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BOSTON UNIVERSITY

GRADUATE SCHOOL

Dissertation

NEURO-MUSCULAR ACTIVITY IN THE
PEDAL WAVES OF HELIX

by

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submitted in partial fulfilment of the
requirements for the degree of

Doctor of Philosophy

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CONTENTS

CHAPTER I	Introduction	Page 1
CHAPTER II	Purpose of this investigation	5
CHAPTER III	Review of the literature	10
CHAPTER IV	Method and procedure	19
CHAPTER V	Results and discussion	26
A.	Creeping during vertical ascension without loads	26
B.	Comparison of vertical creeping with and without loads	33
C.	The effect on locomotion of de-eying <u>Helix</u>	43
D.	Behavior of the detached foot	49
E.	Effect of mechanical stimulation and of Ringer's solution on locomotion	51
F.	Action of adrenalin on the neuro-muscular activities during locomotion	62
G.	Effect of strychnine sulphate on locomotion	98
CHAPTER VI	Summary and conclusions	115
CITATIONS		118
INDEX OF FIGURES		123

Chapter I.

Introduction

Precise measurement of vital processes is necessary in the investigation of many of the problems arising in the study of vital dynamics. Results based on series of facts obtained upon the intact living organism prevent confusions which arise when less exact methods are employed. The philosophical approach into the realm of vital phenomena has not been a step forward because the introduction of philosophical ideas, such as adaptation, behavior, emergence, psychobiology and purpose have resulted only in methods of vagueness and inexactness.

Huxley (1854) predicted that the science of the biologist would be as deductive and exact as mathematics. He was of the opinion that since biology is a physical science the methods of this science must be analogous to those followed in other physical sciences. This prediction was fulfilled sooner than he would have believed possible. Loeb (1888) laid the foundations for this method of precise measurement by his contribution of tropistic conduct. Crozier (1928) has taken tropisms as a working tool for the further development of many unexplained activities found in living phenomena. He recognizes the significance of the quantitative aspects of behavior and believes that it is this quantitative treatment of tropistic behavior which is essential if an understanding of conduct is to be furthered. As he has

stated, the biological system presented by a single individual is not a "thing", a single event, but a system of relations. These relations must be defined through investigation and their functional dependence analyzed.

Stier (1928) has mentioned certain aspects of animal conduct other than those considered in the tropism doctrine; that is, motor activity and fluctuations of sensitivity may be based on principles originating in the fundamental laws of chemistry and physics.

Rose (1929) emphasized the fact that it is only by more precise measurement that the problem of vital dynamics can be cleared up, and its progress can be furthered only by the use of quantitative methods and rigorous analyses. By delicate measurements scientists have obtained just such desired results, which have completely revolutionized researches in the field of plant tropisms and at the same time have opened up new aspects of the physiology of plants.

Furthermore, various related phenomena of animal reactions cover a wide field. Myogenesis (Carey, 1919-20), oxidations as a function of temperature (Crozier, 1924-25), tonic immobility (Hoagland, 1927), spontaneous movement (Stier, 1928) and inheritance (Crozier and Pincus, 1929-30) are a few activities taken from the field of animal conduct that have led to important results by the application of the method of precise measurement. For example, Crozier and Pincus have arrived at a definition of gene differing from the usual one; for them it is a definition of the

effect in inheritance as a function of some controlling, independent variable.

In the investigation of the dynamics of histogenesis, Carey (1919-20) has shown by the method of precise measurements (the number of contractions made in a unit of time, measurement of intra-vesicular hydro-dynamic pressure and volume of stimulus) that the formation of muscular tissue is due to a definite active process, not a passive one, as the term self-determination denotes. He proved by his experiments that the intensional stimulus is the necessary factor in myogenesis. From his dynamic point of view the muscle types represent differences in the amount of work that has been done on the undifferentiated mesenchyme by the differentially growing parts of the embryo during the active growth period. He demonstrated that it is possible to transform unstriated muscles into striated by varying the velocity of application and the intensity of the tensional stimulus to a higher optimum degree. By this method of procedure his results enabled him to conclude that the variable intensity of the optimum tension determined the muscular type. In other words, the structure of muscular tissue is determined by the function it performs and the work it does, but on the other hand structure does not determine function. He thus reached these conclusions as a result of his experiments employing analysis through tension and pressure.

Therefore, just as analysis through temperature

characteristics plays an important part in the specific control of vital processes, so also analysis through pressure and tension becomes an additional factor which may lead to identification of reacting living matter. The following experiments are approached through this procedure.

Chapter II.

Purpose of this investigation

The purpose of this investigation is the detailed analysis of the factors involved in locomotion, to gain additional information concerning the creeping mechanism of Helix. Inasmuch as the movement of the waves found in the foot of gastropods is inseparable from locomotion it is necessary to describe the types of waves found in these animals; and it is also of importance to consider other forms of periodic waves found in muscular tissue.

Various kinds of periodic waves occurring in muscular tissue have been investigated to determine, if possible, the cause of neuro-muscular activity. The waves which are present in the peristaltic action of the intestine, the rhythmic pulsation of the cloaca in holothurians, the pedal waves found in larvae of slug-moths, the waves found in Thyone briareus, and the peristaltic locomotor waves of the tent caterpillar may be cited as examples.

The balance of opinion as to the cause of the rhythmic contractions of the intestine has been in favor of neuro-genesis, but there is now considerable evidence of myogenic origin, offered by Magnus (1905), Gunn and Underhill (1914), Alvarez and Mahoney (1922), Cowie, Parsons and Lashmet (1929), Ascanio and Alvarez (1929). Alvarez agreed with some of these investigators as to myogenic origin of peristaltic action and believed that a gradient of irritability has been

found which may be an important factor in the direction of normal peristalsis.

The anal pulsating mechanism of holothurians might be regarded as constituting another unit in the series of independent effectors, such as pedicellariae and spines, which go to make up the echinoderm neuro-muscular equipment. Crozier (1916) has investigated the physiological characteristics of cloacal pulsations in Stichopus moebii Semper. The results of these experiments are in essential agreement with the data derived from many previous studies in pulsating structures, such as those of medusae, ctenophores, the arthropod heart, and the vertebrate heart and intestine, i.e., the rhythm has a temperature coefficient of the order of magnitude of that for chemical processes and the relation of pulsation to the salts of sea water is essentially like that in other well-known pulsating systems.

The pedal waves found in the larvae of the slug-moths (Cochlidiidae) are similar in many respects to those on the pedal surface of gastropods. Crozier (1923-24) has found that the speed of these pedal waves corresponds almost exactly with the speed of the pedal wave in Chiton tuberculatus and is therefore in this respect more comparable to the molluscan foot than to the peristalsis of the body in the earthworm or in caterpillars. He has concluded that the peristaltic pedal waves of these animals are to be regarded as "a derivative of the general peristalsis of ordinary caterpillars, and as in the 'myenteric reflex' of the vertebrate intestine

it implies reciprocal innervation." (p.328)

When the peristaltic locomotor waves of the tent caterpillar were studied by Crozier and Stier (1925-26) it was found that the frequency of the abdominal waves during vertical ascension was controlled by temperature according to the Arrhenius equation.

Stier (1928) has come to the conclusion that a proprioceptive mechanism in the body wall seems to control the initiation of the locomotor waves in Thyone.

Lutz (1930) studied the effect of low oxygen tension on the pulsations of the isolated holothurian cloaca (isolated strips from the cloaca of Stichopus moebii Semper and ring preparations from the cloaca of Cucumaria frondosa) because oxygen deficiency has often been associated with periodicity and augmentation of response in various tissues. He concluded that a certain degree of oxygen lack results in increased activity of the tissue.

Although the exact cause of the initiation of the rhythmical waves (referred to in the preceding paragraphs) is not known, it can be seen that by means of investigations of this nature new factors are brought to light that play an important part in the control of periodic waves.

To investigate rhythmical waves further the gastropods offer excellent opportunities, and the same principle when found may apply to other tissues where periodic waves occur. The action of the waves found in the foot of gastropods has frequently been compared to the peristaltic waves occurring

in the stomach and intestine. Jordan (1927) found that the cerebral ganglia of snails have a quantitative influence on the peristaltic action of the foot (with its pedal ganglia) similar to the autonomic innervation of the stomach. However, the use of the foot of this animal is of advantage compared with the use of other tissues such as excised pieces of intestine. In the study of the latter many more factors enter, such as regulation of temperature and of intra-intestinal pressure, and the use of oxygenated Locke's solution, as well as other factors, thus adding to the complication of investigation.

Helix pomatia (Fig. A1, a and b) and Helix lactea (Fig. A1, c and d), common Mediterranean species, were used in these experiments, which extended over a period of three years. As the animals possess great tenacity of life and are unaffected by extremes in temperature they are adapted to experiments continuing over long periods of time. Another advantage is their precise negative geotropism. In the experiments of vertical creeping with and without loads this tropistic quality insures upward creeping. Still another advantage of using Helix is the type of wave it possesses, spreading over the entire foot. Therefore, the adhesive power of the foot does not present such a complication as in the foot that does not form pedal waves over its whole breadth. Helix is moreover very suitable for analysis through precise measurements on the

intact organism.¹

Just as investigation through temperature characteristics has thrown light on the specific control of vital processes, so analysis through pressure and tension becomes an additional factor that may lead to the identification of reactions in living matter. As the central nervous interplay governing the mechanism of locomotion and the exact cause of the waves is unknown in gastropods, the subsequent experiments were undertaken to investigate further these waves and other factors involved during locomotion under varying conditions. The quantitative measurements of the activities investigated in these animals have been studied and their functional dependence analyzed.

¹ Crozier and Pilz (1923-24) have stated that it should be possible to utilize these waves for the study of neuromuscular physiology in the intact animals, avoiding in this way effects due to lack of proper circulation and the like when isolated organs are used.

Chapter III.

Review of the literature

The pedal waves found in the foot of the gastropods have been described and in some cases classified by Carlson (1904-05), Biederman (1905, 1906), Vles (1907), Parker (1911, 1914), Olmstead (1917-18), van Rijnberk (1918-19), Crozier (1918-19); 1919-20; 1922-23 a and b), and ten Cate (1923). The creeping of Limax maximus has been described by these writers as a result of a rhythmic succession of evenly spaced, progressive wave-like deformations of the pedal surface.

Vles (1907) has grouped the waves that appear on the foot of mollusks into two general types, i.e., the "direct" waves that pass from the posterior to the anterior end of the foot when the animal moves forward, and the "retrograde" waves which pass over the foot from front to back as the animal moves forward.¹ In both types of movement several subtypes can be distinguished as determined by the lateral extent of the pedal wave, i.e., "monotaxic", "ditaxic" and "tetrataxic" subtypes. The direct waves may be monotaxic, ditaxic and tetrataxic types, the retrograde waves either

¹ With the exception of Chiton gastropods always move forward and never backwards, regardless of the type of waves passing over the foot. Parker (1914) has found that Chiton can reverse its locomotion and creep backwards a few millimeters. This is the first instance of backward locomotion to be recorded in gastropods (verified later by Crozier and Navez).

monotaxic or ditaxic. The direct monotaxic waves consist of a single system of waves traversing the foot as found in Limax and Helix.¹ Direct ditaxic waves consist of two systems of waves occupying each one of the lateral halves of the foot and alternating regularly on the two sides of the median line which is not affected by these waves. Parker (1911) has added Tectarius nodulosus and Nerita tessellata as ditaxic gastropods and has found that in these animals the waves on the two sides of the foot usually alternate and are so extensive that never more than two waves can exist on one side at one time. As a result of this the foot moves forward in alternate steps, first on the right and then on the left, the motion resembling that of a person walking in a sack. Vles (1907) has described the direct tetrataxic waves, i.e., the foot which is traversed by four systems of waves is broadly fissured on the median line and each lateral sole is overrun by two systems of alternate waves such as are found in the ditaxic foot. The retrograde waves may be monotaxic or ditaxic. In considering the theories advanced to explain the locomotion of gastropods only the first group (direct monotaxic) is considered by the following authors.

The investigation of the activity of these waves has resulted in various conclusions concerning both the mechanism controlling them and other factors in locomotion.

¹ In Helix the waves are monotaxic, but they pass over the entire breadth of the foot (helicine foot), whereas in Limax they appear only in the central longitudinal area of the foot.

Contraction of the longitudinal muscles, the action of the dorso-ventral muscles (transverse and oblique muscles), the pressure of substances in the body fluids, and other factors have been advanced to explain the controlling mechanism.

Simroth (1878) accounted for locomotion by a theory that he called "extensile muskulatur". He concluded that the waves cannot be produced by the separate or the combined contractions of the oblique and transverse muscular strands, but that the cause of the extension of the foot is the active extension of the longitudinal musculature. He believed that the extensile muscle fibers in all or nearly all snails are the important factors in its movement from place to place.

Jordan (1901) advanced a theory which he later modified. He did not accept the theory of "extensile muskulatur" in accounting for locomotion in the marine gastropod Aplysia. He attributed the relaxation or the extension of the longitudinal muscle of the foot to pressure of isolated bodies of the visceral fluid or blood.

Bohn (1902) attributed the production of the waves to direct excitation of the muscle fibers and to a sort of progressive "induction". He explained that this "induction" would be produced every time the muscle fibers were placed on level cylindrical or conical surfaces and the waves would be distributed in bands or rings, parallel, narrow and identical with each other. He called them organic waves and postulated the theory that by the study of these

and other organic waves other facts of biologic induction may be brought to light and may play an important role in an explanation of the kinetogenetics of evolution and perhaps even of heredity.

Carlson (1904-05) has described the external mechanics of locomotion and has differed in some respects from other authorities. He did not accept Simroth's theory of "extensile muskulatur" or Jordan's theory of the pressure of isolated bodies in the visceral fluid. Carlson tested the longitudinal muscle in the foot of several gastropods and his results showed that there is no difference between the physiology of the muscle and that of any other muscle. He concluded that during ordinary progression the animal assumes its greatest length and smallest diameter, due to the contraction of the transverse and the oblique muscles of the dorsal and lateral parts of the body. The waves of locomotion in the foot are diminutive representations of the waves of relaxation and contraction. At the areas of relaxation the sole of the foot adheres closely to the ground and between these points the sole is slightly elevated. Although von Uexküll believed that the foot is provided with some mechanical device such as the setae of the earthworm, Carlson believed that the area of contact of the foot with the ground in any region serves as a fixed point through friction, and acting on this the contraction of the longitudinal muscles of the foot pulls the neighboring portion of the body forward.

Parker (1911) has described most satisfactorily the external mechanics of locomotion in Helix pomatia. He stated that the forward movement takes place in the dark waves, and quiescence is characteristic of the intermediate lighter portions of the foot. Each wave is a pulse of forward motion and the rest of the foot is momentarily quiescent. He stated, "the area covered by the wave is probably a fourth or fifth of the whole foot. At any moment, therefore, three-fourths to four-fifths of the surface of the foot is stationary and about one-fourth to one-fifth is moving forward. In other words, the snail stands on the greater part of its foot while it moves forward with a much lesser part." (p. 102). From experiments performed he believed that each wave on the underside of the foot (of Helix pomatia) is a slight concavity. When the muscle of the foot relaxes the portion of that foot that was elevated is returned to its former level and the muscle recovers its original length and position. This action of the dorso-ventral muscles takes place from behind forward and thus a concave wave runs on the surface of the foot from tail to head. The forward movement of that portion of the foot which is temporarily lifted from the substrate is accomplished by the action of the longitudinal muscles. The contraction of each longitudinal fiber serves to move the foot forward as the relaxing wave passes over the foot. It also extends the relaxing posterior fibers. An important point in this description is that the dorso-ventral muscles

play an active part in lifting the foot from the substrate due to the fact that their dorsal ends, being more firmly set than the ventral ends, serve as relatively fixed points and therefore the ventral ends move. The action of the ventral end lifts the foot locally and overcomes adhesion in the given region. In this way each point of the foot is lifted, moved forward and set down again and thus the foot, and with it the animal as a whole, moves forward.

Although Vlès and Bathellier (1920) did not arrive at a conclusion in regard to the action of the pedal waves of gastropods they deduced that certain numerical laws held for them.

ten Cate (1923) was of the opinion that the wave of contraction is caused by the nervous peripheral net-work.

Crozier and Pilz (1923-24) agreed with the description of the external mechanics of locomotion as given by Parker (1911). It appears from Crozier's investigation of the neuro-muscular activity of the foot that he did not have at first conclusive evidence of nerve-net transmission. However, from the experiments (Crozier and Pilz, 1923-24) performed on the temperature coefficient for pedal activity in Limax these investigators found that the velocity of a single wave must have very nearly the same temperature characteristic which is found also in another case of nerve-net transmission (Renilla). They found that when work was done at a constant rate the frequency of the pedal waves is influenced by the temperature according to the Arrhenius equation, with $\mu = 10,700$ (Q_{10} for 11° to $21^{\circ}\text{C.} = 2.1$).

Magnus (1924) has shown from the "half animal" experiments performed by Jordan and Uexküll that stretching of the musculature in snails can react on the condition of the pedal ganglia.

Crozier and Federighi (1924-25c) concluded that the pedal organ of the slug, although under the control of central nervous impulses, is essentially an independent effector. (See further, page 49 of this thesis.)

Cole (1925-26) considered the stimulus for geotropic orientation and locomotion in Helix aspera to be the tension of the body muscles produced by the downward pull of gravity. The stimulus is received by the proprioceptors of these muscles.

Jordan (1927) explained the rhythmical transmissions of stimulation in the snail's foot according to von Uexküll's law (p.243). The foot of the snail, with its nerve net, is not sufficiently autonomous to be able to carry on creeping without the help of a ganglion. The stimulation which produces locomotion is conducted in the beginning through the nerve-net and through the pedal ganglia with the nerves radiating from them to the periphery. As to the manner in which the movement itself comes into existence, nothing exact is known. A true antagonism such as appears in arthropods, annelids and vertebrates is lacking in these animals, and instead the phenomenon of "viscosoid" tonus is present.

Jordan considered "viscosoid" tonus as a static phenomenon and as an attribute of a colloid system. In his

distension experiments on the snail's foot he stated that the characteristics of these muscles can be recognized as those of a fluid colloidal phase. The musculature of these animals possesses attributes which in many connections have the characteristics of protoplasm. For instance, phagocytosis is present in considerable degree in the same animal in which "viscosoid" tonus plays so important a role in the outer muscle layer. In amoeboid movements and phagocytosis the work is connected with the shifting of the parts and thence with an alteration of the form. With regard to muscle, therefore, a new form of contraction problem arises. As soon as the musculature is shortened through stimulation nothing more is observed of the fluid phenomenon. The contracted muscle is an almost pure elastic body. In this connection Freundlich, as referred to by Jordan, has stated that in dead colloidal stuffs the conversion of the fluid-viscoid condition into the elastic state occurs. Hypothetically one may think, in the case of the muscle of a conversion from the sol to the gel condition. In the latter the form-alteration would no longer be explicable through the shifting of the component parts but through a particular reversible elastic form-alteration of them.

ten Cate (1927-28) has stated that the movement of the appendages of Aplysia limacina is determined directly by the pedal ganglia and is not produced by a chain of ganglia.

As real geotropic orientation of the Helix type of gastropod takes place only during active progression,

orientation, because of its close relationship to locomotion, can be included in this discussion.¹

Geotropic orientation rather than locomotion has been studied by Crozier and Navez (1930). They concluded that orientation is not governed either as to direction or as to latent period by the involvement of the statocyst. Gravitationally excited orientation is probably controlled by the proprioceptive stimulation through impressed tensions (Crozier and Navez, 1930). As orientation and progression are so closely united these investigators have stated that it is still a question as to whether (1) the creeping movement and postural movement are released together or (2) the operation of the pedal wave is the necessary factor to act on the stimulus for orientation. Therefore it can be seen from various theories existing with regard to this subject that it is important to investigate the question further. Helix, rather than Limax was chosen, as more work has been done on Limax (Crozier and Pilz, 1923-24; Crozier and Federighi, 1924-25c) and very few data have been obtained for Helix.

¹ The muscular mechanism for locomotion and that for orientation is structurally distinct, i.e., creeping is brought about by means of a pedal organ, while orientation is accomplished by means of muscles in the dorso-lateral regions of the body wall.

Chapter IV

Method and Procedure

Specimens of Helix lactea and Helix pomatia used in these experiments were kept at room temperature in separate jars which were kept slightly moist. Lettuce was fed every other day and the jars were thoroughly cleaned. In this way the animals lived for six or seven months, the vigor of creeping showing no diminution at the end of that period.

The following experiments were carried on within the temperature limits of 19.5° and 24.8°C . The animals were subjected to varying environmental conditions, which will be described subsequently. The required measurements were obtained as follows: the animal was placed in a glass jar one and one half feet high¹ and when the snail began to creep vertically upwards, the sides of the jar being kept moist to promote active creeping, the distance travelled while ten waves were passing over the foot from posterior to anterior end was measured in millimeters on the outside of the jar. (Fig. 1). The time was recorded in seconds or minutes. This constituted one run, and ten runs made up a series (see Table I). Several series for each animal were obtained and the conditions for each series were the

¹ In later experiments a glass plate set in a frame similar to a picture frame was used, as this enabled handling the animal more easily.

same. The length of the foot during each run was also measured in millimeters.

From these data it is possible to determine:

- (1) The frequency of the pedal waves (F).
- (2) The velocity with which a single wave traverses the foot (v).
- (3) The speed of creeping (V).
- (4) The advance due to a single wave (A).

An example of the way in which the data observed have been used to obtain the absolute values of the frequency of waves and the velocity of progression¹ is presented in detail in Table I, and a description of the way these absolute values are used in plotting one relation to another is given in the text.

As the ultimate purpose of this series of experiments was to analyze the relation between the frequency of waves and the velocity of progression it was necessary to obtain from the data thus observed the values of the velocity of progression, which are given in Table II. With these measurements it is possible to show by their actual scatter and by comparative graphs the linear relationships between the different activities involved in locomotion. The method of plotting was to take the computed value of the frequency of waves and the velocity of progression (Table II)

¹ The same method was followed to obtain the measurement for the advance per wave and the velocity of a single pedal wave.

same. The values of the above ratios were also found

measured in similar cases.

From these data the following results were obtained:

- (1) The frequency of the waves is proportional to the square of the velocity.
- (2) The velocity of the waves is proportional to the square of the frequency.

The results are:

- (3) The speed of the waves is proportional to the square of the frequency.
- (4) The frequency of the waves is proportional to the square of the speed.

An example of the way in which the data were treated has been used to obtain the results of the above.

of waves and the velocity of propagation. The results are given in detail in Table I, and a description of the way in which the data were treated is given in the Appendix.

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As the velocity of the waves is proportional to the square of the frequency, the results of the above are given in Table I, and a description of the way in which the data were treated is given in the Appendix.

and the velocity of propagation is proportional to the square of the frequency, the results of the above are given in Table I, and a description of the way in which the data were treated is given in the Appendix.

From the data thus obtained the results of the above are given in Table I, and a description of the way in which the data were treated is given in the Appendix.

proportional, while the results of the above are given in Table I, and a description of the way in which the data were treated is given in the Appendix.

measurements, it is possible to obtain the results of the above are given in Table I, and a description of the way in which the data were treated is given in the Appendix.

and by comparison with the linear relationship between the frequency of the waves and the velocity of propagation, the results of the above are given in Table I, and a description of the way in which the data were treated is given in the Appendix.

The above method was used to obtain the results of the above are given in Table I, and a description of the way in which the data were treated is given in the Appendix.

and plot the actual scatter, the ordinates representing the frequency of waves and the abscissae the velocity of progression. After presenting the actual scatter in this way their average points were obtained. The best fitted line was then drawn through these average points. The graph resulting from the data just given in detail is shown in Fig. 3 for animal No. 7. Graphs showing the same relationship have been compared in subsequent experiments when the data are derived under varying conditions.

Tables IIa and IIb represent the data for a complete experiment when each one of the absolute values was determined, i.e., velocity of progression, frequency of waves, velocity of a single pedal wave and the advance per wave. From these data the various graphs were plotted as described above.

TABLE I

Observed data ¹						Calculated values of frequency of waves from observed data
No. of runs	No. of series	No. of waves	Actual time in seconds	Actual distance in mm.	Length of foot during each run ²	Frequency of waves = $\frac{\text{waves}}{\text{time}}$
1	= 1	10	16.0	7.5	49.0	0.624
2		10	10.8	7.5	51.0	0.925
3		10	13.9	7.0	51.0	0.719
4		10	13.9	8.0	52.0	0.719
5		10	12.4	8.5	54.0	0.806
6		10	11.2	7.5	53.0	0.892
7		10	12.2	10.0	53.0	0.819
8		10	12.0	7.5	54.0	0.832
9		10	11.4	8.5	53.0	0.878
10		10	10.4	10.0	54.0	0.961

¹ Animal No. 7.

² These measurements are needed when the velocity of a single wave is to be determined, i.e., $V = \frac{\text{Length of foot}}{\text{Time for 1 wave}}$.

TABLE I

Continued
from page 10

Continued

No. of runs	Secs. per run	Secs. per run	Secs. per run	Secs. per run	Secs. per run
1	10.0	10.0	10.0	10.0	10.0
2	10.0	10.0	10.0	10.0	10.0
3	10.0	10.0	10.0	10.0	10.0
4	10.0	10.0	10.0	10.0	10.0
5	10.0	10.0	10.0	10.0	10.0
6	10.0	10.0	10.0	10.0	10.0
7	10.0	10.0	10.0	10.0	10.0
8	10.0	10.0	10.0	10.0	10.0
9	10.0	10.0	10.0	10.0	10.0
10	10.0	10.0	10.0	10.0	10.0

Initial No. 1

These measurements were taken from the results of a single run in an unobstructed, i.e., 1 x 1 ft. duct.

TABLE II

Observed data ¹						Calculated values of progression obtained from observed data
No. of runs	No. of series	No. of waves	Actual time in seconds	Actual distance in mm.	Length of foot during each run	Velocity of progress = $\frac{\text{distance (mm.)}}{\text{time (sec.)}}$
1	= 1	10	16.0	7.0	49.0	0.437
2		10	10.8	7.5	51.0	0.694
3		10	13.9	7.0	51.0	0.503
4		10	13.9	8.0	52.0	0.574
5		10	12.4	8.5	54.0	0.685
6		10	11.2	7.5	52.0	0.669
7		10	12.2	10.0	53.0	0.819
8		10	12.0	7.5	54.0	0.624
9		10	11.4	8.5	53.0	0.745
10		10	10.4	10.0	54.0	0.961

¹ Animal No. 7

TABLE 1

UNITED STATES
DEPARTMENT OF AGRICULTURE
BUREAU OF PLANT INDUSTRY
WASHINGTON, D. C.

PLANT INDUSTRY

PLANT INDUSTRY	UNITED STATES	DEPARTMENT OF AGRICULTURE	BUREAU OF PLANT INDUSTRY	WASHINGTON, D. C.
1	2	3	4	5
6	7	8	9	10
11	12	13	14	15
16	17	18	19	20
21	22	23	24	25
26	27	28	29	30
31	32	33	34	35
36	37	38	39	40
41	42	43	44	45
46	47	48	49	50
51	52	53	54	55
56	57	58	59	60
61	62	63	64	65
66	67	68	69	70
71	72	73	74	75
76	77	78	79	80
81	82	83	84	85
86	87	88	89	90
91	92	93	94	95
96	97	98	99	100

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TABLE IIa

Observed data

No. of the run	No. of series	No. of waves	Actual time in sec.	Actual distance in mm.	Length of foot (mm.) during each run
1		10	10.0	9.5	46.0
2		10	9.9	9.0	46.0
3		10	8.6	6.5	46.0
4		10	9.0	8.0	45.0
5		10	9.0	8.5	47.0
6		10	9.8	10.0	45.0
7		10	9.0	10.5	45.0
8		10	10.0	7.0	45.0
9		10	7.8	7.5	46.0
10		10	9.6	8.5	46.0

= 1 Series

The foot was measured during each run with a flexible millimeter ruler.

TABLE IIb

Treatment of data

Taking the measurements obtained as given in Table I the absolute values for the velocity of progression (V), the frequency of waves (F), the velocity of a single pedal wave (v) and the advance per wave (A) can be obtained for each run.

No. of the run	V $\frac{\text{dist. mm.}}{\text{time sec.}}$	F $\frac{\text{waves}}{\text{time (sec.)}}$	v $\frac{\text{length of foot (mm.)}}{\text{time for 1 wave}}$	A $\frac{\text{dist. (mm.)}}{\text{waves}}$
1	0.950	1.000	46.0	0.950
2	0.909	1.010	46.4	0.900
3	0.755	1.161	53.4	0.650
4	0.898	1.111	50.0	0.800
5	0.944	1.111	52.3	0.850
6	1.020	1.020	45.9	1.000 = 1 Series
7	1.166	1.111	50.0	1.050
8	0.700	1.000	45.0	0.700
9	0.961	1.280	58.9	0.750
10	0.885	1.041	47.9	0.850

Chapter V.

Results and Discussion

A. Vertical ascension without added load.

In a series of experiments extending over a period of several months eleven animals, Helix lactea, of varying weights were used. Measurements were obtained for a total of 36 series, each series made up of ten runs, and each run made up of ten waves; thus 3600 waves were observed.

Data were obtained from observing 400 waves in each of the animals when creeping vertically upwards carrying no load. The relations between the velocity of a single wave and the velocity of progression, and between the frequency of waves and the velocity of progression, as well as between the advance per wave and the velocity of progression were analyzed.

Fig. 2¹ shows the relation between the velocity of a single pedal wave and the rate of creeping ($v \frac{V}{v}$).

It can be seen that a linear relationship exists regardless of the weight of the animal.

¹ The actual observations were plotted taken from data obtained from 4 series of 40 runs, each run consisting of the record of the time and distance covered for 10 waves. In order to obtain the average points of Fig. 2 the mean was taken of the actual observations. This was the way in which the points were determined in the other figures. It is possible to obtain the average deviation from the mean for any of the points given in the figures.

	Weight	Slope of line ¹
Animal No. 1	6.5 gm.	0.20
" " 4	8.6 "	0.36
" " 7	7.9 "	0.34

Fig. 3 shows the relation between the frequency of waves and the velocity of progression ($F \frac{\text{ }{V}$). It can be seen also that a linear relation exists regardless of the weight of the animal.

	Weight	Slope of line
Animal No. 1	6.5 gm.	0.20
" " 7	7.9 "	0.18
" " 10	3.8 "	0.20

Fig. 4 shows the relation between the advance per

¹ To find the slope of the line as given in Fig. a, the distance AB expressed in mm. is subtracted from the distance CD expressed in mm. and divided by the distance represented by line AC. The same unit for AC is used in each case, i.e., 50 mm.

Animal No.	Weight	Height
1	1.5	1.2
2	1.5	1.2
3	1.5	1.2

Fig. 3 shows the relation between the weight of the animal and the weight of the brain. The weight of the brain is given in grams. The weight of the animal is given in kilograms. The relation is shown in the following table:

Animal No.	Weight of Brain (g)	Weight of Animal (kg)
1	1.5	1.2
2	1.5	1.2
3	1.5	1.2

Fig. 4 shows the relation between the weight of the brain and the weight of the body. The weight of the brain is given in grams. The weight of the body is given in kilograms. The relation is shown in the following table:

Animal No.	Weight of Brain (g)	Weight of Body (kg)
1	1.5	1.2
2	1.5	1.2
3	1.5	1.2

wave and the velocity of progression ($A \frac{\text{ } }{V}$). The results show that in this relationship also a linear relationship exists regardless of the weight of the animal.

	Weight	Slope of line
Animal No. 4	8.6 gm.	0.3
" " 7	7.9 "	0.3
" " 10	3.8 "	0.3

Further experiments of this nature were performed on a number of different animals to see whether consistent results would be obtained. The following summary shows the scope of the experiments from which data were obtained for the three different relationships: $V \frac{\text{ } }{V}$, $F \frac{\text{ } }{V}$, $A \frac{\text{ } }{V}$, for animals 1, 3, 4, 5, 6, 7, 8, 10 and 11 during vertical ascensions without load. For these nine animals a total number of 3200 waves was observed and measurements taken. From these data graphs were plotted of the actual scatter for each animal showing the relation between the velocity of a single pedal wave and the rate of progression ($V \frac{\text{ } }{V}$), between the frequency of waves and the rate of creeping ($F \frac{\text{ } }{V}$) and between the advance per wave and the velocity of progression ($A \frac{\text{ } }{V}$). The average points of each graph were obtained and the best fitted line drawn. Fig. 4A shows a comparative graph of these lines, each line illustrating for each animal the relation between the velocity of a single wave and the

velocity of progression. A straight line relationship is found to exist under these conditions. The slope of the line for each animal was determined. The same method of comparison and analysis was employed in the investigation of the relation between frequency of waves and the velocity of progression (Fig. 4B), and between the advance per wave and the velocity of progression (Fig. 4C). These results are given in Table III.

Helix pomatia was used in a series of experiments to determine whether the laws derived from observations of Helix lactea would be confirmed. The conditions and method of these experiments were the same as for Helix lactea. The limits of temperature were 16.2 to 21.0°C., but constant for each experiment. The length of the foot of Helix pomatia when creeping is from 60 to 100 millimeters (Figs. Ala and Alb), and that of Helix lactea from 25 to 40 millimeters (Figs. Alc and Ald). Helix pomatia has the helicine foot, i.e., it forms waves over the whole breadth of its foot, so that adherence and progression is not attended to by functionally separate divisions of the pedal surfaces. Table IIIa¹ shows some average results of these experiments. Fig. 4D illustrates that for Helix pomatia a linear proportionality exists between the velocity of a single wave and the rate of creeping. Fig. 4E shows that the frequency of waves is directly proportional to

¹ Time is recorded in minutes.

TABLE III

Animal	Weight	Relationship	Slope of line	Relationship	Slope of line	Relationship	Slope of line
1	6.5 gm.		0.20		0.20		0.28
3	7.7 "		0.52		0.18		0.34
4	8.6 "		0.36		0.18		0.30
5	7.1 "		0.48		0.20		0.38
6	6.7 "		0.82		0.18		0.20
7	7.9 "		0.34		0.18		0.30
8	8.5 "		0.52		0.18		0.34
10	3.8 "		0.54		0.20		0.30
11	5.0 "		0.24		0.22		0.20

the rate of creeping. Fig. 4F demonstrates that the advance per wave is directly proportional to the rate of creeping.

Analysis of the experimental results obtained in this series yields certain information regarding the condition of the foot during vertical ascensions without load. A study has been made of the relations between the velocity of a single pedal wave and the velocity of progression, between the frequency of waves and the velocity of progression, and between the advance per wave and the velocity of progression. The slope of the line for these relationships has been determined. A comparison of these slopes shows the deviation of the linear proportionality, if any. Variations from strictest proportion found occasionally may arise in large part from the complex character of the pedal musculature. In general the linear proportionality of these relationships to each other is unmistakable. The relation between the velocity of progression and the velocity of a single wave shows the greatest alteration during a given number of runs under the same conditions for a number of animals of varying weights. The relation between the frequency of waves to the velocity of progression exhibits the least alteration within a number of series of one animal or when several animals are compared with each other. A study of the graphs and tables shows that the slope of the line in a given relation may be the same regardless of the weight of the animal.

Therefore, these results show that the speed of

TABLE III a

Average results for vertical creeping of Helix pomatia¹ under normal conditions.

Animal	Velocity of progression mm. per min. V	Frequency of waves per min. F	Velocity of single waves mm. per min. v	Advance per wave, mm. A	Temperature (C) degrees
No. 41	18.7	56.6	4866	0.15	17.7
No. 43	7.17	33.3	2013	0.21	16.4
No. 44	15.1	84.0	8100	0.19	17.7
No. 45	7.3	46.7	3188	0.13	17.7
No. 46	8.5	45.8	3950	0.18	17.7
No. 47	11.6	77.7	4200	0.30	17.7
Maximum	18.7	84.0	8100	0.30	17.7
Minimum	7.17	33.3	2013	0.13	16.4

¹ The waves are the same type as found in Helix lactea, i.e., monotaxic, which spread over the entire surface of the foot. At any one moment 10 to 15 waves traverse the foot.

creeping for Helix lactea and Helix pomatia without load on a vertical surface during upward progression is directly proportional to the velocity with which a single average wave courses over the foot. It is independent of the weight of the animal and the length of the foot. The velocity of progression is directly proportional to the frequency of waves and to the advance of a single wave.

B. Vertical ascension with added load.

The technique for adding the loads was to attach to the shell lead weights held on by pieces of adhesive tape. The animals (Helix lactea) were first tested to determine the weight necessary for complete exhaustion. This was for the purpose of gaining an indication of the probable number of weights to be attached and to select individual weights that could be added conveniently. Table IV represents the results when the animals were tested for complete exhaustion.

TABLE IV

No. of Animal	Complete Exhaustion with weight	Weight of Animal
1	4.3 gm.	6.5 gm.
2	0.7 "	7.1 "
3	3.5 "	7.7 "
4	4.8 "	8.6 "
5	3.0 "	7.1 "
6	4.8 "	6.7 "
7	4.8 "	7.9 "
8	3.8 "	8.5 "
9	2.0 "	3.5 "
10	4.8 "	3.8 "
11	3.0 "	5.0 "

Animals No. 2 and No. 9 showed the most rapid exhaustion with the least load. Some inherent weakness may have accounted for this as both animals died after three months. Animal No. 2 crept poorly from the beginning and it was possible to obtain only half the number of runs as compared with the other animals when creeping without loads. The same was true for No. 9, except that great activity was shown for the series that was obtained, though only half the series as compared with the other animals was obtained. The results show that exhaustion caused by added weights was independent of the weight of the animal. The load which caused exhaustion for the majority of the animals was 3.0 gm. or more up to 4.8 gm. Consequently individual lead weights of 0.5 gm. were chosen as the most suitable to obtain effects of small added loads up to 4.3 or 4.8 gm.

The method of applying the weights was as follows: the weight for each load was gradually increased but these increased loads were not added in immediate succession. A run without any added load or a load smaller than the load just used was made between the various increases. Table V illustrates this method. The advantage of this method was to insure the fact that any effects of fatigue caused by the additional weights could be avoided by having a run of no load or a lighter load than had just been removed before the next heavier weight had been added. This was the method used to obtain data for eleven

animals, the range of temperature for the entire series being from 19.5° to 24.8°C . but constant for each experiment.

The way in which these graphs were made was to take the data from a group of animals, i.e., animals numbered 3, 4, 7 and 10 and use it to plot four different comparative graphs (each graph for each animal) showing the following relations:

- a. Between velocity of progression and velocity of pedal wave.
- b. Between frequency of waves and velocity of progression.
- c. Between advance due to a single wave and speed of creeping.

The measurements obtained when 8,300 waves were observed while eleven animals were carrying varying loads during vertical ascension were used in the analysis of the data. A comparison was made with and without load from the actual scatter of the data. The same relationships as those described for the last experiments were used as functions of one another. Collective graphs were plotted. The following typical graphs illustrate the way the data were utilized throughout these experiments. Fig. 5A shows the actual scatter obtained from direct observations from a series of runs with and without load for animal No. 7. The relation between the velocity of progression and the frequency of waves has been plotted. Fig. 5 also shows the effect of added loads on animal No. 7 when the frequency

TABLE V

Method of obtaining data when varying loads were added to one animal during vertical creeping.

Animal	Series	10 Runs of 10 Waves each	Without Added Load	With Added Load
#1	1	"	"	
"	2	"		0.7 gm. (run 1)
"	3	"	"	
"	4	"		1.2 " (run 1)
"	5	"		0.7 " (run 2)
"	6	"		2.0 "
"	7	"	"	
"	8	"		2.5 "
"	9	"		0.7 " (run 3)
"	10	"		3.0 "
"	11	"		1.2 " (run 2)
"	12	"		3.8 "
"	13	"	"	
"	14	"		4.3 "
				etc. until exhaustion

TABLE V

Report of electrical tests when testing leads were tested
to one relay, leading terminal a-couple.

Relay	Lead	Resistance	Capacitance
1	1	0.1	0.001
2	2	0.1	0.001
3	3	0.1	0.001
4	4	0.1	0.001
5	5	0.1	0.001
6	6	0.1	0.001
7	7	0.1	0.001
8	8	0.1	0.001
9	9	0.1	0.001
10	10	0.1	0.001
11	11	0.1	0.001
12	12	0.1	0.001
13	13	0.1	0.001
14	14	0.1	0.001

of waves is plotted against the velocity of progression. However, this graph has been derived by first plotting the actual scatter for each added load (each load plotted on a separate graph). Then the average points of the actual scatter for each load were determined and the best fitted line drawn. A comparative graph was made of these lines - each line representing an added load. Fig. 5B also illustrates for animal No. 10 the relation between frequency of waves and the velocity of progression when creeping vertically upwards carrying varying loads.

From an analysis of the data obtained under the above conditions it is possible to study the individual effect of each load on the various relations. This individual effect can be observed by comparing the slope of each line for each load. For instance, in Fig. 5 these results show that this relationship (frequency plotted against velocity of progression) is independent of the load carried. This is strikingly illustrated when we analyze these slopes; for example No. 7, without load has a slope of 0.20, with 3.8 gm. added the slope became 0.22, and with 4.3 gm. it was again 0.20.

Fig. 6 illustrates the effect of tension as related to velocity of progression. No definite laws can be stated, but in general the first loads (0.7 gm. to 2.0 gm.) tend to increase the velocity of progression. A load of 2.5 gm. tends to diminish the rate of creeping and with increasing load usually there is a slight increase in the

rate and then no change.

After the results were determined as given in Fig. 6 a comparison was made to determine the probable error when plotted with varying loads. Factors which account for error may be fluctuations in the velocity with which the individual waves traverse the foot and the possibility that the adhesive power of the foot largely depends on its extruded mucous material which may result in a slight error when comparing the effectiveness of the pedal mechanism.

The probable error of any one of the activities may be plotted against varying loads and then compared with the normal. A graph showing the relationship between the probable error for velocity of progression and varying loads is given in Fig. 7¹. The probable error appears to be greatest when weights of 0.7 gm., 1.2 gm. and 2.0 gm. were added, and very slight with 3.0 gm., 3.8 gm. and 4.3 gm. A large probable error in experiments of this kind would not be expected because the factors observed in these experiments are very definite, each wave being readily discernible.

¹ Using Bessel's formula for probable error:

$$0.6745 \sqrt{\frac{\sum v^2}{n(n-1)}}$$

the following values were obtained and plotted against the varying loads. Animal No. 6.

Without loads	3.04	With load 2.5 gm.	3.12
With load 0.7 gm.	4.34	" " 3.0 "	3.43
" " 1.2 "	3.96	" " 3.8 "	3.35
" " 2.0 "	3.50	" " 4.3 "	3.43

After the receipt of these results it was found that a comparison was made in the laboratory and that the results were in good agreement with the theoretical values. The error was found to be less than 1% in the case of the first series of experiments. The results of the second series of experiments are given in Table I. The error was found to be less than 1% in the case of the first series of experiments. The results of the second series of experiments are given in Table I.

The results of the third series of experiments are given in Table II. The error was found to be less than 1% in the case of the first series of experiments. The results of the second series of experiments are given in Table I. The error was found to be less than 1% in the case of the first series of experiments. The results of the second series of experiments are given in Table I.

Table I. Results of the first series of experiments.

Series	Value	Error
1	1.00	0.01
2	1.00	0.01
3	1.00	0.01
4	1.00	0.01
5	1.00	0.01
6	1.00	0.01
7	1.00	0.01
8	1.00	0.01
9	1.00	0.01
10	1.00	0.01

Table VI shows the effect of loads on the individual factors involved in locomotion. Absolute values and percentage of increase or decrease during vertical ascensions with added loads are compared with ascensions without load. In analyzing the effects of these added loads on the individual activities the frequency is found to show the least alteration and velocity of progression the greatest.

The results of the foregoing experiments confirm for the first time for Helix these facts relative to locomotion. The results show that the velocity of a single wave, the frequency of the waves and the advance per wave as related to the rate of creeping are independent of the load carried. These facts show that the foot is an independent effector. This evidence indicates that the intrinsic neuro-muscular mechanism of the foot is the primary factor in locomotion. Cole, on the contrary, has stated (1925-26) that the stimulus for locomotion in Helix is tension of the body muscles produced by downward pull of gravity and that the stimulus is received by proprioceptors of these muscles. This conclusion would indicate that the primary factor in locomotion is the central nervous control which does not accord with the experimental findings of the above experiments. Experiments reported later in this thesis show that the foot is secondarily under the control of central nervous impulses but that primarily it is an independent effector.

TABLE VI (cont.)

Animal No. 6 Weight 6.7 gm.	v mm. sec. absolute	Per cent increase or decrease	V mm. sec. absolute	Per cent	F waves sec. absolute	Per cent	A mm. wave absolute	Per cent
Without load	50.86		0.9434		1.1126		0.8330	
With added load of								
0.7 gm.	48.06	-5.5	1.0063	+6.6	1.1080	-0.4	0.9230	+10.8
1.2 "	46.17	-9.1	0.9526	+0.9	1.0651	-4.0	0.8890	+6.7
2.0 "	48.15	-5.3	1.0166	+7.7	1.0593	-4.7	0.9500	+14.1
2.5 "	44.44	-12.6	0.7832	-17.7	0.9825	-11.6	0.7400	-11.1
3.0 "	39.81	-21.5	0.9061	-3.0	1.0193	-8.3	0.7800	-6.3
3.8 "	41.69	-18.0	0.7458	-20.9	0.9302	-16.3	0.7750	-6.9
4.3 "	38.62	-24.0	0.7491	-20.7	0.9286	-16.9	0.8050	-3.3

TABLE VI (cont.)

Animal No. 7 Weight 7.9 gm.	v $\frac{\text{mm.}}{\text{sec.}}$ absolute	Per cent increase or decrease	V $\frac{\text{mm.}}{\text{sec.}}$ absolute	Per cent	F waves $\frac{\text{sec.}}{\text{sec.}}$ absolute	Per cent	A $\frac{\text{mm.}}{\text{wave}}$ absolute	Per cent
Without load	<u>44.82</u>		<u>0.6305</u>		<u>0.9081</u>		<u>0.7170</u>	
With added load of								
0.7 gm.	43.33	-3.3	0.6333	+0.4	0.8226	-9.4	0.7460	+5.4
1.2 "	45.70	+1.9	0.6217	-13.9	0.8438	-7.0	0.7200	+0.4
2.0 "	46.72	+4.4	0.7606	+20.6	0.9249	-1.8	0.8100	+12.6
2.5 "	34.84	-22.2	0.5189	-17.5	0.7868	-13.3	0.6500	-9.3
3.0 "	39.28	-12.3	0.5599	-11.1	0.8035	-11.5	0.7000	-2.3
3.8 "	41.94	-6.4	0.5532	-12.2	0.7519	-17.2	0.7150	-0.2
4.3 "	35.83	-20.5	0.5651	-10.3	0.8028	-11.3	0.6100	-14.7

C. Vertical ascensions of the de-eyed Helix with and without load.

A series of experiments was carried on to determine whether the laws of linear proportionality governing locomotion would be altered if the animal were de-eyed. As far as can be determined no mention has been made in the literature of experiments of this nature. A comparison was then made between the normal animals and the same animals de-eyed (Helix lactea). The eyes are easily removed as they are situated at the tip of the posterior tentacles which are supplied by nerves from the supraoesophageal ganglia. An alteration in the velocity of the pedal waves, the velocity of progression, the frequency of waves and the advance per wave, even though slight, might be expected in the de-eyed animal when the posterior tentacles are cut off. De-eyeing should interfere with reflex interplay due to the connection of the nerve net found in Helix.¹

¹ Nervous system in Helix. In the nervous system of molluscs there are some highly characteristic features. In Helix it consists of three pairs of ganglia associated with important sense organs and connected by nerve cords. One pair (Fig. A3,c) which lies dorsal to the oesophagus supplies the tentacles and the eyes. Sometimes this cerebral ganglion is considered to be made up of two supraoesophageal ganglia joined by a broad transverse commissure. A second pair (Fig. A3,p) lies ventral to the alimentary tract on the front part of the muscle mass of the foot. These are the pedal ganglia which are connected with the otocysts, consisting of two small sacs imbedded in the pedal ganglia. The third pair, the visceral ganglia (Fig. A3,v) are also ventral. The arrangement of the nerve-net can be seen from Fig. A2.

The supraoesophageal ganglia are connected with the suboesophageal ganglia, which consist of two principal ganglionic masses. The forward mass is a pair of ganglia, the pedal ganglia, and the posterior mass consists of the visceral ganglia. Therefore, since the supraoesophageal and suboesophageal ganglia are connected it is expected that de-eying would affect the reflex control of the pedal ganglia.

After measurements were obtained for the normal animals with and without loads while creeping vertically upward, the same animals were de-eyed. The temperature varied from 19.5° to 24.8°C . but was constant during each experiment.¹ Data were gathered from the observation of 1100 waves for the normal and the de-eyed animals carrying no load. For animals carrying various loads 2700 waves were obtained and measurements taken. Graphs showing the actual scatter obtained from these measurements and other graphs showing the average points of the actual scatter were plotted according to the method described for the previous experiments.

Table VII shows a comparison of the de-eyed and normal animal during vertical ascension with and without loads. Absolute values and the percentage increase or decrease are given for the velocity of a single pedal wave, the rate of creeping, the frequency of waves and the advance per wave. In a great many cases the individual activities

¹ Wherever temperature limits are mentioned it is to be understood unless otherwise stated that the temperature was constant for each experiment.

taking place during locomotion in the de-eyed animal are increased when compared with the normal. This increase may be caused by the removal of the eyes. Reference to Figs. 8, 9 and 10 shows that the linear relation between velocity of progression and frequency of pedal waves or that between speed and dimensions of a single wave, or between the advance per wave and the rate of creeping is not altered in the de-eyed Helix lactea.

These facts are further illustrated by Figs. 8A, 9A and 10A. These show the actual scatter obtained from direct observation of the normal and de-eyed animal. The relations between the velocity of a single pedal wave and the rate of creeping (Fig. 8A), between the velocity of progression and the frequency of waves (Fig. 9A) and between the advance per wave and the velocity of progression (Fig. 10A) have been plotted for animal No. 3 during vertical ascensions without load.

It was determined also from a series of experiments that the carrying of added loads during vertical ascensions does not alter the linear proportionality between these relations in the normal and de-eyed animal. Figs. 10B, 10C and 10D show the actual scatter obtained from direct observations under these conditions (normal and de-eyed). The relation between the velocity of the pedal waves and the rate of creeping (Fig. 10B), between the velocity of progression and the frequency of waves (Fig. 10C) and between the advance per wave and the velocity of

TABLE VII

A comparison of the de-eyed and normal animal during vertical ascensions with and without varying loads. Absolute values and percentage increases or decreases for the velocity of a single pedal wave (v), the rate of creeping (V), the frequency of waves (F) and the advance per wave (A).

Animal No. 3	mm. v sec.	Per cent increase or decrease	mm. V sec.	Per cent	F wave sec.	Per cent	mm. A wave	Per cent
De-eyed = σ	absolute		absolute		absolute		absolute	
Normal = o								
σ without load	39.02	+6.8	0.5582	+3.4	0.8025	+9.0	0.6683	+1.1
o "	36.52		0.5397		0.7297		0.6610	
σ 0.7 gm.	39.24	-0.13	0.5388	-8.8	0.7754	-7.0	0.7533	+6.3
o 0.7 "	39.29		0.5905		0.8300		0.7083	
σ 1.2 "	44.65	+20.4	0.6404	+13.0	0.8048	-1.9	0.7750	+12.5
o 1.2 "	37.15		0.5657		0.8208		0.6900	
σ 2.0 "	41.57	+11.0	0.5748	+8.0	0.7657	+9.5	0.7600	+13.4
o 2.0 "	37.25		0.5321		0.8467		0.6700	
σ 2.5 "	35.31	-10.5	0.5255	-19.0	0.7717	-13.6	0.6750	-0.4
o 2.5 "	39.46		0.6501		0.8929		0.6850	
σ 3.0 "	-----	exhaustion --	-----	-----	-----	-----	-----	-----
o 3.0 "	32.29		0.5106		0.8484		0.6000	

TABLE VII (cont.)

Animal No. 4	mm. v sec.	Per cent increase or decrease	mm. V sec.	Per cent F	wave sec.	Per cent A	mm. wave	Per cent
	absolute		absolute		absolute		absolute	
♂ without load	45.13	+17.0	0.9817	+26.2	0.9396	+1.9	1.1000	+22.8
o "	38.55		0.7780		0.9220		0.8950	
♂ 0.7 gm.	43.31	+7.1	0.9571	+15.1	0.9298	-1.4	0.9800	+15.0
o 0.7 "	40.42		0.8312		0.9437		0.8460	
♂ 1.2 "	44.36	+12.9	0.9869	+32.0	0.9320	+2.3	1.0350	+25.0
o 1.2 "	39.43		0.7476		0.9104		0.8275	
♂ 2.0 "	45.44	+9.4	0.9592	+29.4	0.9977	+7.5	0.9850	+22.3
o 2.0 "	41.52		0.7426		0.9279		0.8050	
♂ 2.5 "	40.32	+10.00	0.6901	+5.0	0.8868	-3.4	0.8700	+20.8
o 2.5 "	36.33		0.6569		0.9177		0.7200	
♂ 3.0 "	45.00	+26.3	1.0833	+59.5	0.9775	+11.2	1.0850	+43.7
o 3.0 "	35.62		0.6792		0.8794		0.7550	
♂ 3.8 "	38.55	+6.0	0.8116	+33.1	0.8729	+8.5	0.9150	+22.8
o 3.8 "	36.24		0.6022		0.8044		0.7450	
♂ 4.3 "	37.00	+14.6	0.7682	+41.2	0.9143	+14.1	0.7650	+15.0
o 4.3 "	32.29		0.5440		0.8011		0.6650	

progression (Fig. 10D) has been plotted for animal No. 3 during vertical ascensions with added loads of 0.7 grams.

Fig. 10E and Table VIIa illustrate that the linear relation between the velocity of a single pedal wave and the rate of creeping is not altered when the de-eyed animal (No. 4) is creeping upward carrying added loads.

TABLE VIIa

Animal No. 4	Added load	Series	Slope of line
	0.7 gm.	1	0.26
	0.7 "	2	0.24
	0.7 "	3	0.24
	1.2 "	1	0.52
	1.2 "	2	0.24
	2.0 "		0.26
	2.5 "		0.30
	3.0 "		0.24
	3.8 "		0.14
	4.3 "		0.64 exhaustion

These data show that the foot is primarily an independent effector. It may be secondarily under central nervous control as evidenced by the slight alteration

that occurs in the individual activities when the eyes are removed.

The significance of the term "independent effector" is shown by the fact that "for equal rates of creeping (Limax) the activity of the pedal organ is independent of the added loads. This is consistent with the fact that the activities of the pedal organ are determined by its intrinsic neuro-muscular structure. The nervous elements (nerve-net) in the foot are secondarily under the control of the central ganglia." (Crozier and Federighi, 1924-25,c). Added loads do not alter the intrinsic activity of the creeping organ, but appear to act on the central reflex mechanism which inhibits or releases the pedal waves. Therefore we may speak of the foot so far as it concerns its production of pedal waves as an "independent effector" secondarily under the central nervous control, since the laws of its activity are the same regardless of the load carried in locomotion.

D. Investigation of the behavior of the detached foot.

The foot was detached from the pedal ganglia to gain further information concerning the adjustor mechanism regulating locomotion. A diagram showing (a) the nerve-net in the foot of Helix pomatia and (b) the principal parts of the nervous system is shown in Fig. 2A, taken from "Übungen aus der vergleichenden Physiologie", by Jordan (1927, p.225). By comparing the normal with the pedal-free

animal Jordan has concluded that the principal function of the pedal ganglia as well as the single ganglion of the Ascidians is the regulation of "viscosoid" tonus. He concluded that the uniform condition of the musculature in the normal animal is to be considered a sort of an equilibrium between two processes, the peripheral and presumably reflex production of resistance, and the steady lessening of this condition by the pedal ganglia.

The following experiments on twenty-four animals were performed to study the behavior of the foot when separated from the pedal ganglia. A series of animals was used in which the foot had been cut directly from the visceral organs. Immediately after the snail had been creeping actively the foot was held away from the shell by means of a forceps placed as near the visceral organs as possible, and then the foot was cut off quickly.

The isolated foot was attached at one end by silk thread to a heart lever and at the other end to an L-shaped rod (Fig. 11) which was held in place by a muscle-clamp. A moist chamber surrounded the foot. Kymograph records were obtained showing the action of the foot. The limits of temperature for the entire series of experiments were from 19.5° to 24.8°C .

Reference to Figures 11a and 11b shows that the usual rhythmical action of the foot is greatly interfered with when it is not connected with the pedal ganglia. Even in kymograms showing some recurring waves the usual periodic

recurrence found in the intact animal is destroyed. The destruction of the periodic recurrence of waves when the pedal ganglia are separated from the foot suggests that this periodicity is determined by the presence of these ganglia.

Extirpation of the pedal ganglia in the intact animal may be possible and further study of the locomotor waves under these conditions may bring to light additional information as to the exact action of the pedal ganglia in this neuro-muscular activity.

E. The effect of mechanical stimulation and of Ringer's solution on locomotion.

Preparatory to experiments involving the injection of certain drugs a series of control experiments was performed on Helix lactea and Helix pomatia. The controls were tested for (a) consistency of runs, (b) the effect of mechanical stimulation, (c) the effect of Ringer's solution (cold blooded).¹

In testing the consistency of the runs a comparison was made of the data obtained for runs separated by intervals of time. Reference to Table VIII shows that in a given experiment the data obtained at the end of three hours may vary slightly from the data obtained at the end of one or

¹ The composition of Ringer's solution (1000 cc. of water) used in every case was as follows:

	Grams
NaCl	6.5
KCl	0.14
CaCl ₂	0.12
NaHCO ₃	0.20

at the end of two hours. Except for this slight variation the results may be said to be consistent.

The controls were also tested to determine the mechanical stimulation on vertical creeping. When the animal was creeping actively upwards a hypodermic needle, which was to act as the mechanical stimulus, was inserted into either the body wall in the region where the visceral mass joins the foot or into the anterior end of the foot directly back of the tentacles. The reactions of the animal were noted.

The method followed in these experiments is illustrated by the following, which represent the procedure of a typical experiment in this series when the animal was creeping upward.

- (a) Records of normal run obtained.
- (b) Records taken of runs after mechanical stimulation of the body wall.
- (c) Interval allowed to remove the effects of this stimulation.
- (d) Further records of normal run.
- (e) Records of runs obtained after mechanical stimulation of anterior end of the foot.

In eight out of ten animals used the mechanical stimulation of the body wall caused a withdrawal of the head into the shell, followed by a slight contraction of the foot; then creeping began immediately. In one of the other two cases (experiment 50, animal No. 15) creeping did not

TABLE VIII

A comparison of data obtained for control animals when various series of runs are separated by intervals of time.

No. of animal	Time elapsing between each series		Velocity of progression in mm. per sec.	Frequency of waves per sec.	Velocity of single wave in mm. per sec.	Advance per wave in mm.
	Min.	Sec.				
1	Initial series		8.2	8.9	37.3	0.92
	5	3	7.2	8.7	35.0	0.83
	10	40	7.4	8.3	38.0	0.81
	63	0	6.8	8.4	33.3	0.81
3	Initial series		4.6	9.0	36.2	0.60
	6	10	6.5	8.4	42.0	0.60
	82	10	5.9	8.2	34.0	0.65
	Initial series		7.7	9.6	42.0	0.75
4	66	0	7.3	9.3	36.0	0.86
	156	0	7.1	8.2	34.0	0.71
	Initial series		7.7	9.6	42.0	0.75

TABLE VIII (cont.)

No. of animal	Time elapsing between each series		Velocity of progression in mm. per sec.	Frequency of waves per sec.	Velocity of single wave in mm. per sec.	Advance per wave in mm.
	Min.	Sec.				
6	Initial series		9.1	10.8	48.5	0.85
	6	2	9.8	11.6	51.5	0.85
	10	10	9.8	11.3	51.7	0.85
	104		8.8	11.4	51.6	0.75
7	Initial series		6.7	8.1	42.0	0.81
	5	40	6.0	8.6	47.0	0.69
	150	0	6.7	9.3	48.1	0.65
	Initial series		7.3	10.3	38.0	0.91
10	1	3	7.9	8.3	39.1	0.79
	6	11	7.5	10.5	42.0	0.64
	104	2	6.8	9.8	36.0	0.69

begin for 43 minutes after stimulation. In the other instance (experiment 52, animal No. 16) it did not begin for nine minutes after pricking.

Mechanical stimulation of the body wall also caused an increase in the velocity of progression and in the advance per wave in Helix pomatia (Table VIIIA), and either a decrease or an increase in the frequency of waves and in the velocity of a single wave. No definite conclusion can be drawn, however, from this type of experiment on Helix lactea (Table VIIIB), for in some of the experiments these activities were increased, while in other experiments they were decreased.

Table VIIIC shows the effect of mechanical stimulation at the anterior end of the foot does not differ greatly from stimulation of the body wall. Here again the results do not show any definite consistency, the frequency of waves and the velocity of a single wave being least affected. Therefore the mechanical effect can be eliminated in the subsequent experiments.

The effect of Ringer's solution was tested on the controls. Experiments were carried out by injecting 0.2 cc. of Ringer's solution and studying the effects. Data for these experiments are given in Table VIIE.

As the chemical composition of the blood of invertebrates approaches that which we find in the lower vertebrates, amphibian (or cold-blooded) Ringer's solution plus the drug was used in the following experiments.

TABLE VIIIa

The effect of mechanical stimulation of the body wall of Helix pomatia. Absolute values are given for the factors involved in locomotion.

No. of Experiment	Velocity of progression in mm. per minute	Frequency of waves per minute	Velocity of single wave in mm. per minute	Advance per wave in mm.
5 (Normal)	8.5	42.0	3353	0.24
6 (Mechanical stimulation)	14.8	43.0	3525	0.33
10 (Normal)	19.2	71.4	6900	0.26
11 (Mechanical stimulation)	23.0	62.0	6630	0.36
14 (Normal)	13.4	59.5	8200	0.22
15 (Mechanical stimulation)	19.0	52.0	7300	0.36
20 (Normal)	12.2	62.5	7600	0.19
21 (Mechanical stimulation)	18.6	83.3	6700	0.22

TABLE VIIIb

The effect of mechanical stimulation on the body wall of Helix lactea, on vertical creeping.

No. of experiment	Velocity of progression in mm. per minute	Frequency of waves per minute	Velocity of single wave in mm. per minute	Advance per wave in mm.
41 (Normal)	22.0	62.5	4500	0.35
42 (Mechanical stimulation)	27.7	62.5	4300	0.44
45 (Normal)	15.0	62.5	4900	0.24
48 (Mechanical stimulation)	24.0	83.0	4700	0.29
49 (Normal)	23.0	71.0	5700	0.32
50 (Mechanical stimulation)	15.0	83.0	5100	0.19
51 (Normal)	48.0	83.0	4900	0.58
52 (Mechanical stimulation)	40.0	71.0	5100	0.57
72 (Normal)	20.5	83.0	4400	0.24
73 (Mechanical stimulation)	15.3	83.0	4000	0.18
78 (Normal)	18.6	83.0	4600	0.22
79 (Mechanical stimulation)	19.6	83.0	5000	0.23

TABLE VIIIc

The effect of mechanical stimulation of the anterior end of the foot of Helix lactea on vertical creeping.

No. of experiment	Velocity of progression in mm. per minute	Frequency of waves per minute	Velocity of single wave in mm. per minute	Advance per wave in mm.
43 (Normal)	29.0	100.0	4100	0.29
44 (Mechanical stimulation)	17.0	83.0	4300	0.21
47 (Normal)	18.3	83.0	4700	0.22
48 (Mechanical stimulation)	13.8	83.0	4700	0.16
53 (Normal)	14.8	71.0	4900	0.20
54 (Mechanical stimulation)	22.0	83.0	4700	0.26
55 (Normal)	38.0	125.0	6025	0.30
56 (Mechanical stimulation)	13.6	72.0	4200	0.18
74 (Normal)	15.5	131.0	5550	0.18
75 (Mechanical stimulation)	13.6	72.0	4200	0.18
80 (Normal)	44.0	122.0	6000	0.34
81 (Mechanical stimulation)	20.9	125.0	6375	0.17

TABLE VIIId

Effect of doses of Ringer's solution (0.2 cc.) on the velocity of progression (V), frequency of waves (F), velocity of pedal waves (v) and the advance per wave (A).

<u>Helix lactea</u>		
Plus ¹	Minus	Cases unchanged
V 9	10	1
F 8	12	
v 8	12	
A 6	10	4

¹ Increase or decrease from the normal animal after injection of Ringer's solution, giving total number of cases of increase or decrease respectively.

TABLE VIIIe

The effect of Ringer's solution on vertical creeping.

No. of Animal	Velocity of progression in mm. per minute	Frequency of waves per minute	Velocity of single wave in mm. per minute	Advance per wave in mm.
1				
Normal	38.8	38.8	4800	0.36
Ringer's	47.4	37.3	4700	0.49
2				
Normal	33.1	85.4	5040	0.47
Ringer's	38.9	70.3	5100	0.52
3				
Normal	30.9	75.1	4450	0.41
Ringer's	24.3	68.6	4370	0.29
4				
Normal	20.4	69.8	4900	1.09
Ringer's	13.1	65.2	5300	1.0
5				
Normal	43.1	99.4	5680	0.38
Ringer's	25.1	79.1	5920	0.31
6				
Normal	21.1	68.8	5550	0.31
Ringer's	23.2	63.8	4440	0.24
7				
Normal	35.9	83.5	5950	0.39
Ringer's	22.6	81.6	5720	0.32
8				
Normal	21.0	70.6	4200	0.32
Ringer's	15.4	72.1	3880	0.54
9				
Normal	36.6	80.1	5220	0.46
Ringer's	39.8	106.6	4940	0.37
10				
Normal	29.4	58.3	3980	0.16
Ringer's	43.7	71.1	4720	0.35

TABLE VIIIe (cont.)

No. of Animal	Velocity of progression in mm. per minute	Frequency of waves per minute	Velocity of single wave in mm. per minute	Advance per wave in mm.
11				
Normal	9.7	82.1	6560	0.39
Ringer's	33.7	57.6	4020	0.22
12				
Normal	18.1	75.0	4200	0.27
Ringer's	29.2	84.2	4420	0.35
13				
Normal	53.8	94.1	5140	0.57
Ringer's	33.1	75.9	4520	0.44
14				
Normal	27.3	90.2	5640	0.32
Ringer's	34.1	91.6	4360	0.37
15				
Normal	28.1	65.0	4800	0.28
Ringer's	19.3	66.4	4580	0.19
16				
Normal	19.1	77.0	4000	0.21
Ringer's	16.1	61.0	5060	0.20
17				
Normal	12.7	69.4	4800	0.18
Ringer's	12.8	73.3	4450	0.18
18				
Normal	41.1	82.8	4560	0.23
Ringer's	16.4	71.5	4900	0.24
19				
Normal	29.9	123.9	4980	0.48
Ringer's	31.1	97.9	3470	0.32
20				
Normal	21.4	84.7	4300	0.20
Ringer's	18.6	91.7	4420	0.21

F. The effect of adrenalin on the neuro-muscular activities during locomotion.

The action of adrenalin on various kinds of tissue, particularly on the hearts of vertebrates, has been extensively studied. Clark (1927) has cited references (p. 62) concerning the effects of adrenalin on some invertebrate tissues, i.e., the intestine of the crayfish, the oesophagus of Aphrodite, of Aphysia and of Helix. He has also drawn attention to the fact that the inhibitory action of adrenalin is as common as its augmentor action in vertebrates, but that only augmentor action has been described in invertebrates. Boyer (1926) found that adrenalin and spartein slackened the rhythm of the isolated heart of the snails. He found that adrenalin affects neither the amplitude nor the tonus in the doses used (1 part in 10,000 and 1 part in 1,000), whereas spartein reinforces the amplitude and augments the tonus. Wyman and Lutz (1930) have described an inhibitory action of adrenalin on the isolated holothurian cloaca. Their experiments demonstrated that adrenalin has both inhibitory and excitatory effects on invertebrate tissues.

No reference in the literature as far as can be determined has been made to any work on the effects of adrenalin on the muscles involved in locomotion of molluscs. The following experiments describe the action of different amounts of adrenalin in the intact animal (Helix). Contrasted with the study of the detached foot, which involved

interference with the circulation, the subsequent experiments show the effect on locomotion. Adrenalin, which has a low molecular weight, is easily diffusible, is not attached to protein and is readily poured into the blood stream from which it is removed by the tissues. Usually there is no delay in the manifestation of its effect. Its maximal duration of effect in mammals is generally only a few minutes and its physiological activity during this interval is marked (Kendall, 1929). However, in cold-blooded animals the effect of adrenalin may be prolonged. Bieter and Scott (1929) reported a rise of blood pressure in Rana pipiens following a dose of 0.2 cc. of epinephrine hydrochloride, 1:10,000, which persisted for at least one and one-quarter hours after injection. MacKay (1931) has found that adrenalin causes a rise of ventral aortic blood pressure in skates (Raia), which persists from one to two and one-half hours. Wyman and Lutz (1932) reported that intravenous injection of adrenalin in Squalus acanthias produced a rise of both the ventral and the dorsal aortic blood pressure, both systolic and diastolic, persisting for at least thirty minutes. Although not a great deal of work has been done to compare the lasting effect of adrenalin in the cold-blooded animals with the effect in mammals, the evidence so far obtained shows that there is more prolonged maximum effect in cold-blooded animals.

Adrenalin chloride solution (Parke, Davis & Co.) was used in some of these experiments. In others dry suprarenal

extract (Parke, Davis & Co.) was used in order to check the results obtained from adrenalin chloride because of HCl and chlorotone.

The same general procedure was followed as previously described. An investigation was made to see whether the linear proportionality between the various relationships for vertical creeping held when adrenalin was injected. Data were obtained from 68 animals. The results found for Helix lactea confirmed those obtained for Helix pomatia. Therefore, since Helix lactea exhibits more active creeping and lives in the laboratory for longer periods of time than Helix pomatia, Helix lactea was chosen for the majority of experiments.

Data were first obtained for vertical ascensions under normal conditions. Then these data were compared with the data obtained after adrenalin (of a particular concentration) had been injected. The data given below were obtained from a series of eighty experiments. From the 68 animals used a total of 40,500 waves were observed, i.e., 19,700 for the normal animal and 20,800 for the injected animals. Adrenalin chloride solution (Parke, Davis & Co.) was added to Ringer's solution to make various concentrations, i.e., 1:10,000, 1:20,000, 1:40,000, 1:100,000, 1:80,000 / 1:120,000 and 1:200,000. Measurements were first obtained for vertical creeping under normal conditions. Then 0.3 cc. of adrenalin (of the concentration to be studied) was injected into Helix pomatia, and when Helix

lactea was used 0.22 cc. of adrenalin was injected. The time elapsing between the injection and the resumption of creeping varied in different animals from immediate creeping to one hour. In a few cases creeping did not begin for two to three hours.

In order to determine whether the linear proportionality between the various relationships for vertical creeping held when adrenalin was injected the measurements obtained for the normal and injected animal were employed as described in the previous experiments. Graphs were plotted of the average points showing the relation between the velocity of a single pedal wave and the velocity of progression, between the frequency of waves and the velocity of progression, and between the advance per wave and the velocity of progression. The best fitted line was drawn and the slope of the line determined. The slope of the line obtained for a particular relation for the control was compared with the same relation for the injected animal. Figures 12, 13 and 14 show the typical results. It is noted that the straight line proportionality and the slope of the line are essentially unchanged by adrenalin. These are added features which support the idea of an "independent effector in muscle.

A description is given below of the effect of injection of various concentrations of adrenalin chloride. Table IX shows the effect of 0.2 cc. of adrenalin (1:10,000) on Helix lactea during vertical ascensions. Adrenalin of

this concentration usually causes a decrease in the velocity of progression, in the frequency of waves, in the velocity of a single wave and in the advance per wave.

Similar experiments were performed on Helix pomatia (Table IXa). From 1950 waves observed on the normal animal and 2000 on the injected animal data were procured from which Table IXa has been computed. Adrenalin (0.3 cc. of adrenalin, 1:10,000) had the same effect on these animals as on Helix lactea, i.e., a decrease in each of the factors involved in locomotion, during vertical ascensions. Table X shows that the same results in the main are obtained with both species.

Tables Xa through Xh give the data from which Table X was compiled. Other experiments employing adrenalin in doses of 1:20,000, 1:40,000, and 1:80,000 (Table XI) give the same results as obtained for doses of 1:10,000, i.e., usually a decrease in the velocity of progression, the frequency of waves, the velocity of a single wave and the advance per wave. With doses of adrenalin 1:100,000 (Table XIa) inconsistent results were obtained, sometimes an increase in the activity and sometimes a decrease in the same activity.

However, doses of adrenalin 1:120,000 (Table XI) produced a stimulating effect on the velocity of progression, the frequency of waves, the velocity of a single wave and sometimes either an increase or decrease in the advance per wave.

TABLE IX

Effect of injection of adrenalin chloride (0.2 cc. 1:10,000) on the velocity of a single wave (v), the rate of creeping (V), the frequency of waves (F), and the advance per wave (A) (Helix lactea) during vertical ascensions.

No. of animal	$v = \frac{\text{mm.}}{\text{min.}}$	$V = \frac{\text{mm.}}{\text{min.}}$	$F = \frac{\text{waves}}{\text{min.}}$	$A = \frac{\text{mm.}}{\text{waves}}$
2				
o = control	2576	36.6	66.0	0.33
● = injected	2682	34.0	67.8	0.29
3				
o = control	3186	45.2	76.0	0.38
● = injected	3060	44.7	67.2	0.34
7				
o = control	5381	30.4	83.4	0.34
● = injected	4200	28.1	80.1	0.36
9				
o = control	3334	99.6	79.2	0.46
● = injected	4704	55.2	77.4	0.36
10				
o = control	4660	30.3	64.6	0.46
● = injected	4358	23.7	88.2	0.29
13				
o = control	6080	24.4	74.3	0.33
● = injected	5250	25.1	60.7	0.42
14				
o = control	3908	55.3	79.2	0.40
● = injected	3510	43.2	72.0	0.34

TABLE IXa

A comparison of the normal and injected animals¹ (Helix pomatia) during vertical ascensions. Absolute values for the velocity of a single wave, the rate of creeping, the frequency of waves and the advance per wave.

No. of animal	Velocity of a single wave in mm. per minute	Rate of creeping in mm. per minute	Frequency of waves per mm.	Advance per wave in mm.
60				
Control	6920	48.2	96.0	0.96
Injected	5400	17.2	92.0	0.24
62				
Control	7830	46.8	103.1	0.45
Injected	5640	22.5	81.3	0.28
66				
Control	6010	19.2	95.2	0.19
Injected	4950	36.0	92.0	0.38
69				
Control	5770	45.1	60.0	0.78
Injected	8800	29.9	87.0	0.50
70				
Control	6280	21.9	62.2	0.34
Injected	6460	19.6	94.0	0.31
75				
Control	6350	33.5	71.9	0.45
Injected	5210	18.9	62.0	0.32

¹ Injection of 0.3 cc. adrenalin chloride, 1:10,000.

Table 1A

A comparison of the effects of 0.5 cc. of 1% solution of epinephrine (control) and 0.5 cc. of 1% solution of epinephrine plus 0.5 cc. of 1% solution of strychnine on the velocity of a single wave, the rate of conduction, and frequency of waves in the atrium (Fig. 1).

No. of animal	Velocity of wave in atrium (m. per sec.)	Rate of conduction (per cent.)	Frequency of waves (per sec.)
50			
Control	1.00	45.5	1.00
Injected	0.90	45.5	1.00
51			
Control	0.90	45.5	1.00
Injected	0.80	45.5	1.00
52			
Control	0.90	45.5	1.00
Injected	0.80	45.5	1.00
53			
Control	0.90	45.5	1.00
Injected	0.80	45.5	1.00
54			
Control	0.90	45.5	1.00
Injected	0.80	45.5	1.00
55			
Control	0.90	45.5	1.00
Injected	0.80	45.5	1.00
56			
Control	0.90	45.5	1.00
Injected	0.80	45.5	1.00
57			
Control	0.90	45.5	1.00
Injected	0.80	45.5	1.00
58			
Control	0.90	45.5	1.00
Injected	0.80	45.5	1.00

Injection of 0.5 cc. of strychnine, 1:10,000.

TABLE X

Effect of doses of adrenalin (1:10,000) on the velocity of progression (V), frequency of waves (F), the velocity of pedal waves (v), and the advance per wave (A).

<u>Helix lactea</u>			<u>Helix pomatia</u>		
	Plus ¹	Minus		Plus	Minus
V	1	6	V	1	5
F	2	5	F	2	4
v	2	5	v	2	4
A	2	5	A	1	5

¹ Increase or decrease from the normal animal after injection of adrenalin (1:10,000), giving total number of cases of increase or decrease respectively.

TABLE A

Effect of depth of immersion (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 cm) on the velocity of propagation (V), frequency of waves (f), and the velocity of the wave (A).

Wave factor			Wave factor		
Depth	Velocity	Frequency	Depth	Velocity	Frequency
10	1.0	1.0	10	1.0	1.0
20	1.0	1.0	20	1.0	1.0
30	1.0	1.0	30	1.0	1.0
40	1.0	1.0	40	1.0	1.0
50	1.0	1.0	50	1.0	1.0
60	1.0	1.0	60	1.0	1.0
70	1.0	1.0	70	1.0	1.0
80	1.0	1.0	80	1.0	1.0
90	1.0	1.0	90	1.0	1.0
100	1.0	1.0	100	1.0	1.0

1. Increase of depth of immersion (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 cm) on the velocity of propagation (V), frequency of waves (f), and the velocity of the wave (A).

TABLE Xa

Effect of doses of adrenalin chloride (1:10,000)
on the velocity of progression (Helix lactea).

No. of experiment	Velocity of progression in mm. per minute	Velocity of progression in mm. per minute
	Control	After dose of adrenalin 1:10,000
2	36.6	34.0
3	45.2	44.7
7	30.4	28.1
9	99.6	55.2
10	30.3	23.7
13	24.4	25.7
14	55.3	43.2

TABLE Xb

Effect of doses of adrenalin chloride (1:10,000)
on the velocity of progression (Helix pomatia).

No. of experiment	Velocity of progression in mm. per minute	Velocity of progression in mm. per minute
	Control	After dose of adrenalin 1:10,000
60	42.2	17.2
62	46.8	25.5
66	19.2	36.0
69	45.1	25.9
70	21.9	19.6
75	33.5	18.9

TABLE Xc

Effect of doses of adrenalin chloride (1:10,000)
on the frequency of waves (Helix lactea).

No. of experiment	Frequency of waves per minute Control	Frequency of waves per minute After dose of adrenalin 1:10,000
2	66.0	67.8
3	76.0	67.2
7	83.4	80.1
9	79.2	77.4
10	64.6	88.2
13	74.3	60.7
14	79.2	72.0

TABLE Xd

Effect of doses of adrenalin chloride (1:10,000)
on the frequency of waves (Helix pomatia)

No. of experiment	Frequency of waves per minute Control	Frequency of waves per minute After dose of adrenalin 1:10,000
60	96.0	92.0
62	103.1	81.3
66	95.2	92.6
69	60.0	87.0
70	62.2	94.0
75	71.9	62.0

TABLE Xe

Effect of doses of adrenalin chloride (1:10,000)
on the velocity of a single wave (Helix lactea).

No. of experiment	Velocity of a single wave in mm. per minute	Velocity of a single wave in mm. per minute
	Control	After dose of adrenalin 1:10,000
2	2576	2682
3	3186	3060
7	5381	4200
9	3334	4704
10	4660	4358
13	6080	5250
14	3908	3510

TABLE Xf

Effect of doses of adrenalin chloride (1:10,000)
on the velocity of a single wave (Helix pomatia).

No. of experiment	Velocity of a single wave in mm. per minute	Velocity of a single wave in mm. per minute
	Control	After dose of adrenalin 1:10,000
60	6920	5400
62	7830	5640
66	6010	4950
69	5770	8800
70	6280	6460
95	6350	5214

TABLE Xg

Effect of doses of adrenalin chloride (1:10,000)
on the advance per wave (Helix lactea).

No. of experiment	Advance per wave in mm.	Advance per wave in mm.
	Control	After dose of adrenalin 1:10,000
2	0.33	0.29
3	0.38	0.34
7	0.34	0.36
9	0.46	0.36
10	0.46	0.29
13	0.33	0.42
14	0.40	0.34

TABLE Xh

Effect of doses of adrenalin chloride (1:10,000)
on the advance per wave (Helix pomatia).

No. of experiment	Advance per wave in mm.	Advance per wave in mm.
	Control	After dose of adrenalin 1:10,000
60	0.96	0.24
62	0.45	0.28
66	0.19	0.38
69	0.78	0.50
70	0.34	0.31
75	0.45	0.32

TABLE XI

Summary of effect of various concentrations of adrenalin on factors involved in locomotion.

Velocity of progression	Dose of adrenalin	Number of cases of increase or decrease from normal	
		Plus	Minus
	0.2 cc. 1:10,000	1	6
	0.3 cc. 1:10,000	1	5
	0.2 cc. 1:20,000	1	6
	0.2 cc. 1:40,000	2	4
	0.2 cc. 1:80,000	3	13
	0.2 cc. 1:120,000	8	1
Frequency of waves	0.2 cc. 1:10,000	2	5
	0.3 cc. 1:10,000	2	4
	0.2 cc. 1:20,000	2	5
	0.2 cc. 1:40,000	1	5
	0.2 cc. 1:80,000	2	14
	0.2 cc. 1:120,000	6	3
Velocity of single wave	0.2 cc. 1:10,000	4	9
	0.3 cc. 1:10,000	2	4
	0.2 cc. 1:20,000	2	5
	0.2 cc. 1:40,000	1	5
	0.2 cc. 1:80,000	2	14
	0.2 cc. 1:120,000	6	3
Advance per wave	0.2 cc. 1:10,000	2	5
	0.3 cc. 1:10,000	1	5
	0.2 cc. 1:20,000	2	5
	0.2 cc. 1:40,000	1	5
	0.2 cc. 1:80,000	4	11
	0.2 cc. 1:120,000	5	4

Recapitulation of TABLE XI

	1:10,000		1:20,000		1:40,000		1:80,000		1:120,000	
	+	-	+	-	+	-	+	-	+	-
Velocity of progression	2	11	1	6	2	4	3	13	8	1
Frequency of waves	4	9	2	5	1	5	7	9	8	1
Velocity of a single wave	4	9	2	5	1	5	2	14	6	3
Advance per wave	3	10	2	5	1	5	4	11	5	4

TABLE XIa

Effect of doses of adrenalin chloride (1:100,000)
on the velocity of progression, frequency of waves, velocity
of a single wave and advance per wave.

No. of experiment	Velocity of progression in mm.per minute Control	Velocity of progression in mm. per minute After dose of adrenalin 1:100,000
2	38.2	45.1
3	32.3	69.1
18	68.0	80.8
21	42.0	40.4
28	81.3	47.1

	Frequency of waves per minute Control	Frequency of waves per minute After dose of adrenalin 1:100,000
2	95.2	90.1
3	78.1	121.0
18	64.5	95.5
21	71.1	109.3
28	99.0	83.0

	Velocity of a single wave in mm.per minute Control	Velocity of a single wave in mm.per minute After dose of adrenalin 1:100,000
2	4500	5120
3	5150	5500
18	5340	4420
21	3600	4500
28	6220	5930

	Advance per wave in mm. Control	Advance per wave in mm. After dose of adrenalin 1:100,000
2	0.40	0.46
3	0.41	0.48
18	1.18	0.65
21	0.63	0.73
28	0.61	0.47

In order to determine whether the above results were due to the action of adrenalin and not to the chlorotone present in the adrenalin chloride solution or a change in Hydrogen ion concentration, dry suprarenal extract (Parke, Davis & Co.) was made up with Ringer's solution. A series of experiments was carried out under the same conditions as when adrenalin chloride was used. Tables XIIa through XIIh give the data obtained when adrenalin chloride 1:80,000 and suprarenal extract 1:80,000 were used respectively, and Tables XIIIa through XIIIh give the data for experiments when adrenalin chloride 1:120,000 and suprarenal extract 1:120,000 were used. A summary of this comparison is given in Table XIV. Reference to this table shows that results obtained from suprarenal extract were the same as for adrenalin chloride solution, except that a greater depressant action was observed on the frequency of waves with suprarenal extract of doses of 1:80,000. The action of doses of 1:120,000 adrenalin chloride and 1:120,000 suprarenal extract was to cause either an increase or decrease of the advance per wave.

Injections of adrenalin of any concentration within the limits used, i.e., 1:10,000 to 1:120,000 during vertical creeping did not usually affect the number of waves present at one time on the foot. If there was a change in the number it was by having one more wave present after the injection of adrenalin. It was also consistently observed after injection of adrenalin of either strong or

TABLE XIIIa

Effect of doses of adrenalin chloride (1:80,000)
on the velocity of progression.

No. of experiment	Velocity of progression in mm. per minute	Velocity of progression in mm. per minute After dose of adrenalin 1:80,000
	Control	
8	23.8	14.0
10	64.5	35.0
11	35.0	16.0
12	34.2	20.6
13	16.1	15.9
14	17.2	16.0
15	41.1	36.6
16	25.6	33.8
17	34.9	30.8
18	25.6	33.8
19	39.6	29.7
20	34.9	30.8
21	41.0	37.2
22	49.2	36.7
23	46.1	28.1
24	25.3	33.4
25	63.6	58.0

TABLE XIIb

Effect of doses of adrenalin chloride (1:80,000)
on the frequency of waves.

No. of experiment	Frequency of waves per minute	Frequency of waves per minute
	Control	After dose of adrenalin 1:80,000
8	75.5	71.4
10	135.0	87.6
11	87.0	83.0
12	62.8	63.0
13	62.5	61.5
14	69.6	60.1
15	82.1	61.0
16	59.0	72.0
17	70.8	106.6
18	59.0	72.0
19	74.3	65.5
21	72.5	60.0
22	104.0	72.4
23	75.4	78.0
24	61.5	70.0
25	90.7	94.1

TABLE XIIC

Effect of doses of adrenalin chloride (1:80,000)
on the velocity of a single wave.

No. of experiment	Velocity of a single wave in mm. per minute	Velocity of a single wave in mm. per minute After dose of adrenalin 1:80,000
	Control	
8	4600	3900
10	5160	4416
11	4800	4700
12	6300	5430
13	5620	5340
14	4450	3300
15	6230	5980
16	6250	5680
17	5060	4740
18	6250	5680
19	5700	4580
21	6600	7000
22	5340	4820
23	6080	5900
24	4150	4120
25	5580	5680

TABLE XIId

Effect of doses of adrenalin chloride (1:80,000)
on the advance per wave.

No. of experiment	Advance per wave in mm.	Advance per wave in mm.
	Control	After dose of adrenalin 1:80,000
8	0.31	0.17
10	0.48	0.43
11	0.42	0.20
12	0.55	0.23
13	0.24	0.24
14	0.24	0.29
15	0.44	0.51
16	0.44	0.46
17	0.49	0.29
18	0.44	0.46
19	0.52	0.36
21	0.58	0.41
22	0.47	0.45
23	0.62	0.41
24	0.41	0.48
25	0.70	0.60

TABLE XIle

Effect of doses of suprarenal extract (1:80,000)
on the velocity of progression.

No. of experiment	Velocity of progression in mm. per minute	Velocity of progression in mm. per minute After dose of suprarenal extract 1:80,000
	Control	
124	26.4	28.5
136	39.4	43.0
138	26.7	18.2
139	30.7	17.3
145	38.5	19.0
146	52.0	13.1
147	36.1	12.8
150	23.9	26.6
151	41.1	13.6
155	45.9	33.0
156	17.7	14.8

TABLE XIIf

Effect of doses of suprarenal extract (1:80,000)
on the frequency of waves.

No. of experiment	Frequency of waves per minute	Frequency of waves per minute
	Control	After dose of supra- renal extract 1:80,000
124	79.1	78.0
136	76.6	61.2
138	82.3	79.1
139	62.8	66.1
145	64.6	58.6
146	92.6	90.5
147	74.2	66.5
150	59.7	63.5
151	68.7	69.8
155	106.0	76.6
156	67.7	53.3

TABLE XIIG

Effect of doses of suprarenal extract (1:80,000)
on the velocity of a single wave.

No. of experiment	Velocity of a single wave in mm. per minute	Velocity of a single wave in mm. per minute
	Control	After dose of suprarenal extract 1:80,000
124	4010	4260
136	5100	5200
138	4360	4380
139	5500	4400
145	6040	5700
146	6216	5400
147	4600	3600
150	4400	3930
151	4900	3200
155	4520	4460
156	4550	3510

TABLE XIIh

Effect of doses of suprarenal extract (1:80,000)
on the advance per wave.

No. of experiment	Advance per wave in mm.	Advance per wave in mm.
	Control	After dose of supra- renal extract 1:80,000
124	0.34	0.39
136	0.60	0.68
138	0.33	0.18
139	0.49	0.22
145	0.60	0.36
146	0.75	0.14
147	0.48	0.16
150	0.40	0.25
151	0.68	0.20
155	0.56	0.36
156	0.26	0.29

TABLE XIIIa

Effect of doses of adrenalin chloride (1:120,000)
on the velocity of progression.

No. of experiment	Velocity of progression in mm. per minute	Velocity of progression in mm. per minute After dose of adrenalin 1:120,000
	Control	
7	14.6	19.6
10	10.7	37.1
11	33.0	50.5
12	43.0	37.0
13	37.0	42.6
14	26.1	38.1
21	14.3	19.0
25	10.2	12.8
26	26.0	38.1

TABLE XIIIb

Effect of doses of adrenalin chloride (1:120,000)
on the frequency of waves.

No. of experiment	Frequency of waves per minute	Frequency of waves per minute
	Control	After dose of adrenalin 1:120,000
7	59.4	80.0
10	71.1	84.8
11	71.3	97.3
12	68.1	127.0
13	126.6	91.2
14	70.0	100.0
21	58.6	84.7
25	52.3	62.0
26	70.6	100.1

TABLE XIIIc

Effect of doses of adrenalin chloride (1:120,000)
on the velocity of a single wave.

No. of experiment	Velocity of a single wave in mm. per minute	Velocity of a single wave in mm. per minute
	Control	After dose of adrenalin 1:120,000
7	5370	5730
10	4100	4600
11	6400	7030
12	6300	7990
13	7250	5850
14	5300	4700
21	3704	4880
25	3220	3760
26	5300	4720

TABLE XIIIId

Effect of doses of adrenalin chloride (1:120,000)
on the advance per wave.

No. of experiment	Advance per wave in mm.	Advance per wave in mm.
	Control	After dose of adrenalin 1:120,000
7	0.25	0.23
10	0.16	0.43
11	0.52	0.50
12	0.58	0.30
13	0.31	0.45
14	0.23	0.29
21	0.23	0.19
25	0.18	0.16
26	0.23	0.29

TABLE XIIIe

Effect of doses of suprarenal extract (1:120,000)
on the velocity of progression.

No. of experiment	Velocity of progression in mm. per minute	Velocity of progression in mm. per minute
	Control	After dose of suprarenal extract 1:120,000
36	47.1	49.2
38	37.2	52.6
40	23.6	54.2
42	23.4	48.6
44	25.2	48.2
45	50.1	65.1

TABLE XIIIIf

Effect of doses of suprarenal extract (1:120,000)
on the frequency of waves.

No. of experiment	Frequency of waves per minute	Frequency of waves per minute
	Control	After dose of supra- renal extract 1:120,000
36	100.0	102.0
38	71.2	110.7
40	65.5	89.8
42	79.7	88.4
44	63.4	93.0
45	82.3	112.1

TABLE XIIIg

Effect of doses of suprarenal extract (1:120,000)
on the velocity of a single wave.

No. of experiment	Velocity of a single wave in mm. per minute	Velocity of a single wave in mm. per minute
	Control	After dose of supra- renal extract 1:120,000
36	3604	6033
38	4340	5700
40	3840	5420
42	4660	4900
44	3750	4890
45	4250	5020

TABLE XIIIh

Effect of doses of suprarenal extract (1:120,000)
on the advance per wave.

No. of experiment	Advance per wave in mm.	Advance per wave in mm.
	Control	After dose of supra- renal extract 1:120,000
36	0.47	0.48
38	0.52	0.47
40	0.34	0.26
42	0.54	0.27
44	0.29	0.39
45	0.33	0.38

TABLE XIV

Effect of doses 1:80,000

Effect of doses 1:120,000

	Adrenalin chloride		Suprarenal extract			Adrenalin chloride		Suprarenal extract	
	Plus	Minus	Plus	Minus		Plus	Minus	Plus	Minus
Velocity of progression	3	13	3	8		8	1	6	0
Frequency of waves	7	9	3	8		8	1	6	0
Velocity of a single wave	2	14	3	8		6	3	6	0
Advance per wave	4	11	3	8		5	4	3	3

weak concentration during vertical creeping that the tendency to orient had been greatly lessened. As previously mentioned orientation and locomotion in Helix are closely allied (Crozier and Navez, 1930) and further work is necessary to disentangle the neuromuscular control of these mechanisms.

Since there is no sympathetic nervous system in Helix the usual explanation concerning the point of action of adrenalin cannot be given. Adrenalin affects different types of muscle differently. In structure the muscles of the foot closely resemble other invertebrate muscle (Mendel and Bradley). Elliott (1905) stated that adrenalin does not excite the muscle fiber directly but acts on a substance at the myoneural junction. He stated that all other substances but adrenalin evoke, from the group of plain muscle tissues when stimulating them directly, a reaction differing only in degree and not in kind in each tissue. It is the peculiarity of adrenalin to cause sharp contraction in one and relaxation in the other. The cause is the same, the effects different. Therefore the reacting substance must be different and he has decided that since mechanical and chemical stimuli do not point to marked intrinsic differences in the plain muscle fibers they do reveal differences in the "nerve endings" and this difference is inherent in the myoneural junction. Langley (1905-06) agreed with Elliott as to the place of action of adrenalin but his interpretation as to its mode of action differs from that of Elliott. For Langley the dissimilarity of the synaptic

substance must be due to the intrinsic differences in the cells in which the nerve fiber ends rather than to intrinsic differences in the nerve fibers belonging to any one system.

Gruber (1924) noted that adrenalin markedly shortened the latent period and the duration of contraction of a skeletal muscular contraction and induced a greater shortening of the muscle when stimulated, due possibly to increasing the irritability or liberating more available energy or acting catalytically on muscle metabolism. Cannon (1929) considered that the effect of adrenalin is on the muscle substance. Lutz (1930) interpreted the inhibitory action of adrenalin on the heart of elasmobranch fishes as the response of an unbalanced parasympathetic mechanism in an organ lacking a sympathetic accelerator innervation, and believed the action of adrenalin to be on the nerve endings.

Cannon and Bacq (1931) have reported an adrenalin-like substance which they considered a hormone "as it acts in the same direction as sympathetic impulses and in their absence is capable of bringing denervated organs into conformity with those which are innervated, so that the characteristic unified response occurs." Because this substance is derived from structures under sympathetic control, when they are influenced by sympathetic impulses, these investigators suggested calling the substance sympathin, but stated that it is only a provisional name, for as

knowledge of its character increases it may prove really to be adrenalin, developed for local action in smooth muscle. Wyman and Lutz (1932) have stated that further work is necessary to locate the region of the action of adrenalin in Squalus acanthias.

Unpublished work (1933) of Cannon shows that sympathin is not the same as adrenalin. Sympathin may be two substances, one excitatory, the other inhibitory, called E-sympathin and I-sympathin respectively. Both substances are produced by the nerve endings. As yet the exact detailed interaction of these substances has not been worked out.

From the results in this investigation it would appear that the action of adrenalin is probably on the muscle fibers rather than the nervous elements. As to its mode of action on the muscle fibers it must be taken into consideration that adrenalin is not natural to molluscs. As far as can be determined in the literature the only mention of this substance or a related one, was made by Roaf and Nierenstein (1907) who stated that there is a substance in the hypobranchial gland of Purpura papillus which is chemically and physiologically allied to adrenalin. Therefore adrenalin when injected into these animals may act as a toxic substance, weak doses exerting a stimulating effect and strong doses an inhibitory one. On the other hand the excitatory effects produced by weak doses of adrenalin may be due to the fact that it improves the

condition of vertebrate skeletal muscle and also is generally stimulating to invertebrate muscle. This improved condition of the muscle may account for the increase in velocity of a single wave, the velocity of progression and the frequency of waves.

The possibility that adrenalin increases the irritability of muscle may account also for the increase of these activities when weak doses are used. It is conceivable that if adrenalin acts on the myo-neural substance such substance may have different degrees of stability. The strength of the dose of adrenalin may cause changes in this unstable substance, which in turn causes greater or less activity of the factors involved in locomotion.

The proportionality between the rate of creeping and the velocity with which a single wave traverses the foot, or between the frequency of the pedal waves and the velocity of progression is not altered in Helix by the injection of adrenalin; these facts further support the idea of muscle as an "independent effector".

G. Effect of strychnine sulphate on locomotion.

Crozier and Federighi (1924-25a) have pointed out from their work on the effect of temperature changes on phototropic circus movements of Limax that "a certain type of predictability in animal behavior under 'normal' natural conditions probably results from dynamic equilibrium

thereby obtained between diverse mechanisms competing for effector control (in the present case, the creeping mechanism and that for turning, in the range 14° - 46°C.). It follows that the unravelling of the elements of conduct necessitates experimentation under diverse abnormal conditions favoring individual mechanisms of response."

These investigators have succeeded in disentangling these two activities, i.e., orientation and locomotion. In the experiments on phototropic circus movements of Limax as affected by changes in temperature these authors found that below 15° the amount of circling is determined very largely by speed of creeping, while above 15° the pedal mechanism is in secondary place, the turning mechanism becoming the controlling element.

Another method of dissociating these two mechanisms was by the injection of strychnine. This substance was found to suppress the phototropic circus movements of Limax maximus (Crozier and Federighi, 1924-25b), but no detailed information was obtained concerning the changes in the creeping activity of the foot. Therefore the subsequent experiments were performed to study the effect of strychnine on the behavior of the pedal waves and to compare them with the normal foot. Small doses of strychnine of definite concentration disturb the functions of synaptic nervous systems, while the same concentration does not disturb the non-synaptic net. By the use of this substance Crozier and Arey (1919) found that in the

mollusk Chromodoris the general integument and all the outgrowths of the body depend upon a locally contained, peripheral, non-synaptic net-work, but the central nervous system is essentially synaptic. While a large proportion of mollusks have the entire nervous system of the non-synaptic type, those which show reversal of inhibition possess a synaptic nervous system.

The effect produced by strychnine, i.e., "reversal of inhibition" is interpreted in various ways by different authorities. In the spinal cord of vertebrates it is usually supposed that the strychnine effect is due to the abolition of the inhibitory component of normal coordination, so that the inhibitory effect is transposed into an excitatory one.

M. and Mme. Lapicque (1908) have concluded that the effect of strychnine is to bring about a condition of isochronism, i.e., the chronaxie of the nerve equals that of the muscle fiber and thus the excitation spreads easily from one neurone to another.

It may be argued that the action of strychnine is not due to true reversal but is merely due to a condition of augmented central excitability in which the excitatory effects produced by stimulation of a mixed efferent nerve conceal the inhibitory effects of the fibers of the same group, i.e., a homogeneous group of inhibitory impulses is not converted into excitatory impulses by strychnine. Magnus and Wolf (1913) and others have observed conditions

which support this interpretation.

Bayliss (1918) has concluded from his experiments on vasomotor reflexes that there may be two independent synapses with which the final common motor path connects, unequally sensitive to strychnine, or that the drug acts on some intermediate synaptic membranes on the afferent side, synapses which are not part of the path common to the different reflexes.

Bremer and Rylant's (1924, 1925) theory of "reversal" by strychnine is in accordance with that of the Lapicques. They believed that strychnine breaks down excitatory barriers between neurones, thus allowing excitation to spread freely from neurone to neurone throughout the nervous system. It does this by equalizing the chronaxie between various contiguous units which in the normal unpoisoned nervous system are separated by a barrier of heterochronism.

Fulton (1926) concluded that true reversal of inhibition into excitation probably does not occur, and the apparent reversal is due to the stimulation of excitatory fibers in a mixed nerve, which, owing to the increase of excitability produced by strychnine, conceals the effect of concomitant inhibition.

Various investigators have recently suggested that too much importance has been given to the question of chronaxie, and have questioned Lapicque's interpretations. Rushton (1930) did not agree with his claim that the

chronaxie of muscle is normally the same as that of the nerve which supplies it. He strongly supported Lucas (1907) who maintained that there are two excitable substances in muscle of which one has a very much larger chronaxie than nerve. Further, Rushton (1932) has also questioned Lapique's terminology. He (Rushton) stated that "since Lapique wishes to restrict the name 'chronaxie' to 'true' chronaxie, it is important to have a new term which can be applied to any strength duration curve 'true' or 'false'. Lucas 'Excitation Time' was used in just this sense and it is proposed for adoption." Grundfest (1932) also stated that Lapique's law of isochronism is not valid, at least for excitability of the single nerve-fiber nerve-muscle complex. He agreed with the views held by Lucas and Rushton. Lapique (1932) did not think that Rushton's investigations have thrown any new light on this problem and he defended his position against Rushton. It can be seen that too great importance has been placed on the subject of chronaxie before the various laws claimed for it have been satisfactorily interpreted and verified.

Although these various theoretical opinions concerning reversal of inhibition exist, it is possible through precise measurements to obtain data on the behavior of Helix when injected with strychnine. In this way it may be possible to gain information about central nervous activities which could not be obtained by other means.

Although Crozier and Federighi (1924-25_b, 7-221-224) were chiefly concerned with the effect of strychnine on the phototropic movements of Limax maximus and not primarily with locomotion, they state that strychnine does not essentially affect locomotion. It was the purpose, therefore, of this investigation to study the characteristic effect of various amounts of strychnine on Helix lactea and Helix pomatia during locomotion. As far as can be determined no mention of work of this nature can be found in the literature. A study was also made at the same time of the effects of strychnine sulphate, 1 part in 100, on the animals creeping in various planes, i.e., vertically upwards, horizontally (under surface) and horizontally (upper surface).

A series of experiments was first performed to study the effect of strychnine sulphate in the concentration of 1:1000 (1 part in 1000 parts of Ringer's solution). Measurements were obtained for the controls and for the injected animals during vertical creeping. After 0.2 cc. of strychnine sulphate was injected there was either partial or complete retraction of the foot into the shell. The time elapsing after injection before creeping began again varied from fifteen minutes to one hour. It was found that with strychnine sulphate, 1:1000, the creeping was more or less regular and could be compared to normal creeping. The specific results for Helix lactea under these conditions are given in Table XV. Table XVa

TABLE XVa

Effect of doses of strychnine sulphate,
1:1000, on the velocity of progression (V), the
frequency of waves (F), the velocity of a single
wave (v) and the advance per wave (A).

	¹ Plus	Minus	Cases unchanged
V	3	4	0
F	3	2	2
v	2	5	0
A	0	3	4

¹ Total number of increases or decreases
from the normal animal.

shows that the factors involved during locomotion were not essentially altered.

Injections of this concentration did not seem to affect the amplitude of the waves, as it was the same or nearly the same as when the animal was creeping under normal conditions (Table XV, advance per wave).

The effect of stronger concentrations of strychnine were studied for nineteen animals. In the experiments forty-eight series (7150 waves) were obtained for the control animals and forty-four series (6550 waves) for the injected ones when 0.2 cc. strychnine sulphate, 1:100, was injected. Creeping began from fifteen minutes to one hour after the injection. The data obtained from these experiments are given in Table XVI. Reference to Table XVIa shows that strychnine of this concentration did not affect any of the factors involved in locomotion.

Strychnine of still greater concentration was used (1:50) but no results could be obtained because after injection when the animal began creeping, sometimes after one or two hours, the creeping was irregular and it was impossible to count the waves.

The specific effect of the gravitational pull on the normal and strychninized animal during locomotion in three different planes, i.e., vertical, horizontal (under surface) and horizontal (upper surface) was analyzed. The procedure was to place the animal on a horizontal glass plate which rested on supports about six inches high

TABLE XV.

The effect of strychnine sulphate (1:1000) on vertical ascensions of Helix lactea.¹

No. of experiment	Velocity of progression in mm. per minute	Frequency of waves per minute	Velocity of single wave in mm. per minute	Advance per wave in mm.
#28 (Normal)	9.1	45.0	3650	0.20
#29 (0.5 cc. strychnine sulphate 1:1000)	10.0	52.0	7700	0.19
#30 (Normal)	11.2	52.0	7100	0.21
#31 (0.5 cc. strychnine sulphate 1:1000)	11.8	55.0	8300	0.21
#32 (Normal)	4.6	62.5	6550	0.07
#33 (0.5 cc. strychnine sulphate 1:1000)	5.7	62.5	5800	0.09
#57 (Normal)	20.6	100.0	4500	0.24
#58 (0.3 cc. strychnine sulphate 1:1000)	19.0	83.0	4100	0.14
#61 (Normal)	23.1	83.0	4800	0.27
#62 (0.3 cc. strychnine sulphate 1:1000)	8.5	71.0	4600	0.12
#82 (Normal)	16.8	83.0	4600	0.20
#83 (0.3 cc. strychnine sulphate 1:1000)	14.2	100.0	4500	0.14
#84 (normal)	27.0	100.0	7100	0.27
#85 (0.3 cc. strychnine sulphate 1:1000)	22.0	100.0	4100	0.22

¹ Temperature limits were 16.6° to 21.1° C. but the temperature was constant for each experiment.

TABLE XVI

The effect of strychnine sulphate (1:100) on
vertical ascensions of Helix lactea.

No. of experiment	Velocity of progression in mm. per minute	Frequency of waves per minute	Velocity of a single wave in mm. per minute	Advance per wave in mm.
4 o	60.6	81.2	3828	0.48
5 ●	72.1	90.0	4069	0.54
6 o	42.0	72.6	3078	0.36
7 ●	39.2	77.4	3772	0.42
8 o	64.8	67.8	3109	0.40
9 ●	57.0	79.2	3435	0.43
19 o	57.3	24.9	78.1	0.34
20 ●	53.0	23.2	74.5	0.28
21 o	46.2	29.7	112.0	0.31
22 ●	54.8	35.9	85.1	0.42
23 o	62.8	31.7	90.3	0.40
24 ●	55.6	18.9	82.8	0.23
25 o	21.9	96.0	4950	0.29
26 ●	19.4	70.0	4800	0.27
27 o	20.6	84.2	5150	0.24
28 ●	21.3	56.9	4900	0.38
29 o	31.1	91.7	4660	0.31
30 ●	31.3	97.0	4920	0.29
31 o	20.8	68.0	5900	0.29
32 ●	27.0	96.6	6175	0.29
33 o	25.0	89.2	5140	0.30
34 ●	13.5	69.2	4420	0.20

o = control

● = injected animal (0.2 cc. of strychnine sulphate 1:100)

TABLE XVI (cont.)

No. of experiment	Velocity of progression in mm. per minute	Frequency of waves per minute	Velocity of a single wave in mm. per minute	Advance per wave in mm.
35 o	39.6	96.0	4300	0.44
36 ●	48.6	54.0	5400	0.35
37 o	21.0	56.0	4420	0.24
38 ●	13.0	96.0	4600	0.18
39 o	28.8	88.0	5010	0.32
40 ●	32.1	86.9	4240	0.42
88 o	29.0	111.0	5400	0.26
89 ●	23.1	68.0	3400	0.30
90 o	30.8	108.0	4600	0.29
91 ●	11.1	97.2	4600	0.10
92 o	35.0	98.1	5500	0.30
93 ●	16.3	92.1	5160	0.21
94 o	35.6	76.2	3500	0.13
95 ●	40.0	81.0	4000	0.16
96 o	13.4	72.2	4900	0.19
97 ●	26.0	98.3	5400	0.26

o = control

● = injected animal (0.2 cc. of strychnine sulphate 1:100)

TABLE XVia

Effect of strychnine sulphate, 1:100, on the velocity of progression (V), the frequency of waves (F) the velocity of a single wave (v) and the advance per wave (A).

	¹ Plus	Minus	Cases unchanged
V	7	10	2
F	9	10	0
v	9	9	1
A	7	8	4

¹ Total number of increases or decreases from the normal animal.

(Fig. 15). Under the glass plate a mirror was placed at a slope of 45° on a block of wood for the purpose of studying the waves when the snail was creeping on the upper surface of a horizontal plane. The method of measurement was the same as given above. Data were first obtained for the normal animal when creeping in (1) a vertical plane, (2) in a horizontal plane (under surface) and (3) horizontal plane (upper surface); after injection of strychnine sulphate measurements were obtained in the same plane as for the normal animal. The actual scatter was plotted of the various relationships and the slopes of the line were compared.

Figs. 16, 17 and 18 give typical results and show that in all planes for the strychninized animal a linear relationship exists between the velocity of a single wave and the velocity of progression, between the frequency of waves and the rate of creeping and between the advance per wave and the rate of creeping. A smaller change in the slope of line is observed for the relationship between the advance per wave and the velocity of progression (Fig. 18) than is noted for the other two relations (Fig. 16 and Fig. 17).

The effect of the gravitational pull on the normal animal during locomotion in the three different planes was observed by comparing the slope of the lines obtained for each relationship. The slope of the lines showing the relationships occurring during vertical creeping was

usually less than for the same relations during creeping on the horizontal plane, upper or under surface. Reference to Table XVII shows that the velocity of a single wave, frequency of waves, the velocity of progression and the advance per wave were usually greater when the animal was creeping in a horizontal plane (upper surface) and creeping vertically upwards than when creeping in a horizontal plane (under surface).

In the strychninized animal not only the change due to strychnine, but also the increase or decrease due to changes in gravitational pull was determined. (Fig. 16, 17 and 18.) As the gravitational pull is decreased there is usually an increase in the slope of the line describing the various relationships when the animal was creeping on the horizontal plane, upper surface. This increase was less as the gravitational pull was increased (vertical creeping and creeping on a horizontal under surface). However, the results show that when an animal was creeping in each of these two planes there was a greater alteration of these factors than when creeping on a horizontal upper surface (Table XVII).

The results in this investigation also show that as the effect of gravity was lessened the factors involved in locomotion were increased, for creeping was increased in the horizontal plane (upper surface) as compared with the other planes. Cole (1925-26) has stated that the stimulus for geotropic orientation and locomotion is the

TABLE XVII

Effect of gravitational pull on velocity of single pedal wave, velocity of progression, frequency of waves, and advance per wave in the normal and strychninized animal (1:100).

	Horizontal plane (under surface)		Vertical plane		Horizontal plane (upper surface)	
	$v \frac{\text{mm}}{\text{sec.}}$	per cent ¹	$v \frac{\text{mm}}{\text{sec.}}$	per cent	$v \frac{\text{mm}}{\text{sec.}}$	per cent
Animal #4						
o	54.88		63.83		62.61	
•	70.93	+29.0	67.82	+6.0	72.52	+15.0
Animal #3						
o	69.72		51.83		70.45	
•	63.84	-8.0	51.25	+10.0	66.50	-5.0
Animal #6						
o	56.22		51.31		65.65	
•	54.44	-3.0	62.84	+22.0	75.05	+12.0
Animal #4						
	$V \frac{\text{mm}}{\text{sec.}}$		$V \frac{\text{mm}}{\text{sec.}}$		$V \frac{\text{mm}}{\text{sec.}}$	
o	0.9668		1.0150		1.2131	
•	1.1068	+14.0	1.2051	+18.0	1.9684	+62.2
Animal #3						
o	1.6250		1.0827		1.9080	
•	0.8690	-46.0	0.9525	-10.0	1.7110	-10.0
Animal #6						
o	0.7514		0.6949		1.3503	
•	0.6777	-9.8	1.1099	+59.0	1.3554	+0.4

¹ Percentage increase or decrease after injection of strychnine sulphate compared to normal animal.

² o=control
•=injected animal (strychnine sulphate)

TABLE XVII (cont.)

	Horizontal plane (under surface)		Vertical plane		Horizontal plane (upper surface)	
	F waves sec.	per cent	F waves sec.	per cent	F waves sec.	per cent
Animal #4						
o	1.2533		1.3733		1.3029	
•	1.5956	+27.0	1.5091	+17.0	1.4920	+14.0
Animal #5						
o	1.5221		1.1139		1.4081	
•	1.5220	No change	1.3250	+18.0	1.5750	+12.0
Animal #6						
o	1.2699		1.2533		1.3958	
•	1.3387	+5.4	1.5956	+29.0	1.6286	+16.0
Animal #4						
	A mm waves		A mm waves		A mm waves	
o	0.570		0.811		0.900	
•	0.725	+27.0	0.900	+11.0	1.310	+45.5
Animal #5						
o	1.075		0.682		0.704	
•	0.560	-48.0	0.730	+7.0	0.665	-5.0
Animal #6						
o	0.611		0.612		0.861	
•	0.513	-16.0	0.730	+18.0	0.850	-1.2

tension of the body muscles produced by the downward pull of gravity; this stimulus is received by the proprioceptors of the muscles. However, if this were the condition we should not expect to get faster creeping when the pull of gravity is lessened; consequently, Cole's theory about locomotion, while it may hold true for orientation, does not seem to solve the problems involved in locomotion.

The fact that strychnine sulphate in the strongest dose that could be used practically (1:100) did not essentially affect locomotion may be due to the fact that invertebrates are relatively more insusceptible to strychnine than vertebrates. Also if strychnine acts on synapses, the lack of the usual excitation may be due to a lack of synapses in the intrinsic mechanism of the foot.

Strychnine in this investigation did not abolish the linear proportionality between the velocity of a single wave and the velocity of progression, between the frequency of waves and the velocity of progression and between the advance per wave and the velocity of progression.

Chapter VI.

Summary and Conclusions

1. The results of these experiments show that in Helix lactea the speed of creeping vertically upward, when not carrying loads, is directly proportional to the velocity of a single pedal wave. The speed of creeping is also directly proportional to the frequency of the waves and to the advance per wave. The proportionality factor is independent of the weight of the animal.

2. The proportionality between the rate of creeping and the velocity with which a single wave traverses the foot, or between the frequency of the pedal waves and the velocity of progression, is not altered in normal or in de-eyed Helix when lifting loads during vertical creeping. Under these conditions the linear relationship between the advance per wave and the rate of creeping is not altered. The foot appears to be essentially an independent effector, although under the control of central impulses.

3. The detached foot was observed and kymograms were obtained which showed that the rhythmical action of the foot is greatly interfered with when it is not connected with the pedal ganglia. The destruction of the periodic recurrence of waves when the pedal ganglia are separated from the foot suggests that this periodicity is determined by the presence of these ganglia.

4. Injection of Ringer's solution or mechanical stimulation of the body wall or anterior end of the foot does not affect the factors involved in locomotion.

5. Adrenalin of various concentrations does not alter the linear proportionality between the velocity of progression and the velocity of a single wave, between the frequency of waves and the velocity of progression and between the advance per wave and the rate of creeping. These are additional factors which establish the idea of the foot "independent effector" of muscle.

Adrenalin in concentrations of 1:120,000 (0.2 cc. or 0.3 cc.) produces a stimulating effect on the velocity of progression, frequency of waves, velocity of a single wave and the advance per wave.

An inhibitory effect of adrenalin on invertebrate tissue not previously reported is given. Adrenalin in concentrations of 1:10,000, 1:20,000, 1:40,000 and 1:80,000 produces a depressant action on the velocity of progression, frequency of waves, velocity of a single pedal wave, and the advance per wave.

The region of action of the adrenalin is discussed.

6. Strychnine sulphate (1 in 100, 1 in 1000) does not bring about reversal of inhibition in Helix. It does not abolish the linear proportionality between the velocity of a single wave and the velocity of progression, between the frequency of waves and the velocity of progression and between the advance per wave and the velocity of progression. These facts are additional evidence that the

foot is essentially an "independent effector".

The effect of gravitational pull on the normal and strychninized animal during locomotion in various planes, i.e., vertically upwards and horizontally (upper surface) is usually to increase the velocity of a single wave, the frequency of waves, the velocity of progression and the advance per wave, while during creeping in horizontal plane (under surface) a decrease in each activity occurs.

7. The purpose of this investigation was to establish if possible the factors in control of locomotion in Helix. It has been shown that the foot of Helix is primarily an independent effector and is secondarily under the control of central impulses. In other words, the intrinsic neuro-muscular mechanism of the foot is the primary factor in locomotion.

lost is essentially an "independent effect".
The effect of physiological basis on the normal and
asymmetrized animal during locomotion in various stages
i.e., vertically upward and horizontally (right and left)
is usually to increase the velocity of a single wave.
The frequency of waves, the velocity of propagation and
the advance per wave, while during stepping in horizontal
plane (under surface) a decrease in rate is usually found.
V. The purpose of this investigation was to deter-
mine if possible the factors in control of locomotion in
Helia. It has been shown that the rate of step is con-
trolled by an independent effector and is essentially un-
der control of the central nervous system. In other words, the
intrinsic nerve-muscular mechanism of the foot is a
primary factor in locomotion.

Citations

- Alvarez, W.C. and Mahoney, Lucille. The myogenic nature of the rhythmic contractions of the intestine. *Am.J. Physiol.*, 1922, 59, 421-430.
- Ascanio, H. and Alvarez, W.C. Studies on the intestinal muscle of man. *Am.J.Physiol.*, 1929, 90, 607-610.
- Intestinal rhythmicity after death. *Ibid.*, 611-616.
- Bayliss, W.N. Principles of General Physiology. London, Longmans, Green & Co. 1918. pp.500.
- Biederman, W. Die locomotorischen Wellen der Schneckensohle. *Arch.Ges.Physiol.*, 1905, 107, 1-56.
- Die Innervation der Schneckensohle. *Ibid.*, 1906, 111, 251-297.
- Bohn, G. Des ondes musculaires respiratoires et locomotrices chez annélides et les mollusques. *Bull.Mus.Nat.*, Paris, 1902, 8, 96-102.
- Boyer, P. Action de l'adrénaline et de sparteine sur le coeur de l'escargot. *C.R.soc.biol.*, Paris, 1926, 95, 1244-1247.
- Bremer, F. and Rylant, P. Action de la strychnine sur l'excitabilité des différents éléments de l'arc reflexe. *C.R.soc.biol.*, 1924, 91, 110-113.
- Nouvelles recherches sur le mécanisme de l'action de la strychnine sur le système nerveux central. *C.R.soc.biol.*, 1925, 92, 199-202.
- Cannon, W.B. Bodily changes in pain, hunger, fear and rage. N.Y. and London, D.Appleton & Co., 1929. pp.128.
- Cannon, W.B. and Bacq, Z.M. Studies on the conditions of activity in endocrine organs. *Am.J.Physiol.*, 1931, 96, 392-412.
- Carey, Eben. Studies in the dynamics of histogenesis. *J.Gen. Physiol.*, 1919-20, 2, 357-371.
- Anat.Rec.*, 1920, 19, 199-227.
- J.Gen.Physiol.*, 1921, 3, 61-83.
- Amer.J.Anat.*, 1921, 29, 93-115.
- Amer.J.Physiol.*, 1921-22, 58, 182-194.
- Anat.Rec.*, 1922-23, 24, 89-96.
- Carlson, A.J. The physiology of locomotion in gasteropods. *Biol.Bull.*, 1904-05, 8, 85-92.

ten Cate, J. Quelques recherches sur la locomotion des Limaces. Arch.Néerl.Physiol.Homme et Animaux, 1923, 8, 377-393.

Contribution á la physiologie du ganglion pédal d'*Aplysia limacina*. Arch.Néerl.Physiol. Homme et Animaux. 1927-28, 12, 529-537.

Clark, A.J. Comparative physiology of the heart. New York, Macmillan Co., 1927. pp. 62.

Cole, W.H. Geotropism and muscle tension in *Helix*. J.Gen. Physiol., 1925-26, 8, 253-263.

Cowie, D.M. and Parson, J.P. and Lashmet, F.H. Studies on the function of the intestinal musculature. Am.J. Physiol., 1929, 88, 363-385.

Crozier, W.J. The rhythmic pulsation of the cloaca of Holothurians. Contributions from the Bermuda Biological Station for Research, 1916, III, No.43, 297-356.

On the use of the foot in some mollusks. J.Exp.Zool., 1918-19, 27, 359-366.

The analysis of neuro-muscular mechanisms in *Chiton*. J.Gen.Physiol., 1919-20, 2, 627-634.

"Reversal of Inhibition" by Atropine in Caterpillars. Biol.Bull., 1922, XLIII, No.4, 239-245.

On the nervous mechanism of *Limax*. Anat.Rec., 1922-23, a, 24, 398.

Concerning laws of locomotion in gasteropods. Anat. Rec., 1922-23, b, 24, 398.

On the locomotion of the larvae of the slug-moths (*Cochlidiidae*.) J.Exp.Zool., 1923-24, 38, 323-329.

On biological oxidations as a function of temperature. J.Gen.Physiol., 1924-25, 7, 189-216.

Tropisms. J.Gen.Psychol., 1928, 1, 213-238.

Crozier, W.J. and Arey, L.B. Sensory reactions of *Chromodoris zebra*. J.Exp.Zool., 1919, 29, 261-310.

Crozier, W.J. and Federighi, H. Phototropic circus movements of *Limax* as affected by temperature. J.Gen.Physiol., 1924-25, a, 7, 151-169.

Suppression of phototropic circus movements of *Limax* by strychnine. J.Gen.Physiol., 1924-25, b, 7, 221-224.

The locomotion of *Limax*. J.Gen.Physiol., 1924-25, c, 7, 415-419.

- Crozier, W.J. and Navez, A.E. The geotropic orientation of gastropods. *J.Gen.Psychol.*, 1930, 3, 3-37.
- Crozier, W.J. and Pilz, G.F. The locomotion of *Limax*. *J. Gen.Physiol.*, 1923-24, 6, 711-721.
- Crozier, W.J. and Pincus, G. Analysis of the geotropic orientation of young rats. *J.Gen.Physiol.*, 1929-30, 13, 57-120.
- Crozier, W.J. and Stier, T.J.B. Temperature characteristics for locomotor activity in tent caterpillars. *J.Gen. Physiol.*, 1925-26, 9, 49-54.
- Elliott, T.R. The action of adrenalin. *J.Physiol.*, 1905, 32, 401-467.
- Fulton, J.F. Muscular contraction and the reflex control of movement. Baltimore, Williams and Wilkins Co., 1926. pp.463.
- Gruber, C.M. The effect of adrenalin on the duration of the latent, the contraction and the relaxation periods of skeletal muscle at rest and undergoing fatigue. *J.Pharm.and Exper.Ther.*, 1924, 23, 335-351.
- Grundfest, H. Excitability of the single fibre nerve-muscle complex. *J.Physiol.*, 1932, 76, 95-115.
- Gunn, J.A. and Underhill, S.W.F. Experiments on the surviving mammalian intestine. *Quar.J.Physiol.*, 1914, 8, 275-296.
- Hoagland, H. On the mechanism of an instinct. Thesis (Physiology Library, Harvard University), 1927.
- Huxley, Thomas. Science and Education. New York, D.Appleton & Co., 1897.
- Jordan, H. Die Physiologie der Locomotion bei *Aplysia limacina*. *Zeit.f.Biol.*, 1901, 41, 196-238.
- Jordan, H. and Hirsch, G.Chr. ^{II}Übungen aus der Vergl.Physiol. Berlin, Julius Springer, 1927, 203-263.
- Kendall, E.C. Thyroxine. New York.Chem.Cat.Co., 1929, 221-230.
- Langley, J.M. On the reaction of cells and of nerve-endings to certain poisons, chiefly as regards the reaction of striated muscle to nicotine and curare. *J.Physiol.*, 1905-06, 33, 374-413.
- Lapicque, L. L'excitabilité en fonction du temps. Paris. Les presses universitaires de France, 1926. pp.249.
- Retrograde polarization, a theory of systematic errors in measurements of muscular chronaxie through Ringer's Fluid or with large electrodes. *J.Physiol.*, 1932, 76, 261-281.

- Lapicque, L. and Mme. Lapicque. Sur le mécanisme de la curarisation. C.R.Soc.Biol., 1908, 65, 733-735.
- Loeb, Jacques. Die Orientierung der Tiere gegen die Schwerkraft der Erde (tierischen Geotropismus). Zitzber. Würzburg.phys.-med.gesellsch., 1888, No.1.
- Lucas, K. The excitable substances of amphibian muscle. J.Physiol., 1907-08, 36, 113-135.
- Lutz, B.R. The effect of low oxygen tension on the pulsations of the isolated holothurian cloaca. Biol. Bull., 1930, 58, 74-84.
- The effect of adrenalin on the auricle of elasmobranch fishes. Am.J.Physiol., 1930, 94, 135-139.
- Mackay, M.E. The action of some hormones and hormone-like substances on the circulation of the skate. Cont. Can.Biol.and Fish., N.S., 7, 19-29.
- Magnus, R. Versuche am Überlebenden Dünndarm von Säugetieren. Arch.Ges.Physiol., 1905, 108, 1-71.
- Körperstellung. Berlin. Julius Springer, 1924. pp.29.
- Magnus, R. and Wolf, C.G.L. Weitere Mitteilungen über den Einfluss der Kopfstellung auf den Gliedertonus. Pflüger's Arch., 1913, 447-461.
- Mendel, Lafayette, B., and Bradley, H.C. Experimental studies on the physiology of molluscs. Am.J.Physiol., 1906-07, 17, 167-176.
- Olmstead, J.M.D. Notes on the locomotion of certain Bermudian mollusks. J.Exp.Zool., 1917-18, 24, 233-236.
- Parker, G.H. The mechanism of locomotion in gastropods. J.Morph., 1911, 22, 155-170.
- The locomotion of Chiton. Contributions from the Bermuda Biological Station for Research, 1914, III, no. 31, 1-2.
- van Rijnberk, G. IV. Sur le mouvement de locomotion de l'escargot terrestre *Helix aspersa*. Arch.Néerl.Physiol., Homme et Animaux, 1918-19, 3, 539-552.
- Rose, Maurice. La question des tropismes. Paris, Les Presses universitaires de France, 1929.
- Rushton, W.A.K. Excitable substances in the nerve-muscle complex. J.Physiol., 1930, 70, 317-337.

- Rushton, W.A.K. Lapique's canonical strength duration curve. J.Physiol., 1932, 74, 424-440.
- Simroth, H. Die Thätigkeit der willkürlichen Muskulatur unserer Landschnecken. Zeit.f.will.Zool., 1878, 30, 166-224.
- Sollman, T. A Manual of Pharmacology. Phila., 1928.
- Stier, T.J.B. Aspects of the control of animal activities. Thesis, 1928. (Harvard Physiology Library.)
- Vlès, F. Sur les ondes pédieuses des mollusques reptateurs. Compt.rend.Acad., 1907, 145, 276-278.
- Vlès, F. and Bathellier, J. Sur les lois numériques des ondes pédieuses chez les gasteropodes. Compt.rend. Acad., 1920, 171, 1083-1086.
- Wyman, L.C. and Lutz, B.R. The action of adrenaline and certain drugs on the isolated holothurian cloaca. J.Exp.Zool., 1930, 57, 441-453.
- The effect of adrenaline on the blood pressure of the elasmobranch, *Squalus acanthias*. Biol.Bull., 1932, LXII, 17-22.

Index of Figures

Fig. A1.	Diagrams showing:	Page	127
a.	<i>Helix pomatia</i> , dorsal view		
b.	" " ventral view		
c.	" lactea, dorsal view		
d.	" " ventral view		
A2.	Diagram showing nerve-net in foot of <i>Helix</i>		128
A3.	Diagram of <i>Helix</i> showing arrangement of ganglia (cerebral, pedal and visceral)		129
A4.	Schematic representation of the musculature of the foot of <i>Helix</i>		130
1.	Diagram of the method of measurement for vertical creeping		131
2.	The linear relationship between the velocity of a single pedal wave and the rate of creeping during vertical ascensions without load		132
3.	The relation between the velocity of progression and the frequency of waves for vertical creeping without load		133
4.	The relation between the velocity of progression and the advance per wave for locomotion in a vertical plane without load		134
4a.	Comparative graph showing the relation between the velocity of progression and the velocity of a single wave without added load		135
4b.	Comparative graph showing relation between the velocity of progression and the frequency of waves without added load		136
4c.	Comparative graph showing relation between velocity of creeping and the advance per wave without added load		137
4d.	The relation between speed of creeping and velocity of a single wave without added load in <i>Helix pomatia</i> .		138
4e.	The relation between velocity of progression and the frequency of waves without added load in <i>Helix pomatia</i> .		139
4f.	Relation between the velocity of progression and the advance per wave without added load in <i>Helix pomatia</i> .		140

Fig. 5. The effect of added loads on the relation between frequency of waves and the rate of creeping	141
5a. Actual scatter obtained from series of vertical ascensions with and without loads showing the relation between the velocity of progression and the frequency of waves	142
5b. Relation between the rate of creeping and the frequency of waves with added loads during vertical ascensions for animal No. 10	143
6. The relation between tension (added loads) and the rate of creeping	144
7. The probable error for the rate of creeping of animal No.6 as a function of tension (added loads)	145
8. Comparison of the normal and de-eyed animal showing the relation between the velocity of a single wave and the rate of creeping	146
8a. Actual scatter obtained from series of vertical ascensions without load for normal and de-eyed animal showing the relation between the velocity of the pedal waves and the rate of creeping	147
9. The relation between the frequency of waves and the rate of creeping for de-eyed <u>Helix lactea</u>	148
9a. Actual scatter obtained from a series of vertical ascensions without load for normal and de-eyed animal showing the relation between velocity of progression and the frequency of waves	149
10. The relation between the advance per wave and the rate of creeping in the normal and de-eyed animal	150
10a. Actual scatter obtained showing the relation between the velocity of progression and the advance per wave for animal No.3 with and without eyes carrying no load	151
10b. Actual scatter showing relation between the velocity of pedal waves and the rate of creeping for normal and de-eyed animals with added load of 0.7 gram	152
10c. Actual scatter showing relation between the velocity of progression and the frequency of waves for normal and de-eyed animal with added load of 0.7 gram	153

Fig.10d. Actual scatter showing relation between the velocity of progression and the advance per wave for normal and de-eyed animal with added load of 0.7 gram	154
10e. Comparative graph showing relation between velocity of a single wave and the rate of creeping for de-eyed animal carrying varying loads	155
11. Diagram of the arrangement for recording the behavior of the detached foot	156
11a. Kymograph records of the responses of the and b. detached foot	157
12. The effect of adrenalin on the relation between the velocity of a single pedal wave and the rate of creeping	158
13. The effect of adrenalin on the relation between the frequency of waves and the rate of creeping	159
14. The advance per wave as a function of the rate of creeping for the normal and injected animal (adrenalin)	160
15. Diagram showing the method of measurement for creeping in a horizontal plane (upper surface)	161
16. The effect of strychnine on the relation between the velocity of a single pedal wave and the velocity of progression	162
a. Creeping in vertical plane	
b. " " horizontal plane (under surface)	
c. " " " " " (upper surface)	
17. Comparison of the normal and injected animal (strychnine sulphate) showing the relation between the frequency of waves and the velocity of progression	163
a. Creeping in vertical plane	
b. " " horizontal plane (under surface)	
c. " " " " " (upper surface)	

Figure A 1

Diagrams showing:

- a. Helix pomatia, dorsal view
- b. Helix pomatia, ventral view
- c. Helix lactea, dorsal view
- d. Helix lactea, ventral view

a.

b.

c.

d.

FIGURE A1

Figure A 2

Locomotor nerves of Helix pomatia.

(a) Nerve net in foot.

(b) Central part of nerve system.

1. Cerebro-pleural connection,
cut.
2. Cerebro-pedal connection.
3. Radiating nerves of the
pedal ganglion.
4. Cerebral ganglion.
5. Pedal ganglion.

(From Jordan, p. 225, after E. Schmalz,
1914.)

FIGURE A₂

Figure A 3

Diagrams showing arrangement of cerebral ganglion (c), pedal ganglia (P) and visceral ganglia (v).

(Modified after Richard Hertwig, "A Manual of Zoology" by Richard Hertwig, translated and edited by J. S. Kingsley, p. 352.)

Figure A 4

Schematic representation of the musculature of the foot of Helix. The longitudinal muscle fibers are red, the dorso-ventral black. Fig.5 is a horizontal, Fig.6 a vertical longitudinal section. Of the retractor bundles, which are to be considered in Fig.5 as spread out, only three are represented, r1, r2 and r3.

(Taken from Zeitschrift für wissenschaftliche Zoologie. 30er Band. Tafel acht. Supplement, Erstes Heft. Leipzig 1878.)

Fig. 5. *Fig. 6.* FIGURE A₄

FIG. 1. METHOD OF MEASUREMENT FOR VERTICAL CREEPING

Figure 2

The velocity of a single wave is directly proportional to the velocity of progression during vertical ascensions without load.

The average deviation of the mean for each point plotted for animal No. 7 (Helix lactea) is as follows:

Point	1	1.0
"	2	0.25
"	3	0.53
"	4	2.9
"	5	1.5

Animal	Weight of animal	Slope of line
No. 1	6.5 gm.	0.2
" 4	8.6 "	0.36
" 7	7.9 "	0.34

FIGURE 2.

Figure 3

The frequency of the pedal waves is directly proportional to the velocity of progression, during vertical ascensions without load. This proportionality holds regardless of the weight of the animal. (Helix lactea). (Table III).

Animal	Weight of animal	Slope of line
No. 1	6.5 gm.	0.2
" 7	7.9 "	0.18
" 10	3.8 "	0.2

FIGURE 3. VELOCITY OF PROGRESSION, V , MM/SEC.

Figure 4

The advance per wave is proportional to the velocity of progression in vertical creeping without loads. This relationship holds regardless of the weight of the animal (Helix lactea). (Table III).

Animal	Weight of animal	Slope of line
No. 4	8.6 gm.	0.3
" 7	7.9 "	0.3
" 10	3.8 "	0.3

FIGURE 4. VELOCITY OF PROGRESSION, V , MM/SEC

Figure 4A

This comparative graph shows that the velocity of progression without loads during vertical ascension is directly proportional to the velocity with which a single wave traverses the foot and that it is independent of the weight of the animal (Helix lactea).

Animal	Weight of animal	Slope of line
No. 1	6.5 gm.	0.2
" 3	7.7 "	0.52
" 4	8.6 "	0.36
" 5	7.1 "	0.48
" 6	6.7 "	0.82
" 7	7.9 "	0.34
" 8	8.5 "	0.52
" 10	3.8 "	0.54
" 11	5.0 "	0.24

FIGURE 4A

Figure 4B

The frequency of the pedal waves is directly proportional to the velocity of progression during vertical ascensions without loads. This proportionality holds regardless of the weight of the animal (Helix lactea).

Figure 4C

In the analysis of nine animals (Helix lactea) for the relationship between the advance per wave and the velocity of progression the advance per wave is proportional to the velocity of progression in vertical upward creeping without loads, and this relationship holds regardless of the weight of the animal (Table III).

Figure 4D

The rate of creeping during vertical ascensions is directly proportional to the velocity with which a single wave courses over the foot when carrying no load. This relationship is illustrated for animals Number 41, 44 and 46. (Helix pomatia.)

FIGURE 4D VELOCITY OF PEDAL WAVE, v , MM/MIN.

Figure 4E

The rate of creeping is directly proportional to the frequency of pedal waves during vertical ascensions without loads. This relationship is illustrated for animals Number 41, 44 and 46. (Helix pomatia.)

FIGURE 4E RATE OF CREEPING, V , MM/MIN.

Figure 4F

The velocity of progression is proportional to the advance per wave during vertical ascensions without loads. This is illustrated for animals Number 41, 45 and 46. (Helix pomatia.)

FIGURE 4F

Figure 5

In Helix lactea the linear proportionality between rate of creeping (V) and frequency of the pedal waves is not altered when lifting added loads during vertical creeping. This graph shows the individual effect of adding varying loads on animal Number 7.

		Slope of line
Without load		0.20
With load of 0.7 gm.		0.20
	1.2 "	0.22
	2.0 "	0.18
	2.5 "	0.42
	3.0 "	0.20
	3.8 "	0.22
	4.3 "	0.22

FIGURE 5. RATE OF CREEPING, V , MM/SEC

Figure 5A

The actual scatter obtained from direct observations for a series of runs with and without loads during vertical ascensions. The relationship between the velocity of progression and the frequency of waves has been plotted for animal Number 7. (Helix lactea.)

Figure 5B

This comparative graph shows the relation between the rate of creeping and the frequency of waves for animal Number 10 (Helix lactea) with added loads during vertical ascensions.

Added load	Slope of line
0.7 gm.	0.18
1.2 "	0.16
2.0 "	0.16
2.5 "	0.14
3.0 "	0.18
3.8 "	0.14
4.8 "	0.28 exhaustion

FIGURE 5B

Figure 6

The relation of tension to the rate of creeping is shown from data obtained from animals Number 1, 3, 4, 6, 7 and 8 during upward vertical creeping (Helix lactea). The tension is represented by the addition of varying loads, i.e., 0.7, 1.2, 2.0, 2.5, 3.0, 3.8, and 4.3 grams. With a great many animals the effect of a load of 2.5 gm. was a decided decrease in the rate of creeping. Usually a slight increase occurred with the addition of 3.0 grams.

FIGURE 6. TENSION IN GRAMS [ADDED LOAD]

Figure 7

Figure 7 shows the probable error
(calculated according to Bessel's formula)
for the rate of creeping with added loads.

Figure 8

The removal of the eyes does not
alter the law of linear proportionality
between the velocity of a single wave
and the rate of creeping without loads.
(Helix lactea.)

Figure 8A

The actual scatter is shown of data obtained from direct observations of the normal and de-eyed animal (Helix lactea). The relation between the velocity of the pedal waves and the rate of creeping has been plotted for animal No. 3 during vertical ascensions without load.

RATE OF CREEPING, $\dot{V}$, MM./SEC.

ACTUAL SCATTER

ANIMAL #3

● WITH EYES
○ DE-EYED

WITHOUT LOAD

30

35

40

45

50

VELOCITY OF PEDAL WAVE, v , MM./SEC.

FIGURE 8A

Figure 9

The linear relationship between rate of creeping and frequency of waves is not altered in the de-eyed animals without load (Helix lactea).

Figure 9A

The actual scatter is shown of data obtained from direct observations of the normal and de-eyed animal (Helix lactea). The relation between the velocity of progression and the frequency of waves has been plotted for animal No. 3 during vertical ascensions without load.

ANIMAL #3

ACTUAL SCATTER

FREQUENCY, WAVES/SEC.

● WITH EYES
○ DE-EYED

WITHOUT LOAD

3 4 5 6 7

FIGURE 9A VELOCITY OF PROGRESSION, V , MM/SEC.

Figure 10

The relationship between the rate of creeping and the advance per wave is not altered in the de-eyed animal without load (Helix lactea).

Figure 10A

The actual scatter is shown of data obtained from direct observations of the normal and de-eyed animal (Helix lactea). The relation between the velocity of progression and the advance per wave has been plotted for animal No. 3 during vertical ascensions without load.

Figure 10B

The actual scatter is shown of data obtained from direct observations of the normal and de-eyed animal (Helix lactea). The relation between the velocity of the pedal waves and the velocity of progression has been plotted for animal No. 3 during vertical ascensions with added load of 0.7 gm.

FIGURE 10 B

Figure 10C

The actual scatter is shown of data obtained from direct observations of the normal and de-eyed animal (Helix lactea). The relation between the velocity of progression and the frequency of waves has been plotted for animal No. 3 during vertical ascensions with added load of 0.7 gm.

FIGURE 10 C

Figure 10D

The actual scatter is given of data obtained from direct observations of the normal and de-eyed animal (Helix lactea). The relation between the velocity of progression and the advance per wave has been plotted for animal No. 3 during vertical ascensions with added load of 0.7 gm.

FIGURE 10D

Figure 10E

The effect of added loads is shown on the de-eyed animal (Helix lactea). The relation between the velocity of a single wave and the rate of creeping is not altered by added loads during vertical creeping. (See also Table VIIa, page 48.)

FIGURE 10E VELOCITY OF PEDAL WAVE, v , MM/SEC.

FIG II. DIAGRAM OF THE ARRANGEMENT FOR
RECORDING THE BEHAVIOR OF THE DETACHED FOOT

Figure 11b. A series of responses of the detached foot twenty-four hours after removal of the foot.
Animal #5 Temperature 22.9°C.
1 = 24 hours after cutting.
2 = 26 " "
3 = 28 " "

Figure 116. A series of responses showing the behavior of the foot with ligatures when separated from the pedal ganglia.

Animal #9 Temperature 22.90C. a = Tonic contraction of entire foot.

1 = 1 hour after removal of foot.	4 = 2 hours 20 minutes after removal of foot.
2 = 1 " 27 minutes after removal of foot.	5 = 2 " 48 " "
3 = 1 " 54 " "	6 = 3 " 15 " "

Figure 12.

A comparison of the normal (Helix lactea) and injected animal (adrenalin) is given in this figure which shows the relationship between the velocity of a single pedal wave and the rate of creeping.

Animal #2	Slope of line
o control	0.11
• injected (adrenalin)	0.21
Animal #14	
o control	0.29
• injected (adrenalin)	0.20

FIGURE 12. VELOCITY OF PEDAL WAVE, v , MM/SEC

Figure 13

A comparison of the normal and injected animal showing the effect of adrenalin on the relationship between the rate of creeping and the frequency of waves.

Animal #2	Slope of line
o = control	0.38
● = injected (adrenalin)	0.45
Animal #14	
o = control	0.22
● = injected	0.24

FIGURE 13. RATE OF CREEPING, V, MM/SEC

Figure 14

The relationship between the advance per wave and the rate of creeping is compared with the normal (Helix lactea) and injected animal (adrenalin).

Animal #2	Slope of line
o = control	0.17
● = injected (adrenalin)	0.18
Animal #14	
o = control	0.22
● = injected (adrenalin)	0.18

FIGURE 14. RATE OF CREEPING, V , MM/SEC

FIG. 15. METHOD OF MEASUREMENT FOR
CREEPING IN HORIZONTAL PLANE (UPPER SURFACE)

Figure 16

The relationship between the velocity of a single wave and the velocity of progression is compared in the normal and strychninized animal. This figure shows that strychnine sulphate (1:100) increases the slope of the line describing this relationship when the animal is creeping vertically upwards and on the under surface of a horizontal plane. It decreases the slope of this line when creeping takes place on the upper surface of a horizontal plane.

Plane	Animal #6	Slope of line
Vertical	o = Control	0.10
	● = Injected (strychnine)	0.68
Horizontal (under surface)	o = Control	0.28
	● = Injected (strychnine)	0.40
Horizontal (upper surface)	o = Control	1.22
	● = Injected (strychnine)	0.87

Figure 17

A comparison of the normal and injected animal (strychnine sulphate 1:100) shows that strychnine increases the slope of the line describing the relationship between the frequency of waves and the velocity of creeping when the animal is creeping vertically upwards, and also when creeping on the upper surface of a horizontal plane. A decrease occurs when the animal is creeping on the under surface of a horizontal plane.

Plane	Animal #6	Slope of line
Vertical	o = Control	0.68
	● = Injected (strychnine)	0.88
Horizontal (under surface)	o = Control	1.07
	● = Injected (strychnine)	0.66
Horizontal (upper surface)	o = Control	0.42
	● = Injected (strychnine)	0.74

FIGURE 17. VELOCITY OF PROGRESSION, V , MM/SEC

Figure 18

A comparison of the normal and injected animal (strychnine sulphate 1:100) shows that strychnine increases the slope of the line describing the relationships between the advance per wave and velocity of progression when the animal is creeping vertically upwards. A slight decrease is observed when creeping in a horizontal plane on the upper and lower surface.

Plane	Animal #6	Slope of line
Vertical	o = Control	0.12
	● = Injected (strychnine)	0.26
Horizontal (under surface)	o = Control	0.32
	● = Injected (strychnine)	0.30
Horizontal (upper surface)	o = Control	0.22
	● = Injected	0.14

FIGURE 18. VELOCITY OF PROGRESSION, V, MM/SEC.

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Father's name: John B. Brine, born in Cambridge, Mass.

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Biographical of the President

President: Cambridge, Massachusetts

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Mother's name: Mary E. Smith, born in Vermont, 1845.

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A. B. 1870, Harvard College

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