said that the two groups are allied to each other in the phylogenetic affinity, but a common mode of life or a consistency of environment probably leads to the analogy of neuromuscular mechanism in the course of evolution.

V.   Summary

1.   All motions of *Caridinicola indica* are to be ascribed to three kinds of muscular movements, viz. an extension-contraction, an oscillatory (or a lateral bending) and a twisting movement of the body.

2.   The extension-contraction movement can be manifested without any control of the brain, by antagonistic alternate contraction of the longitudinal and the circular muscles which are presumably connected with each other by the nerve-net system.

3.   The oscillatory motion, being also induced without the brain and the transversal commissure, is effected by alternate contraction of the lateral longitudinal muscles of both sides.

4.   The twisting motion is strictly dependent on the centre in the brain, its most important effector probably being the diagonal muscular system.

5.   The only means of locomotion is a leech-like progression, which consists of extension-contraction movements and antagonistic actions between the anterior (or tentacular) and the posterior suckers. The centre regulating these elements in the normal walking rhythm is situated in the brain.

6. The adhesive ability of either the anterior or the posterior sucker is retained after they are disconnected from the brain. The resting posture is sustained only by a tonic state of the muscles of the posterior sucker.

7. " Seeking movement " is a name given to a series of random movements seeking for food and host, in which the body oscillates in various directions. This motion is often mixed with the twisting movement.

8. The typical righting reflex is nothing but a combination of the expansion-contraction movement and the twisting motion. The brainless worm, however, is capable of righting itself after a long interval of time, using only the expansion-contraction movement.

9. The worm has two kinds of feeding motions, i. e. a protruding-retracting movement of the pharynx to capture the prey and a peristaltic movement of the intestine to transfer the food. The neuromuscular mechanism of these motions is not obvious.

10. The circular motion is rarely seen in an asymmetrically operated worm. Perhaps it is not based on any change of muscle tonus originated in the brain, but merely due to asymmetry of the body shape.

11. The seeking motion is, on the other hand, a positive reaction towards weak mechanical and current stimuli. Against strong ones, either the lateral bending or the longitudinal contraction is effective for avoiding them.

12. The active animal shows a positive phobotaxis to photic stimuli, but does not appear to exhibit any tropistic reaction against the other stimuli.
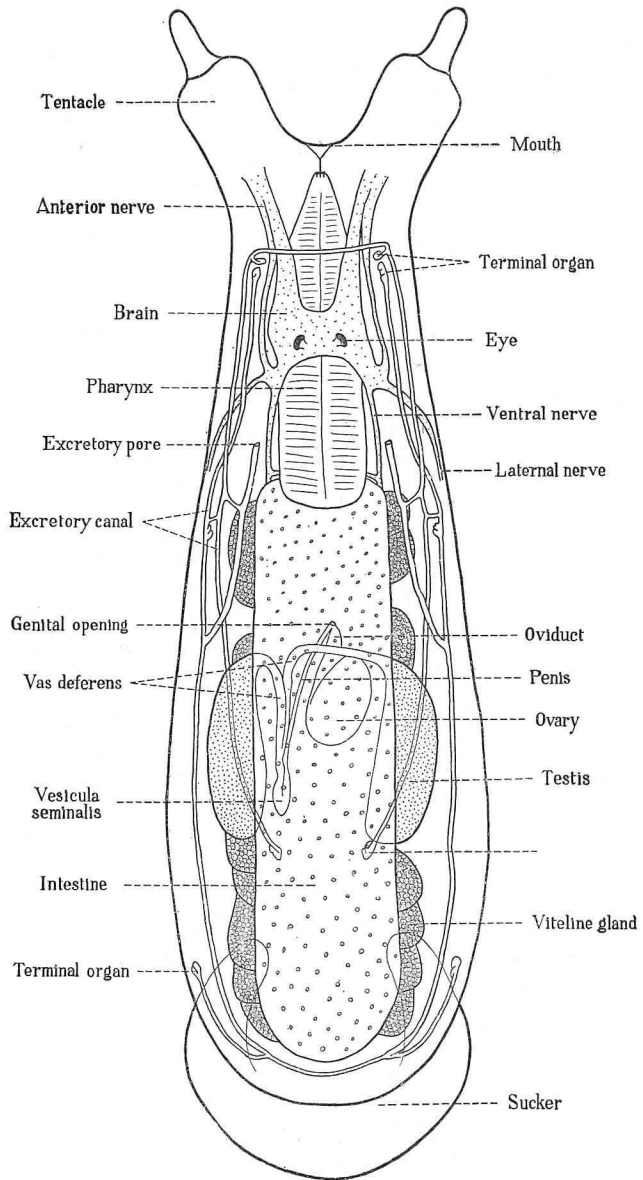
13. The brain is important as a centre of excitation rather than of coordination. The tentacular and the ventral nerves are the essential conductors for the coordinated motions, while the lateral nerves seem to be nothing but sensory in function.

14. Generally speaking, the neuromuscular system of *Caridinicola* is well integrated as compared with other groups of Plathelminthes. From the standpoint of neuromuscular physiology, it is rather on the level of some parasitic forms of Annelida.

(Jan. 29th, 1934).

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