

The Effect of Temperature and the
Critical Thermal Increment
for
Several Species of Amebas

by


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INTRODUCTION

It is the purpose of this paper to show the effect of temperature changes on several species of amebas, to construct their temperature curves from the data obtained and to analyse the underlying protoplasmic activity by comparison with other biological and physiological processes. Since the quantitatively determined effect of temperature on the velocity of a biological process may serve to help identify the nature of the underlying reaction we shall consider (1) the velocity at constant temperatures, (2) the velocity at various temperatures observed on the same ameba, (3) the fall in rate above the optimum, (4) the effect of successive rises and falls in the temperature, (5) rhythmic activity, (6) general effect of temperature on movement, (7) viscosity. The temperature characteristics obtained will be compared with those of other biological and chemical processes.

The analysis here proposed deals with the effect of temperature on forward motion in several species of amebas. This is perhaps the most common act which the animal (ameba) performs, and is therefore at all times available for experiment. It is to be understood that this work in no way attempts to explain the mechanics of ameboid movement, but rather it involves the employment of temperature effects to detect the basic chemical reactions that supply the

energy required for the execution of ameboid movement. Although biologists and physiologists have believed for some time that biological processes are probably chemical in nature, it is only recently that definite progress has been made in analysing the nature of the underlying chemical reactions.

HISTORY

Ameboid movement was first discovered by Rosel V. Rosenhof in 1755. While this peculiar type of movement was interesting it was not considered important because of its seemingly isolated and limited occurrence. Although Dujardin in 1841 from his studies on the pseudopods of amebas and foraminifera made the remarkable discovery that protoplasm is the physical basis of life, he contributed little or nothing to the question of ameboid movement.

It was not until 1774 that Corti noticed a similar streaming of protoplasm in the cells of Chara, the difference being that no locomotion accompanied the streaming in the cells of Chara, but was always found to accompany streaming in amebas. This seemed to be the only stimulus necessary for the large amount of investigation that followed. From these investigations came the generally accepted view that "the really fundamental feature of ameboid movement is the streaming of the protoplasm". (Schaeffer '20)

During these investigations the physiologists soon noticed the ameboid character of the white blood cells of man, and later followed the discovery of ameboid movement among cells of other animals. So widespread has the observation of ameboid movement become that out of it has grown the theory "that even muscular movement is at bottom a specialized sort of ameboid movement, not merely phylogenetically but as it is now known". (Schaeffer '20)

Many theories have been proposed to explain ameboid movement. Most of these theories concern themselves, however, with the accessory phenomena, rather than with the central processes that lie at the bottom of ameboid movement (streaming).

Streaming always occurs in ameboid movement but some of the accessory phenomena such as surface tension and the formation of the ectoplasm that are said to lie at the bottom of ameboid movement do not always occur. We need rather than theories, a large amount of objective data to solve the question of ameboid movement.

In discussing the "third layer" or the surface tension layer in amebas (plasma membrane), Schaeffer (26) questions its importance as a necessary factor in ameboid movement since it does not move the same way in the pseudopods of *Diffugia* or of *Foraminifera* and is entirely lacking as a moving layer in ciliates and plant cells where streaming protoplasm occurs. He nevertheless recognized that its move-

ments indicate a greater surface tension at the ends of active pseudopods than over the rest of the ameba and suggests that "this fact if correctly interpreted, may prove to be a useful guide in further experimental analysis".

When amebas move, free from external stimuli, in a clear fluid, they move in a wavy or sinusoidal path, which represents a helical spiral path on a plane surface. This spiral movement is not confined to amebas but occurs in all animals including man and all motile plants. Larvae of many species of aquatic invertebrates and all motile spermatozoa move in a spiral path. The spiral path of the ameba, having its movement restricted to a space of two dimensions, is the projection of the well known helical spiral path of ciliates and flagellates. (Schaeffer '20)

Bullington ('25) in his study of 165 ciliates and one suctorian concluded that "All ciliates swim in a spiral path and that the spiral is characteristic for each species". The investigation showed no correlation between any visible morphological structure and the direction of turning.

Because so many plants and animals of such widely separated groups move in spirals, Schaeffer ('20) thinks that spiral movement is not an acquired habit to overcome assymetry of the body but believes the cause for its existence to be found phylogeny. He was the first to break away from the older idea that the reactions of animals were due to tropisms or chemical or physical stimuli exclusively. He

thinks instead that there is an "automatic regulating mechanism" present in every living organism, comparable to the brain centers of higher animals.

Rogers ('27) suggests that something may be learned as to the nature of a physiological process from a study of the effect of temperature on that process. He bases this, of course, on the well known law of Van't Hoff which states that the velocity of a chemical reaction is approximately doubled or trebled by a rise in temperature of 10° C. This law has been found to be valid for various kinds of biological processes by a number of investigators. Kantz studied the frequency of contraction in the isolated heart of rabbit and dog. Snyder investigated such phenomena as the rate of transmission of nerve impulses in the pike and frog, and the rhythmic contraction of mammalian small intestine. In all of these cases the velocity was found to be increased two or three times by a rise in temperature of 10° C.

Strumpter reports that ants move twice as fast at 21° C as they do at 11° C. McCutcheon from his studies on the locomotion of leucocytes of *Limulus* found that the velocity of the locomotion shown by neutrophilic polymorphonuclear leucocytes of one individual is doubled by a rise in temperature of 10° C within certain limits of temperature variation.

In the above cases the Van't Hoff law seems to hold and we are justified in thinking with Rogers that "If any

physiological process shows essentially the same modification in rate by changes of temperature as do ordinary chemical reactions the belief would seem to be justifiable that some chemical reaction lies at the basis of the physiological process".

Pantin, (124) in his work on the "Physiology of Amoeboid Movement" showed that the rate of change of state (solZgel) in the protoplasm of amebas is directly related to the velocity. From this it is argued that the velocity of continuous amoeboid movement does not depend directly upon the velocity of some chemical reaction supplying the necessary energy, but on the rate at which the protoplasm can change its state (solZgel).

Pantin thinks that the temperature affects the rate of this change of state as it does the rate of most other biological processes. This does not preclude the possibility that the source of the energy of amoeboid movement is ultimately a chemical reaction and in this respect Pantin and Rogers are in direct agreement.

In view of Crozier's recent work which suggested that the quantitatively determined effect of temperature on the velocity of a biological process may aid in identifying the nature of the underlying reaction, it was deemed of interest also to determine the temperature characteristic of several species of amebas, and to compare their temperature characteristics with those of other biological and chemical pro-

cesses. The chief difficulty in working with amebas lies in handling them, viz., isolating single amebas for study, feeding at proper intervals during the course of the experiment, keeping the glassware, pipettes, lenses etc., free from chemicals, whose dissolution would kill the ameba, selection of food, all requires not only extreme patience but also a special technique that can be developed only after months of practice. There are two methods that have been used extensively to identify the nature of biological processes.

(1). Temperature coefficients of vital processes has attracted a good deal of attention and is centered for the most part around the Van't Hoff rule, which states that the velocity of a chemical reaction is approximately doubled or trebled by a rise in temperature of 10°C . Since biological processes follow the Van't Hoff rule it is assumed that there is at bottom some chemical reaction.

While biological processes are no doubt controlled by an underlying chemical reaction, Crozier ('24) has shown, that the temperature coefficient, Q_{10} , giving the ratio of velocities for an interval of 10° , is an imperfect means of characterizing a process, and little can be learned when the temperature coefficient is used as an index except to separate the chemical from the physical phenomena, as the former were found to have lower or negative temperature coefficients.

(2). Following Arrhenius (1889) who first pointed out by use of his equation that a linear relationship exists between the logarithm of the velocity of a chemical reaction and the reciprocal of the absolute temperature, biologists have been able to show that the velocities of numerous physiological processes are similarly affected by temperature, a fact which gives presumptive evidence of the chemical nature of such phenomena.

Crozier was the first to point out how the equation of Arrhenius could be used for biological processes, and recently has collected from the literature the data on temperature effects that were capable of mathematical treatment.

The Arrhenius equation in the form used by Crozier, and since by Glazier and others states:

$$\frac{K_2}{K_1} = e^{\frac{\mu}{R} \left(\frac{1}{T_1} - \frac{1}{T_2} \right)}$$

In this equation the ratio between the velocity constants K_1 and K_2 of a chemical reaction taking place first at one, then at another temperature is related logarithmically on the naperian base e to the gas constant, the absolute temperature and to a constant (μ), μ , which characterizes a particular reaction.

The temperature characteristic (μ) can easily be determined by transformation and changing the formula from the Naperian to the Briggsian base. In this form the equation reads:

$$\mu = 4.61 \frac{\log K_2 - \log K_1}{\frac{1}{T_1 \text{ absol}} - \frac{1}{T_2 \text{ absol}}}$$

in which K_1 is the velocity of the process under observation at the temperature T_1 and K_2 is the velocity of the same process at temperature T_2 and $\frac{\mu}{2}$ is the critical thermal increment μ , divided by the gas constant 2.

In this form the Arrhenius formula has come into wide usage for determining the character of biological processes, and because of distinct advantage is used instead of the Q_{10} employed by the chemist ($T_2 = T_1 - 10$, then $\frac{K_2}{K_1} = Q_{10}$). As pointed out by Crozier, the value of μ gives $\frac{K_2}{K_1}$ a greater range of values as empirically determined, for whereas Q_{10} for chemical reactions usually varies between 2 and 4, μ has a range between 4000 and 35,000.

Again in some cases at certain points in the temperature scale an abrupt change in the value of μ will occur, indicating that the process is controlled by a new limiting reaction. This change would not be apparent if the usual method of determining Q_{10} is used. The value of Q_{10} is not a constant but varies with the temperature, whereas μ remains a constant as long as the underlying chemical reaction is the same regardless of the temperature.

The chief reasons for obtaining the temperature characteristics (value of μ) for biological processes are:

- (1). To discover the chief underlying reactions of

the biological processes by a comparison with temperature characteristics of simple, known chemical reactions.

(2). A preliminary survey has shown that vital phenomena are to a large degree empirically separable into groups according to the associated value of μ . While the temperature characteristics have been determined for a great many biological processes, similar work on amebas is very fragmentary. (Cole, W. H. 1925; Crozier, W. J. 1925-26; Emmerson, R. *Jo. Gen. Physiol.*, Vol 13, p. 157; Schwitalla, A. M., 1926, *Jo. Morph.*, Vol. 39).

In a study of locomotion in planaria (Cole, W. H., 1926) one finds a process with the lowest increment ($\mu = 7,000$ to $8,000$) assuming control of the locomotor rate at a temperature above 20° - 22° C and that the highest increment ($\mu = 18,000$ to $22,000$) controls below 13° C and one with an intermediate value ($\mu = 11,100$) is in command at the intermediate temperature 13° - 21° C.

Crozier ('24) reports the critical thermal increments for respiratory processes (O_2 consumption, CO_2 production) in various plants and animals. They are characteristically found to be of two, possibly three, types. $\mu = 11,500$ and $16,100$ or $16,700$). The first is commonly encountered above 15° C and the second below that temperature, but these relations may be reversed.

The value $\mu = 16,100$ is associated with the oxidation

of Fe and may be compared with that of respiration in sea-urchin eggs for which iron is a catalyst (Warberg, 1914, Warberg and Myerhof, 1913). Also Crozier ('24) from the data of Hartridge and Roughton ('23) calculated $\mu = 16,525$ for the deoxygenation of hemoglobin. Similar values for μ were not found for oxidative reactions in which iron is not involved.

Clark (1920-21) gives $\mu = 16,000$ for frequency of contraction in the isolated auricle of the rabbit heart.

Taglos and Alvarez ('17) give $\mu = 16,150$ for the frequency of rhythmic movements in strips of rabbit intestine, dependent on O_2 between 30° and $40^\circ C$; from $20^\circ C$ to 30° , $\mu = 8000$. Grey (1923-24) shows that ciliary activity and O_2 consumption in the gill epithelium of *Mytilus* are in constant proportion. Crozier (1924-25) gives $\mu = 16,700$ for 1° to $15^\circ C$., $\mu = 11,500$ for 15° to $35.5^\circ C$. More (1910) gives $\mu = 16,800$ from 14° to $23^\circ C$ for rate of regeneration of polyps in *Tubularia*. Loeb (1891) showed O_2 was necessary for the regeneration.

Snyder (1911) reports that determination of the velocity of the latent period process in strips of turtle ventricle yields $\mu = 16,170$ from 14° to $30^\circ C$ and $\mu = 24,750$ from 0° to $14^\circ C$.

Stewart (1900) gives $\mu = 17,000$ for the velocity of the latent period process in the contraction of strips of

cat bladder.

Hecht (1917-18) gives $\mu = 16,400$ for velocity of conduction of the heart waves in *Ascidia atra*.

Koike (1910) shows $\mu = 16,500$ for the velocity of the process underlying first order periods in the discharge of the electric organ of *Malapterurus*.

Gasser and Erlanger (1922) give $\mu = 16,180$ for the velocity with which the action currents in frog spinal nerves rise to a maximum.

The activities of the nerve net in the body of the colonial coelenterate *Renilla* (Parker 1920) and in the foot of the gastropod *Limax* (Crozier and Piltz, 1923-24) yield $\mu = 11,200$ and $16,100$ (*Renilla*) and $\mu = 10,700$ (*Limax*).

The velocity of the process underlying the first oscillation in the electromyogram of frog gastrocnemius (Judin, 1923) gives $\mu = 11,000$ from 2.4° to 15°C and $\mu = 16,000$ from 15° to 20°C .

The frequencies of respiratory movements in the aquatic *Libellula* larva (Babak and Rocek, 1909) give $\mu = 11,000$ and $16,460$.

The velocity of elongation of the radicle of *Pisum* yields $\mu = 16,450$. (Leitch, 1919).

Arrhenius, in commenting on the similarity of the value of μ for diverse processes does not attempt an explanation (1907-1912) but Crozier (1926) points out that the value

for μ in organic activities is similar to, if not identical with, those given for the oxidation phenomena and goes further to say that "since it is possible to show in certain instances that an activity in question is a function of oxygen tension, or in other cases may safely be presumed to involve cell respiration, the agreement of the values of μ is scarcely to be considered as accidental".

W. H. Cole (1925) determined the rate of pulsation of the anterior contractile vacuole of *Paramecium caudatum* over temperature range of 9° to 31° C. In this case as in others the rate of pulsation was found to be a logarithmic function of the temperature according to the Arrhenius equation. From 9° to 16° C, $\mu = 25,600$; from 16° to 22° C, $\mu = 18,000$; from 22° to 31° C, $\mu = 8,600$. The work of Cole agrees with Crozier's assumption in that an oxidative process may be of fundamental importance in determining the rate of pulsation.

MATERIAL

Six species of amebas were used in this investigation.^{1 & 2}

¹ For description of three (*conipes*, *citata*, *clava*) see Schaeffer, Pub., Carnegie Inst., Wash., March, 1926.

² *Discoides*, *bigenma*. (Schaeffer, 1916d and 1918b)

In some instances reliable culture methods were known which¹ made it possible to get pure line strains of amebas.

Mayorella conipes and *Flabellula citata* were grown in Petri dishes with sea water and small bits of wheat grains. In this way rich cultures were obtained in about two to three weeks, and by employing isolation methods, it was possible to get pure line strains. *Mayorella bigemma* were grown in large culture dishes with cotton, wheat grains and distilled water.

Flabellula citata was grown in Petri dishes in sea water containing the sea weed *Fucus*. This was first boiled to kill off any other ameba, then inoculated with *Flabellula citata*. It grew in large numbers by this method. It was later determined that *Flabellula citata* could be successfully grown by inoculating sterile sea water with two species of bacteria isolated from sea water.

Collections of water samples were made over a wide area from ponds, marshes, watering tanks, etc., until the desired species of amebas were found. Water from this source containing amebas along with some decaying vegetable matter was taken to the laboratory and placed in large culture dishes. In cultures of this sort the amebas continued to grow in

¹ *M. conipes*, *M. bigemma*, *C. diffluens*, *F. citata*.

the laboratory in some cases for several months. The marine amebas (*Mayorella conipes* and *Flabellula citata*) were secured from the east coast in sea water, shipped to the University in 15-gallon containers. Some of the sea-weed *Fucus* was contained in the sea water where the amebas could be found in great abundance. Cultures were made from this.

The culture method is also known for *Chaos diffluens* (Schaeffer) but this ameba did not lend itself at all well to this type of experimentation because of its irregular shape. It was impossible to get an accurate measure of its path and also, when a change of 5°C. was made in the temperature, (especially in the higher ranges, 25⁰ -35⁰C) the ameba released its hold on the substratum and floated off. Since this work is based primarily on uniform movement, sufficient data could not be collected on this form to warrant including it here.

DESCRIPTION OF AMEBAS

The amebas used in this study were: 1. *Flabellula citata*; 2. *Mayorella conipes*; 3. *Mayorella bigemma*; 4. *Trichamoeba clava*; 5. *Metachaos discoides*; 6. *Trichamoeba verrucosa*.

Following is the classification and description of the above organisms, given after Schaeffer.

Regnum Animalia

Phylum Protozoa Goldfuss, 1817

Class Rhizopoda Siebold, 1845

Order Lobosa Carpenter, 1861

Suborder Amoebae Ehrenberg, 1830

Family Mayorellidae Schaeffer, 1926

Genus Mayorella Schaeffer

Species conipes

Description: Ameba, length in locomotion 80 , shape variable, triangular with the base anterior; oblong or rectangular. Blunt and conical determinate pseudopods, mostly on the anterior part. Endoplasm clear along anterior border. A few extremely small, barely optically-active crystals or none at all. Nucleus single ovoidal to spherical with nuclear membrane about 10 - 20 in diameter, and an ovoidal to spherical chromatin mass, 4 to 2 in diameter, varying with the size of the nuclear membrane. Habitat, salt water.

Genus Mayorella Schaeffer

Species bigemma

Description: Ameba in locomotion 100-300 . Forms very changeable; pseudopods, numerous, tapering, blunt, never sharp points. Surface smooth, no fine folds or ridges.

Endoplasm usually contains numerous small twin crystals. Crystals attached to "excretion spheres. Movement rapid, rate 125 per minute. Nucleus, single, spherical or slightly ovoid. 12 in diameter. Contractile vacuoles, small, about 18 in diameter. Endoplasm filled with small vacuoles. Habitat, fresh water.

Genus *Mayorella* Schaeffer

Species *Flabellula citata*

Description: Length in locomotion, 15 to 75 ; width, 25 to 55 . Shape very variable, from a broad fan-shape to a spatula-shape, with the broad end advancing. Pseudopods numerous, of many shapes and sizes, determinate, not directing locomotion. No eruptive waves of endoplasm during locomotion. Anterior third of clear protoplasm, with an occasional ridge. Endoplasm in posterior region granular, and set off definitely from the clear endoplasm by a sharp line. Nucleus single, not conspicuous. Chromatin mass spherical, homogeneous, surrounded by a nuclear membrane. Diameter of chromatin mass, 2 ; of membrane 5 . Uroid of few to many elements always present. Food, bacterial. Food vacuoles, from 1 50 12 or more, frequently present. Habitat, salt water

Family Chaidae

Genus Trichamoeba Schaeffer

Species clava

Description: Size, 75 to 125 long in locomotion. Usually clavate in shape, occasionally with a few short pseudopods. Very fluid endoplasm and thin ectoplasm. Uroid of fine hair-like projections. Color a light ash-gray tinged with blue. Nucleus single, spherical, about 14 in diameter. Chromatin mass in a transparent homogeneous, hollow sphere with a variable number of large granules on it. Contractile vacuoles numerous, maximum size, 10 to 15 diameter. Systole sudden. Rate of movement 150 per minute.

Genus Metachaos (Schaeffer, 1916 arch. f

Protistenk, Vl. 37 pp204-28)

Species discoides

Description: Large amebas, 120 - 15 that move by determinate pseudopods, that is, pseudopods that direct locomotion. The general shape of the body is oblong resembling an antler or stag horn. The mass of the body is divided up into blunt pseudopods which are cylindrical or sub cylindrical in shape, without longitudinal folds. The granular endoplasm fills the entire ameba or all but the extreme tips of the advancing pseudopods, and the granules are very evenly distributed, indicating a unified streaming of the endoplasm which involves the whole of the endopla

plasmic contents. A main pseudopod which leads in locomotion is distinguished at all time. Habitat fresh water.

Family Thecamoebidae

Genus Thecamoeba

Species verrucosa

Description: This species is easily distinguishable from those of other groups and have frequently been referred to as the "ameba with a pellicle", because in locomotion they present the appearance of being invested with a tough outer layer which may readily throw itself into folds or ridges. Their shape during locomotion varies from a circular disc to a flattened ovoid and, when of the latter shape the broader end advances as the anterior end. The dorsal surface has 1-6 longitudinal ridges which continually form at the anterior end, and disappear at the posterior end. No pseudopods are formed. The anterior portion of the ameba is clear protoplasm while the posterior half to three-quarters consists of granular protoplasm.

METHOD

1. APPARATUS

In order to measure the rate of locomotion of amebas under known conditions of temperature, a method for keeping the temperature constant had to be devised. Methods used by other investigators who have worked on constant temperatures were tried out but were found not to be suitable to this par-

ticular problem without radical modification.

The Pflieffer warming stage was first tried. (Figure 1.)

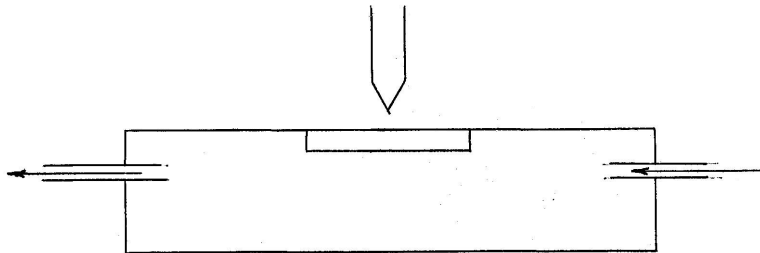


Fig I

This consists of a shallow depression cell which holds the ameba and the cultural fluid with water at the desired temperature circulating beneath it. This method was soon discarded because the depression cell was shallow and it was impossible to observe the ameba for long periods of time without evaporation of the cultural fluid. This was especially injurious when working with sea water as a medium because it became too concentrated and killed the amebas. One never knew exactly the temperature of the medium in the depression cell but had to assume it was the same as the circulating water which may or may not have been correct. In making long observations on the ameba the depression cell was found to be too small. The ameba reach the edge of the cell before the experiment is completed.

Schwitala ('20) however, used the Pflieffer stage with success by measuring the temperature with an electro-thermometer described by Hill ('21) and Rodgers and Lewis ('14).

This method is complicated and since the required apparatus was not at hand another method was used.

This apparatus consisted of two chambers, made of glass and held together with Dekhotinski cement. (Figure 2.)

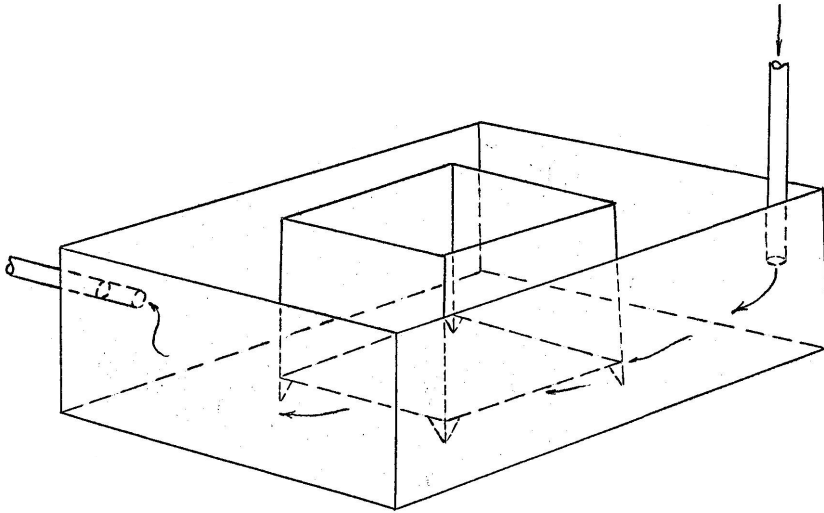


Fig 2

One chamber was designed to fit inside the other and elevated about twelve millimeters. The outer chamber was equipped on the left side with an outlet pipe made of glass. The inlet pipe was on the opposite side (right) leading from a ten-gallon jar. The inflow of water was controlled by a screw clamp. By releasing the screw clamp the water flowed around and under the deep inner chamber which contained the ameba and cultural fluid.

2. MEASURE OF TEMPERATURE

Water was kept constantly running thru the apparatus from the large jar. When temperature above room temperature was desired the water was heated by an electric heater submerged in the large jar and operated by a switch which could easily be turned on or off at the desired temperature. To keep all the water in the large jar at a uniform temperature, an electric stirrer was employed.

This method had several advantages over the others tried. Evaporation of the cultural fluid was no longer a problem. The temperature of the culture fluid was measured directly by a standardized thermometer placed directly in the culture fluid. By magnifying the marks on the thermometer a very slight rise or fall in temperature could be noted, the heat applied for an instant, and in this manner the temperature could be controlled accurately to one tenth degree centigrade.

For temperature below that of room temperature water from melting ice was used. This was gradually warmed as needed. In this manner a range of temperature from 2° C to 60° C or higher was accurately obtained. Lower temperatures were obtained by the use of brine.

3. COLLECTING DATA.

In observing the amebas a compound microscope, with a 10X ocular and a 16 mm. objective was used.

With the aid of a camera lucida drawings were made of

the ameba under observation. The usual method of procedure was first to select from a culture the ameba for observation. In every case an individual was selected that did not exhibit any apparent "abnormal" characters and one that moved at a more or less uniform rate. An attempt was made in each case to select amebas that were fairly uniform in size.

It was found by experiment that more uniform results were obtained when the amebas were placed in their own culture medium for study rather than in a synthesized medium of known chemical composition. In every case, when artificial media of known chemical composition were used the ameba exhibited various abnormal reactions such as forming an excessive number of pseudopods, rolling up in a ball, floating, or they moved in definite circles instead of the characteristic wavy path.

The culture media were either dialyzed or filtered clear, to remove any particles that might interfere with movement of the ameba.

After each change in temperature, a period of about fifteen minutes was always allowed the ameba for acclimatization. Time was allowed for the ameba to settle to the bottom of the dish and again resume normal locomotion (fifteen minutes was found to be sufficient time in most cases).

As soon as the desired temperature became constant, camera lucida drawings were made of the actual path taken

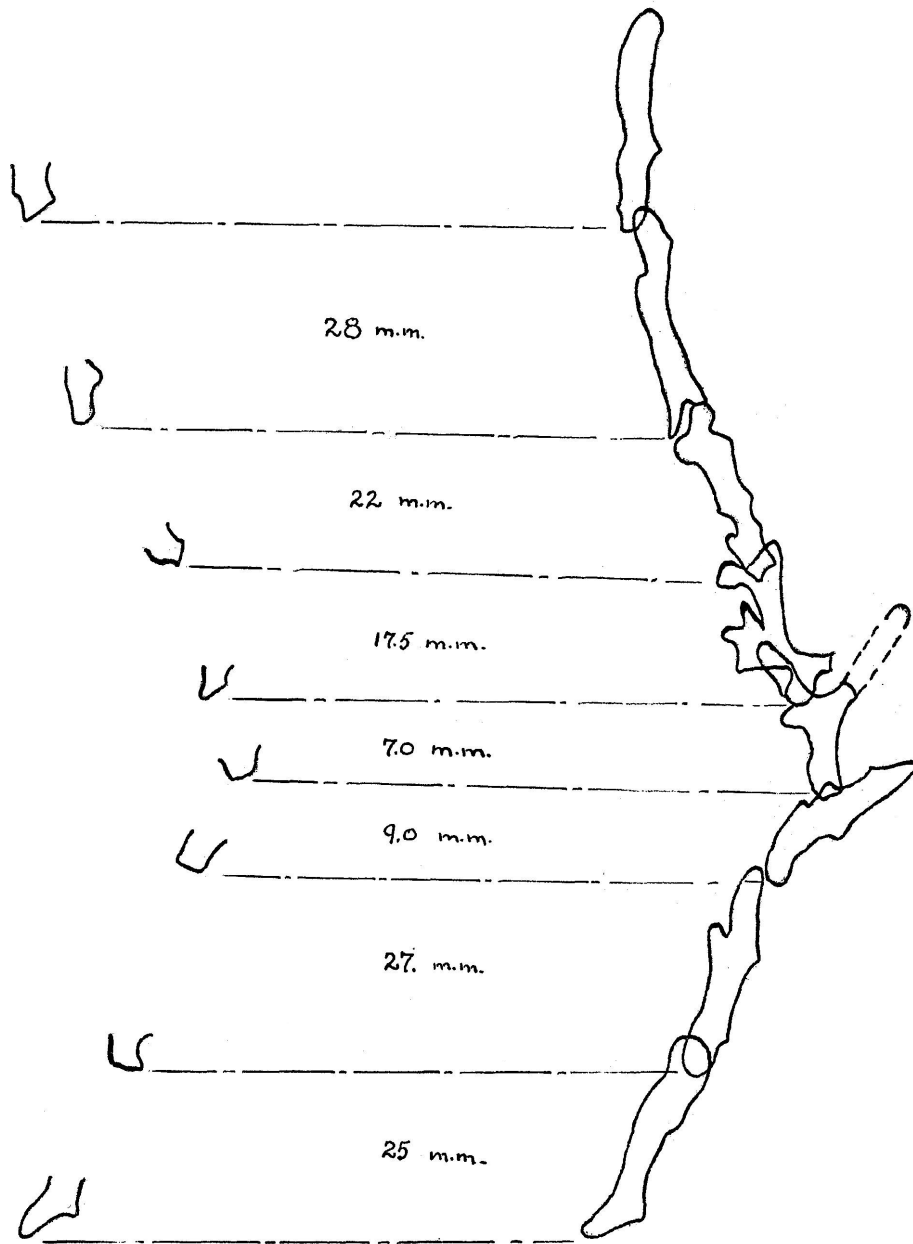
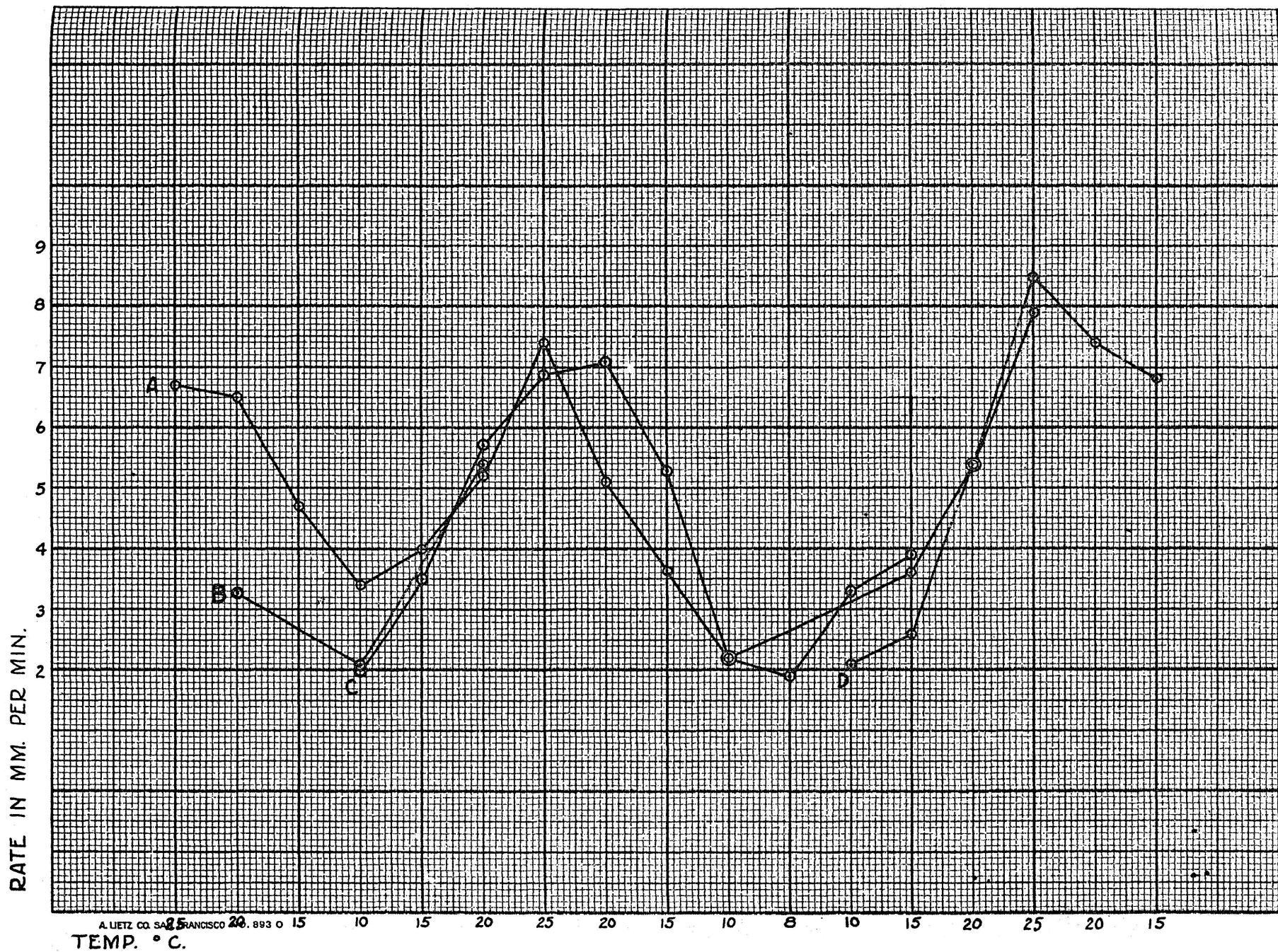


Fig 3



A. LIETZ CO. SAN FRANCISCO 20. 893 O 15 10 15 20 25 20 15 10 8 10 15 20 25 20 15

Fig-4

by the ameba. The drawings were of the whole outline of the ameba rather than of only the posterior end. (Figure 3.) A definite time interval was used between the successive drawings. The length of time that any given ameba was observed and its path drawn for any given temperature varied usually from 30 to 60 minutes. This was found to be sufficient because longer observations of from three to five hours at a constant temperature showed no more variation than did the shorter ones.

After an ameba was "tracked" at a constant temperature for a given time interval the temperature was raised usually two to five degrees centigrade. Again a period of acclimatization was allowed because it was found by experiment (Figure 4.) that the ameba did not respond immediately to the change of temperature and there was a distinct lag. This was overcome in about fifteen minutes at the new temperature.

The amebas were observed in this manner at temperatures of 5, 10, 15, 20, 25, 30, 35, 40, 45 degrees centigrade, and in some cases at temperatures intermediate between these. By measuring the length of the path in mm. of any one individual and dividing this by the time in minutes taken to make the path, the rate of the ameba expressed in mm. per minutes was obtained. When plotted on a graph as shown in Figure 5 it will be noticed that the rate in-

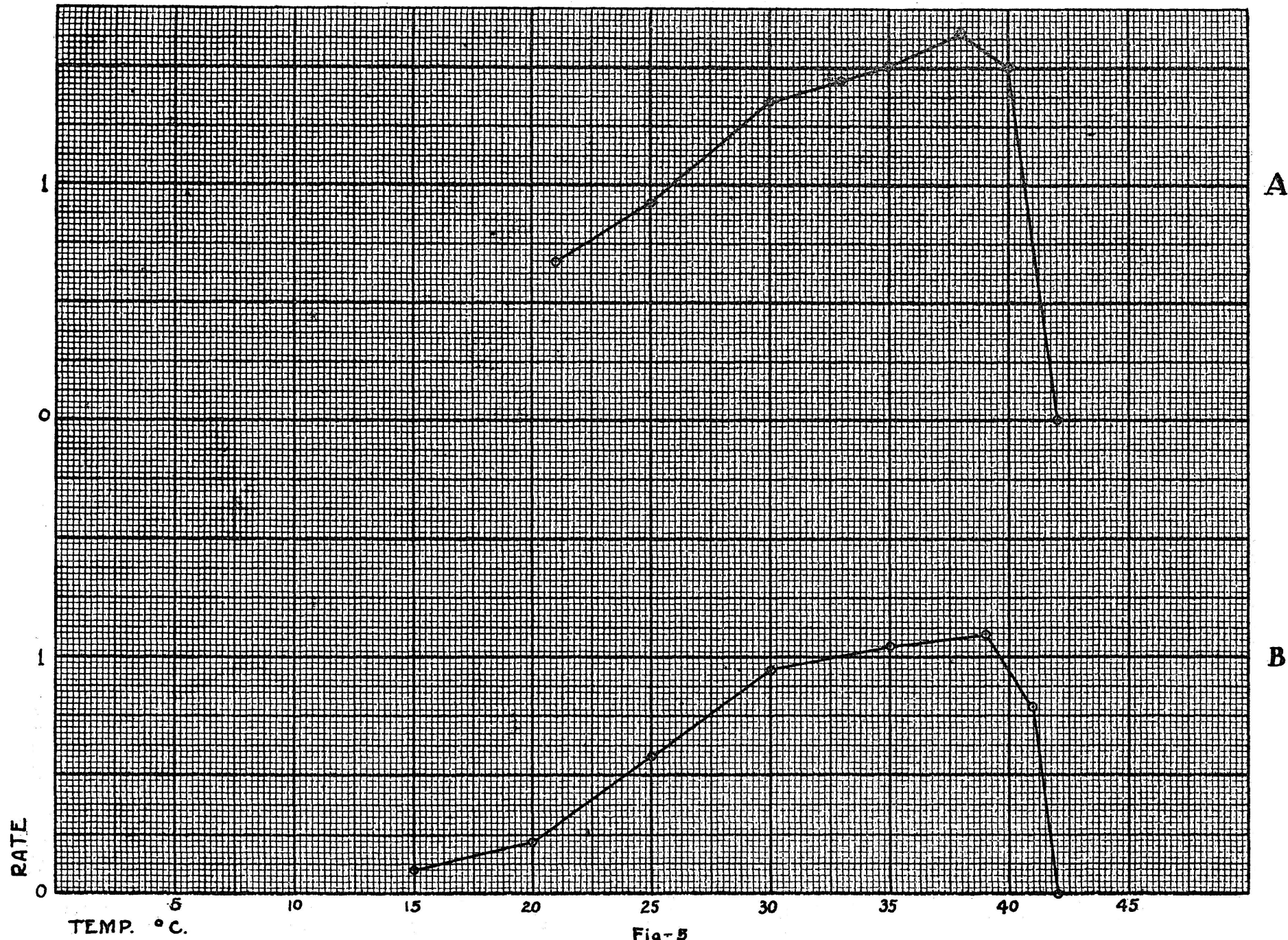


Fig-5

creases with the temperature up to a certain point, then a further increase in temperature is not accompanied by a further increase in rate. Graphs were now made for each ameba, plotting the rate against the temperature in each case.¹ This method was used so that this work would be comparable to similar work on other temperature characteristic curves, where relative changes in rate rather than actual are used.

This shows that the velocity of the biological process under consideration, ameboid movement, varies with the temperature in a manner similar to many other biological processes. Pantin ('24) suggests that at the higher temperatures the check in velocity is probably due to the destruction of a protein. This curve, however, is very similar to an enzyme action curve and in all probability the change in character of the curve is not so much the destruction of a protein as it is a change in the underlying controlling chemical reaction which is probably enzymatic.

Up to this point, however, the rate of movement apparently is controlled by the temperature, and with a rise in tem-

¹ The distances that the ameba moved as expressed on the graphs in millimeters is the apparent distance as it appeared under my optical combination. In order to obtain the actual distance the ameba moved in any case, it is necessary only to multiply by 5.45 (1 mm. on graph = 5.45).

perature of ten degrees centigrade below this point the rate is approximately doubled. This, according to the Van't Hoff law would suggest an underlying chemical reaction.

By applying the Arrhenius formula to these data the probable nature of the controlling reaction can be determined. These graphs bring out some other very interesting processes in connection with ameboid movement that will be taken up in detail. (Page 1, introduction)

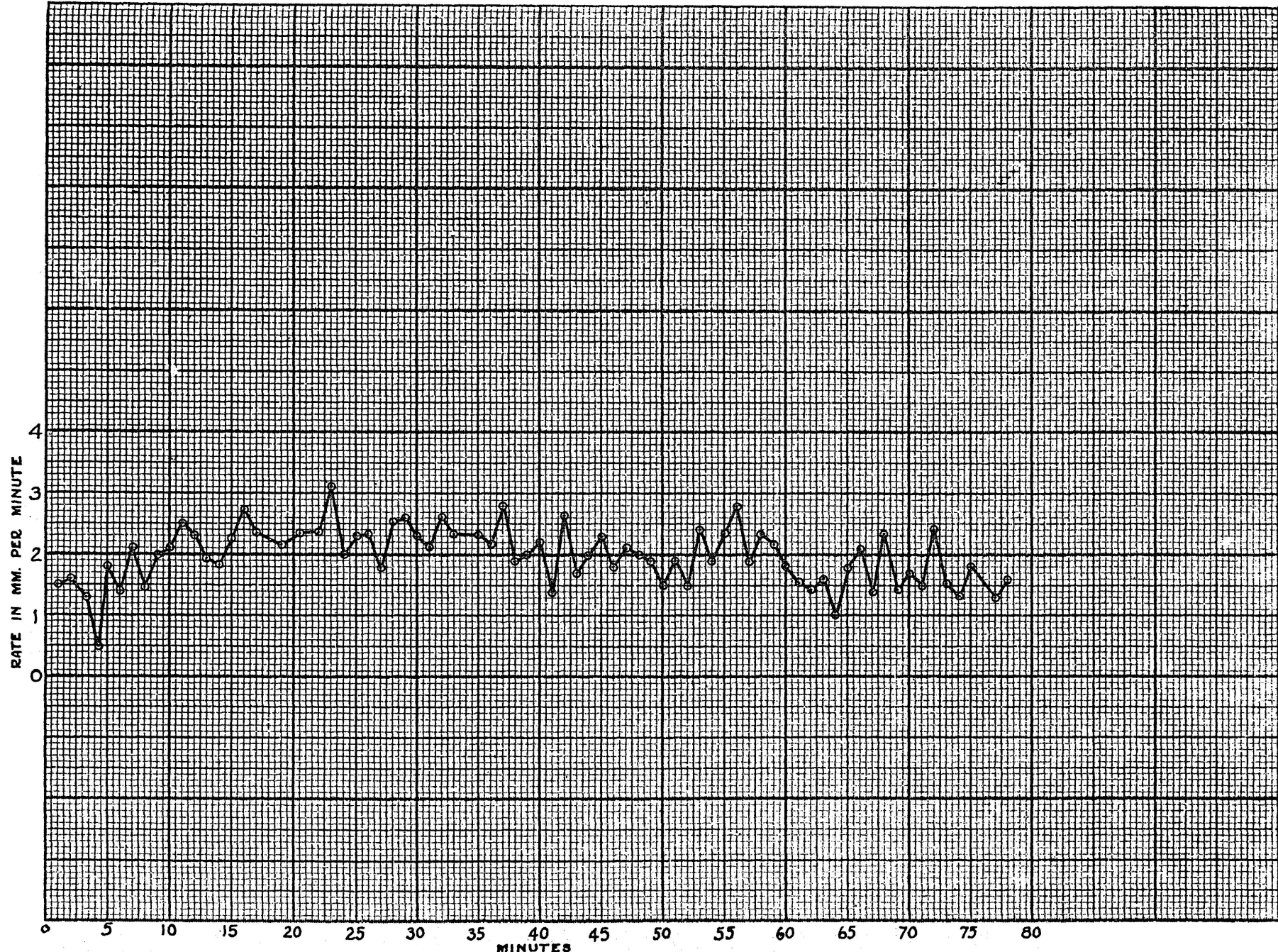
RESULTS

It has been demonstrated by Pantin ('24) working with two limax forms that the velocity varies with the temperature in a manner similar to many other biological processes. Similar results have been demonstrated by Schaeffer ('26) working with *Cochliopodium*. Pantin points out that the velocity is greatest at an optimum temperature above which something is destroyed. That is when the temperature is increased the velocity is increased up to a certain point. Beyond this point (optimum) an increase in temperature tends to destroy something, probably a protein (Pantin), in the ameba and checks its velocity.

Since the limax forms used by Pantin ('24) and the *Cochliopodium* used by Schaeffer belong to widely separated families, but behave so nearly alike, it became desirable as well as interesting, to know what another family of amebas would do under similar conditions.

The family of amebas chosen for the investigation was the Mayorellidae, a group that stands about midway between the limax type (*Chaidae*) and the *Cochliopodium* (*Hyalodiscidae*). The data obtained from *Mayorella conipes* correspond closely to those obtained by Pantin and Schaeffer.

1. REACTION OF MAYORELLA CONIPES AT CONSTANT TEMPERATURES.



INDIV. - 5 AT 14°C.

Fig. 6-

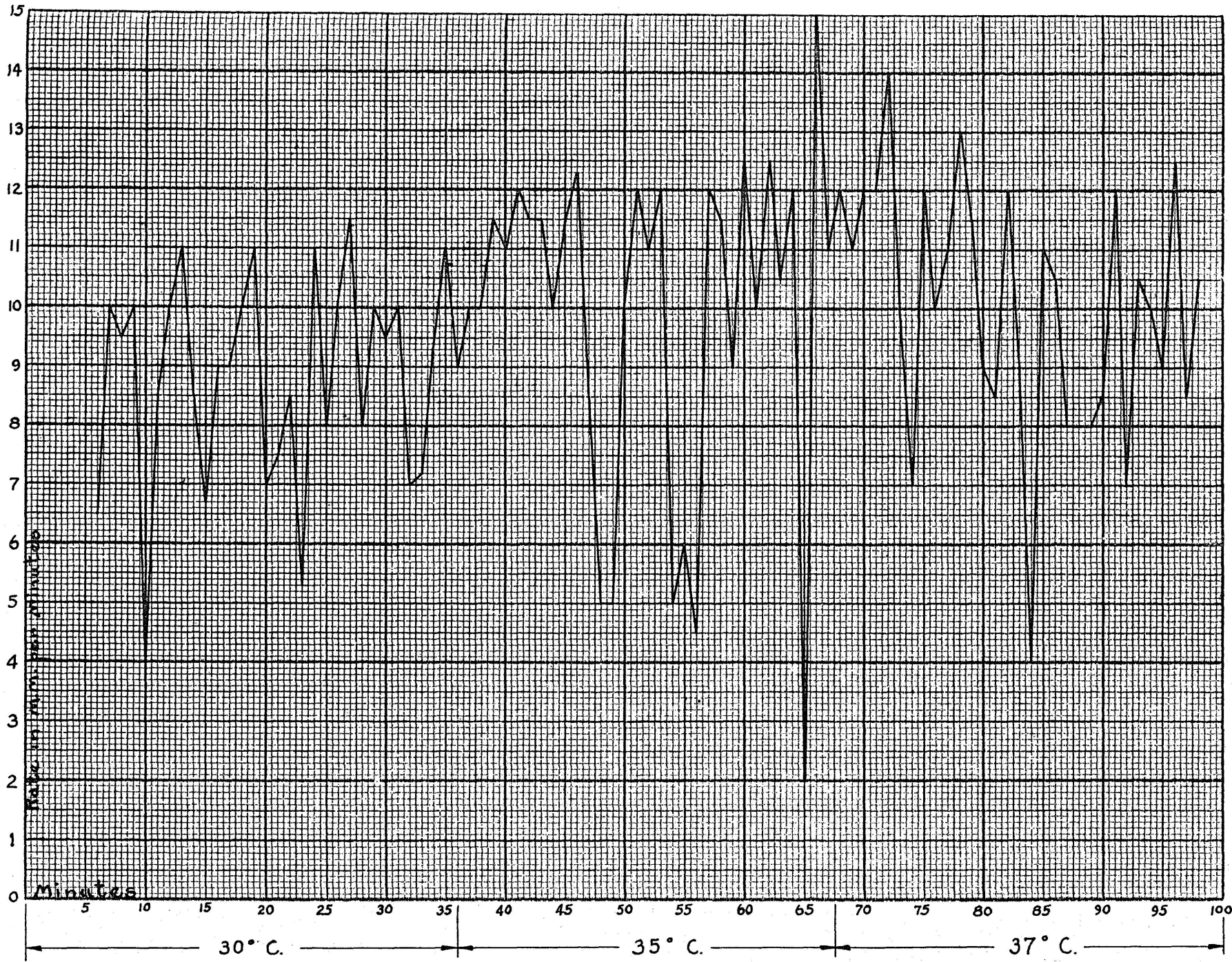


Fig-7-

a. At Low Temperatures.

The rate at which *Mayorella conipes* moves when it is kept at a constant temperature varies considerably depending on the temperature. At low temperatures (14°) the up and down range of the temperature speed curve was found to be from .5 mm. per minute to 3.15 mm. per minute at the extremes. The greatest fluctuation within one minute being at the rate of .5 mm. per minute to 1.75 mm. per minute. Most of the fluctuations, however, were much less. Figure 6 shows the complete graph for individual number 5 observed over a period of 77 minutes at 14°C .

b. At High Temperatures.

At high temperatures (35°C) the up and down range in the curve was found to be much greater. Frequently, in one minute the rate increased from 4.5 mm. per minute to 12 mm. per minute in one minute. In one instance the rate increased from 2.5 mm. per minute to 15 mm. per minute in one minute.

Figure 7 shows the curve for individual number 10 at 30°C , and 37°C , respectively. By comparing this graph with the one shown in Figure 6, the difference in the fluctuations at the high and low temperatures can be seen at once.

This small fluctuation at the low temperatures as compared with the great amount of fluctuation at the high temperatures is an interesting feature of amoeboid movement, chiefly, because it has not before been described. While

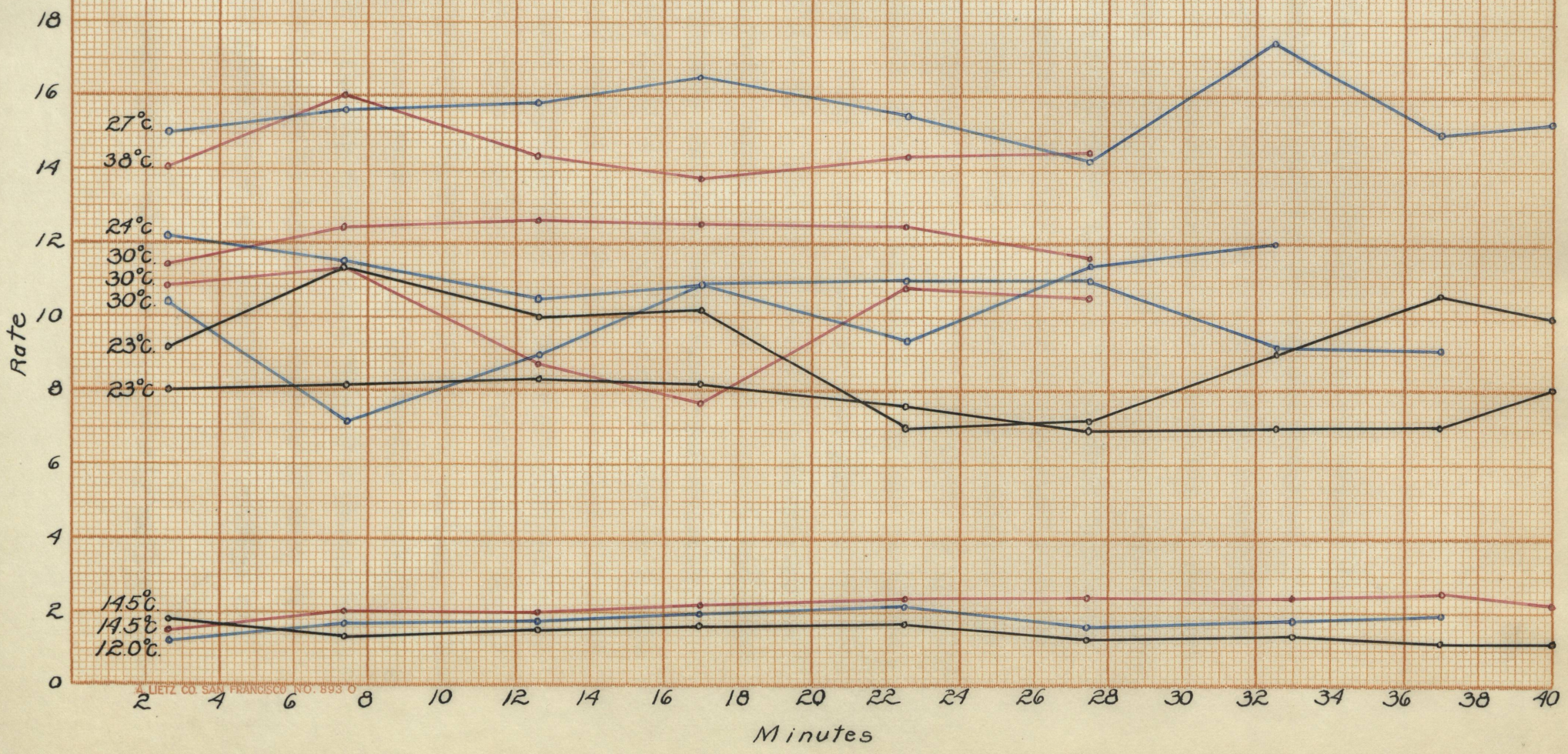


Fig.-8-

these observations are undoubtedly right for amebas they are not necessarily distinctive of ameboid movement. It was noticed, however, that there was a direct correlation between degree of fluctuations and the viscosity of the protoplasm; it being much more fluid at the higher temperatures, and thus more susceptible to changes; viz., from sol to gel according to Pantin ('23-'24) to chemical changes according to Rodgers ('27), Crozier ('25-26-27), and others. It undoubtedly has a direct bearing on the mechanics of ameboid movement, and as yet no theory so far brought out is capable of explaining it. This is just another case showing the futility of establishing a theory of ameboid movement before we know the important facts concerned. Any theory attempting to explain ameboid movement must take into account (1) that an ameba does not move at a uniform rate at a constant temperature and (2) that at high temperature the rate of movement is less uniform than at low temperature.

Figure 8 is offered as proff of this statement, being based on the temperature-speed curve of eleven amebas, picked more or less at random from the data collected. These graphs are exactly like those in Figure 6 and Figure 7 except that they have been condensed by taking an average of each five points on the original graph and plotting that average as one point.

The three lower graphs show the more nearly uniform rate

at low temperatures as compared with the very irregular rate at high temperatures near the optimum.

The rate of movement of *Mayorella conipes*, at constant temperatures, differs considerably from that of the limax forms used by Pantin. He found that when they (limax) were kept at a constant temperature, they maintained a constant velocity within one percent to five percent. This, if plotted would make almost a straight curve. In the limax experiments a ghost micrometer was used to measure the velocity by timing the ameba as it moved over a given number of divisions, with a stop watch. This method was not used in working with conipes because it was found that more accurate measurements could be obtained from a continuous camera lucida path over a long period of time. Had Pantin's method been used the fluctuations in the rate of movement in the ameba would probably not have been detected.

In measuring the velocity of an ameba with a ghost micrometer over a short period of time it is possible to get all "ups" (high velocities) or all "downs" (low velocities) or mostly one or the other, unless a large amount of data is collected (See Figures 6 and 7). It is of course self-evident that camera lucida drawings every minute or every two minutes, over an hour or more give a more accurate account of what the ameba is doing, than would observations only at ten-minute intervals.

2. VELOCITY AT VARIOUS TEMPERATURES OBSERVED ON THE SAME AMEBA.

After having determined the reaction of the ameba at constant temperatures it became of interest to know what the same ameba would do at different temperatures. This necessitated a slightly different method. An ameba was selected and placed in the inner chamber. The usual starting point was 15°C , or 20°C . A track of the ameba was made with the aid of a camera lucida, for a period of thirty minutes. At the end of this time the temperature was raised 5°C and a period of fifteen minutes was allowed to elapse so the ameba could be acclimated to the new temperature. This process was repeated, each time raising the temperature 5°C above the preceding one and allowing time for acclimitization, until such a high temperature was reached that all movement in the ameba stopped.

A graph was made according to the method previously described. This graph can be seen in Figure 5. Figure 9 shows the original of one of the graphs after it was reduced to compare with work done on other forms.

3. SUB-OPTIMAL TEMPERATURES ($15^{\circ}\text{C} - 38^{\circ}\text{C}$), ($15^{\circ}\text{C} - 39^{\circ}\text{C}$)

At temperatures below the optimum the ameba moves in a regular, sinoidal path, with a more or less uniform increase in rate, as the graph will show, (Figure 9) until a temperature of about 30°C is reached. Above this temperature

TEMPERATURE - RATE OF MOVEMENT RELATION
OF 4 SPECIES OF AMEBAS

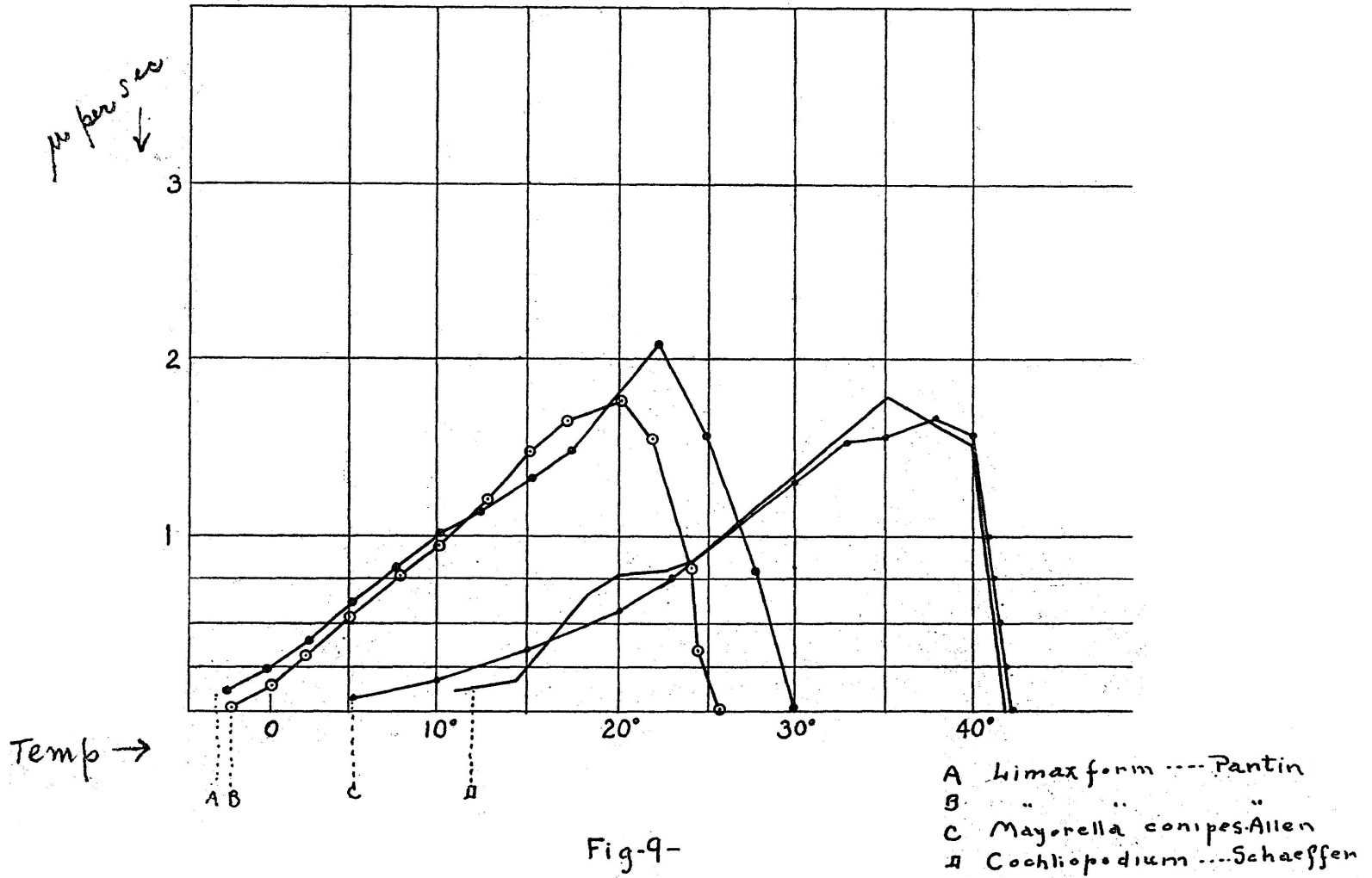


Fig-9-

the rate of increase of velocity falls rapidly until the velocity reaches its optimum at 38°C . It is interesting to compare this optimum with the extremely low optimum of the limax forms, which Pantin found to be 20°C for type B, at 22°C and 25°C for type A. After the optimum had been reached in type B, at 20°C , the velocity fell rapidly to zero at 26°C , but death did not occur until 30°C had been reached. It is extremely interesting to note that *Mayorella conipes* was found to have an optimum temperature actually above the death point of the limax forms.

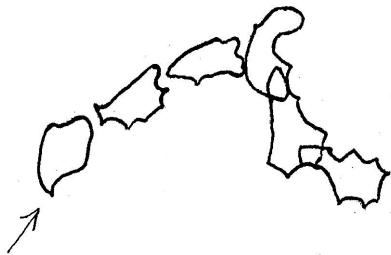
The low optimum of the limax forms does not appear to be general for all cases of ameboid activity. Heart muscle of the frog reaches its optimum activity between 28°C and 30°C and at temperatures above the optimum the activity of the muscle decreases rapidly. McCutcheon has shown that the optimum for human leucocytes occurs at 40°C as might be expected.

There is a possibility that the temperature of the environment may determine the optimum of the amebas as suggested by the human leucocytes. But when we consider that the amebas used in this experiment have been cultured in the laboratory at a room temperature that will average about 25°C , and that they still have the same optimum temperature now as they had when taken directly from the natural habitat, it seems that the temperature of the environment of an

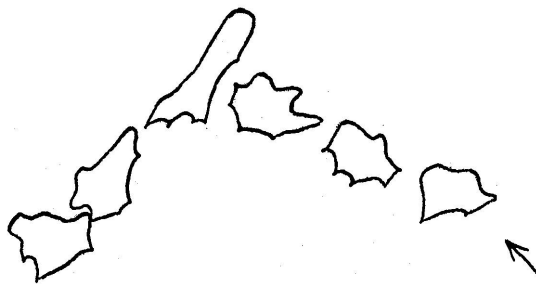
organism is not sufficient to account for its optimum.

In comparing the graphs in Figure 5, it will be seen that the shapes of the graphs in both cases are very similar, and that in both, the velocity increases rapidly up to 30°C, and until the optimum is reached. There is a slight difference in the optimum of the two amebas shown by the graphs in Figure 5. In A the optimum is 38°C, while in B it is 39°C. The fall in the velocity above the optimum in each case is similar. In spite of the fact that these amebas are very much alike in their reactions to the various temperatures, they are consistently different in their rate of movement. Individual 9 is consistently faster than individual 10. At 25°C, individual 9 is going 2.84 mm. per minute faster than individual 10, and at 30°C individual 9 is going 3.4 mm. per minute faster than individual 10, while at 35°C individual 9 is going 3.76 mm. per minute faster than individual 10. This, with additional data, can be seen in the following table. This is not an isolation case but occurred many times throughout the experiment.

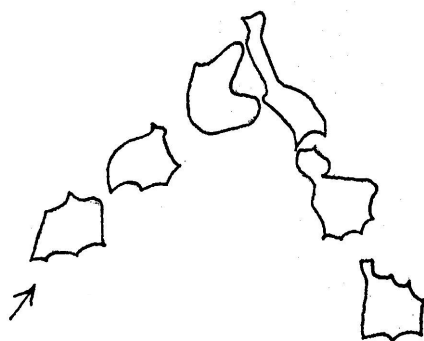
| Figure 5 | 25°C | 30°C | 35°C |
|----------|------|-------|-------|
| A | 8.22 | 12.15 | 13.74 |
| B | 5.33 | 8.75 | 9.68 |
| Diff. | 2.84 | 3.40 | 3.76 |



A



B



C

Fig 10

4. FALL IN RATE ABOVE THE OPTIMUM

After the ameba has reached its optimum, the velocity falls rather rapidly to 41°C and from this point the velocity falls almost immediately to zero at 42°C . At temperatures near the optimum, and some times above, the movement of the ameba differs slightly from that below the optimum, in that at more or less regular intervals, the ameba assumes a very characteristic crescent shape and moves off in every instance at nearly a right angle to its former path. (Figure 10)

This seems to be a racial character rather than an individual one. Pantin noticed in his limax forms a similar irregularity in the movement above the optimum, but it was characterized by the formation of lateral pseudopods rather than crescents. It is interesting to note too, that below the optimum the limax forms studied take a straight path while *Mayorella conipes* takes a wavy one characteristic of most other amebas.

5. THE EFFECT OF SUCCESSIVE RISES AND FALLS IN TEMPERATURES.

After observing *Mayorella conipes* at constant temperatures and at gradually increasing temperatures it became of interest to know if the velocity at any given temperature below the optimum is fixed or dependent upon the previous temperature.

In order to determine this, an ameba was selected and observed for 15 minutes at 25°C . The temperature was now

lowered to 20°C and ten minutes allowed to elapse so the ameba could be acclimated to the new temperature. The ameba was now observed for fifteen minutes more and the temperature this time was raised to 15°C ; acclimated for ten minutes and observed. This process was continued up to 25°C ; allowing first ten minutes at each temperature for acclimatization and observing for fifteen minutes. This has been tried on other amebas, but not on *Mayorella conipes*, and never on any for as long as five hours or longer.

The velocity at each temperature was determined. Figure 4 shows four of the longest observations plotted together on a single graph. For the limax forms, Pantin found a distinct "lag" in the velocity obtained by a rising temperature. That is, he found the velocity at 15°C , for example, to be higher if approached from high temperature, than if approached from a low one.

Graph D, Figure 4, shows a distinct lag. When approached from a low temperature the ameba was going 2.6 mm. per minute at 15°C , but when approached from a high temperature it was going at the rate of 6.8 mm. per minute at 15°C . Again at 20°C when approached from a low temperature it was going 5.4 mm. per minute, but when approached from a high temperature it was going 7.4 mm. per minute. This curve shows only one "up" and one "down", and this is all that has been recorded before by any observer.

In graph A, Figure 4, the "lag" is very definite if we consider only the first "down" and "up". Likewise, in graph C, if we observe only the first "up" and "down" the lag is distinct; but if we observe succeeding "ups" and "downs" over a longer period of time the assumption that the velocity is greater when approached from a high temperature than when approached from a low one does not hold generally.

In these experiments the optimum was never reached, 25°C being the highest temperature, and accordingly nothing should have been destroyed in the ameba, according to Pantin. The only possible explanation for lag is that the acclimatization period was not long enough between successive changes in temperature. But on the other hand, there is evidence to show that in other marine amebas the velocity usually becomes constant in about one minute after a change in temperature.

These experiments show that the previous history of the ameba must be taken into account and that the rate of movement of the ameba at any given temperature cannot be taken solely as an expression of its physical state at that temperature, regardless of its previous temperature history.

5. RHYTHMIC ACTIVITY

In the study of this ameba it becomes necessary to investigate that phase of ameboid movement that has to do with rhythmic activity. Schwitalla ('24) speaks of the "rhythmic

character of the changes in the locomotor rates" in the amebas studied by him. This led to an investigation of this point to see if the rhythmic character that Schwitalla spoke of was present in *Mayorella conipes*.

The word rhythm comes from the Greek ($\rhoυθμός$), meaning "measured motion". We find the word in connection with music, poetry, prose, fine arts, physics, pathology, and various biological processes. Calkins ('04) and Mast ('10), Schwitalla ('24) and Woodruff have called attention to the rhythmic activity of amebas and other protozoa.

While the definitions for rhythm are numerous, there is an idea running throughout them that makes them essentially the same, that being, that for any process to be rhythmical in its action, that action must recur at regular periods and must be predictable as in the case of such biological processes as heart beat and muscle reaction. In each of these cases an action occurs, and recurs regularly, such that it can be predicted fairly accurately. This, according to a composite of all the definitions, can be called rhythm. With this idea of rhythm in mind we will examine the experimental data.

Two species of amebas were used in this phase of the experiment. *Mayorella conipes* was the one used in the previous work and the first one used here. Because *Mayorella*

conipes has no pseudopods,¹ a second species having pseudopods was used as a basis of comparison. (*Chaos diffluens*, Müller)

The amebas were kept and observed at constant temperatures and camera lucida drawings and graphs were made according to the method previously described.

By observing Figure 11 of *Mayorella conipes*, it will be found that the change of the locomotor rate is not rhythmical according to our definition, because throughout the entire curve of 324 minutes in length there cannot be found, even for a small portion of the curve, an action that can be predicted. By comparing this graph of the ameba with a kymograph record of the normal heart beat of a frog, it will be seen at once, by the character of the curve, that there is a difference between a rhythmical (frog heart) and an arrhythmic (ameba) action. Over thirty paths were made of this ameba at constant temperatures and all are similar to the one shown in Figure 11, 324 minutes long. Considering rhythm as it is generally spoken of in connection with other biological processes, (heart action, muscle contraction) and as it has been defined, it can be safely said

¹ *Mayorella conipes* has very indeterminate pseudopods; that is, they do not determine the direction of movement as do the pseudopods in larger amebas which are determinate.

that *Mayorella conipes* moves with alternated acceleration and retarding phases. These phases are seldom of the same duration and at any minute it is impossible to predict the action of the ameba for the next minute.

It was noticed, however, that occasionally *Mayorella conipes* would elongate and assume a crescent shape. This crescent consisted of two pseudopods with protoplasm streaming forward in each. This was accompanied by a slower rate of movement for a time until one pseudopod was retracted and the other predominated. After this, the speed of the ameba increased. In every instance there was a marked correlation between these crescent forms and the decrease in rate of locomotion. From these observations it became evident that the decreased rate was accompanied by the formation of pseudopods. Before one can be certain that this is the case it is necessary to make the same observations on an ameba of different species having a large number of pseudopods. If then, the decrease in rate is due to the formation of pseudopods, and is common to all amebas it should show up very markedly in this ameba. The ameba chosen for this investigation was *Chaos diffluens* (Müller) one of the common large amebas, known to form numerous pseudopods. In order to compare it directly with *Mayorella conipes* it was treated in a similar manner, viz., the same method of constant temperature and camera lucida drawings were made every

minute.

It was found upon examining the path of this ameba, a portion of which is shown in Figure 3, that there was a considerable variation in the rate, and too, just as in *Mayorella conipes* there was a marked positive correlation between the decrease in rate and the formation of pseudopods.

Schwitalla ('24) in his study of the movement of amebas drew only the posterior ends as Figure 3 will show, and he did not record the pseudopods. Since there is a marked positive correlation between retardation and pseudopod formation in the two species of amebas studied here, it is highly probable that the retardation phases observed by Schwitalla were due largely to pseudopod formation.

These crescent forms were more frequently noticed after the ameba had been "tracked" for several hours in a medium free from food. If fed, the ameba would resume normal locomotion and would not form crescent forms until after a considerable time had elapsed after feeding (2 - 3 hours). It would seem then, that these crescents which caused periodic retardations in movement were correlated in some manner with food requirement rather than being fundamentally concerned with rhythmic activity.

6. GENERAL EFFECT OF TEMPERATURE ON MOVEMENT.

The constant μ in the Arrhenius equation represents

theoretically the energy required to transform a given species of molecule from an inactive to an active state. Rice (1923) has developed this idea to mean that since many apparently diverse chemical reactions yield the same value μ , this energy of activation applies to a common molecule; for example the hydrogen ion, or the hydroxyl ion which is concerned as a catalyst. Subsequently Crozier (1924, 1924-25) has shown that a great many diverse protoplasmic activities fall together into a comparatively small number of classes in each of which the process possesses values for μ that are remarkably close together. This fact he has used to support the idea that a given value of μ indicates a common protoplasmic catalyst; and a skilful use of many data has enabled him to make suggestive identifications of some of the catalysts.

It is therefore not without significance that the numerical value of μ which amebas yield is common to a large group of biological activities which are very probably oxidations. More particularly it falls within that group of oxidations which seems to be associated with iron as a catalyst, and for which the value of μ lies between (16,000 - 17,000).

Crozier has brought forward evidence to show that the values grouped around μ (11,500) depend upon a reaction catalyzed by hydroxyl ions whereas those between 16,000 and

17,000 involves some oxidation-reduction system. Values of μ of 16,000 - 17,000 are found to be common for respiratory processes (O_2 consumption and CO_2 production). For reduction of methylene blue by bacteria through removal of H from succinic acid, μ is found to be 16,700. This process at constant temperatures is a function of the hydroxyl ion concentration. The value, $\mu = 16,140$, is associated with the oxidation of iron and may be compared with that of respiration in seaurchin eggs in which iron is a catalyst.

At the extremely low temperatures ($5^\circ - 12^\circ C$) the highest increments are found; in some experiments with frogs (Vernon, 1897) and in Krogh's data in a winter frog 21,000 - 24,000 were found; in the present work with amebas, 30,000.

According to Morgulis (1923) laboratory confinement of *Panulirus* results in the rapid disappearance of glycogen from the blood. It is not difficult to conceive that under these and similar circumstances involving inanition the limiting conditions for oxidation may be profoundly modified.

Crozier suggests the high values of μ to be due to the controlling influence of hydrolytic reactions which prepares the substrate of oxidations.

In finding the temperature characteristic μ for pre-pupal development in *Drosophila* (C. I. Bliss, 1926, *Jo. Gen. Physiol.*, Vol 9, p. 467) show μ 32,210 from $12^\circ - 16^\circ C$;

16,850 from 16°-25°C; 7,100 from 25°-30°C.

From the data given above it seems to be generally recognized that observations illustrating changes in velocities of biological processes (vital activities) with the changes of temperature, are numerous enough and over a wide enough range to show that their corresponding temperature characteristic μ falls into definite classes or groups, and is fully consistent with the idea that the control of vital processes depends not upon one kind of processes but upon a combination of several, interrelated, continuous chemical reactions, each with a variable rate. According to Glazier (1924) "The simplest conception applicable to the controlling reaction is a catenary series O-A-E, in which an original source of supply O is changed into the available form A, whose destruction to the end products E, yields the energy resulting in translation. If O-A has a μ value of 8,000 while A-E has a μ value of 16,000, the depletion of A at higher temperatures would proceed at a faster rate than its replenishment, and hence the increase in the rate of the process as a whole would become less as time went on. If A in the catenary series is considered as a reservoir in the main supplied from O, and E as partly oxidized and partly reversible to O, Myerhof's conception of carbohydrate metabolism in muscle becomes quite applicable."

In muscle, Myerhof points out, the process runs in a

cycle and is composed of two main steps. 1. Glycogen is hydrolyzed to glucose and lactic acid during the anoxidative phase, whereas the oxidative phase results in the oxidation of both these products as well as in the reconversion of three-quarters of the lactic acid to glycogen. If we attach the lower μ value (8,000) to the synthesis of glycogen from lactic acid--an assignment reasonable in view of the large quantities of water produced during this phase, Myerhof's conception should give a fairly well substantiated reaction system capable of the velocity controls demanded for the present case in amebas. Moreover, Myerhof's circular scheme accounts well not only for the transfer of control but allows for the fact that the data can be interpreted as involving at the higher ranges a deficit of A. If we assume for normal temperatures a quantitative adjustment such that the prevailing rate O-A keeps the concentration of A constant at the prevailing rate of oxidation and of the reaction A-E, and then assign μ 16,000 to the oxidative phase, temperatures lower than normal would result in no change of control, but possibly in an accumulation of A. At higher ranges on the contrary, owing to the depletion of A, the rate of the system as a whole would come to depend more and more on the synthetic transformation of E. to O, the forerunner of A, and thus concretely, if amebas were muscle, on the conversion of lactic acid back to glycogen,

and unless the metabolism is radically different the process runs in a cycle and is composed of the two main steps above. Amebas undoubtedly also oxidize carbohydrate since it is definitely known that they digest starch.

When the Arrhenius equation is applied to the temperature-speed curves of amebas a value of μ is obtained which compares favorably with the values of μ for other biological and chemical processes.

By comparing the values of μ thus obtained with the μ value of known chemical and biological reactions it not only becomes apparent that ameboid movement is the result of a series of chemical reactions but that the values for μ may serve to identify the nature of the underlying reaction. The results of the application of the Arrhenius equation are given in the following Figures.

MAYORELLA BIGEMMA

Table I gives the rates of movement in mm. per minute of fourteen different amebas of the species *Mayorella bigemma*. The readings were taken at intervals of five degrees. The averages in the table are the arithmetical means of the readings given at each temperature.

From the Arrhenius equation:

$$\frac{K_2}{K_1} = e^{\frac{\mu}{2} \left(\frac{1}{T_1} - \frac{1}{T_2} \right)}$$

$$\mu = \frac{2 \log_e K_2 - 2 \log_e K_1}{\frac{1}{T_1} - \frac{1}{T_2}}$$

we find:

Examination of the equation in this form shows that it is the slope-form of the equation of a straight line, the type form of which is In
the Arrhenius equation μ is the slope. In plotting the curves we let twice the logarithms of the rates be the y's and the reciprocals of the absolute temperatures be the x's.

In Figure 12 we have plotted twice the logarithms of

the rates against the reciprocals of the absolute temperature readings for each one of the fourteen amebas. The logarithm scale has been moved up sufficiently each time to separate the graph of each ameba from the others. As we have seen the slope of the line in each case represents the μ in the interval. If the lines were exactly parallel the slopes of μ 's would be equal.

In Figure 13 we have plotted the averages of the given rates against the reciprocals of the absolute temperatures. The slope here represents the μ 's of the averages.

MAYORELLA BIGEMMA

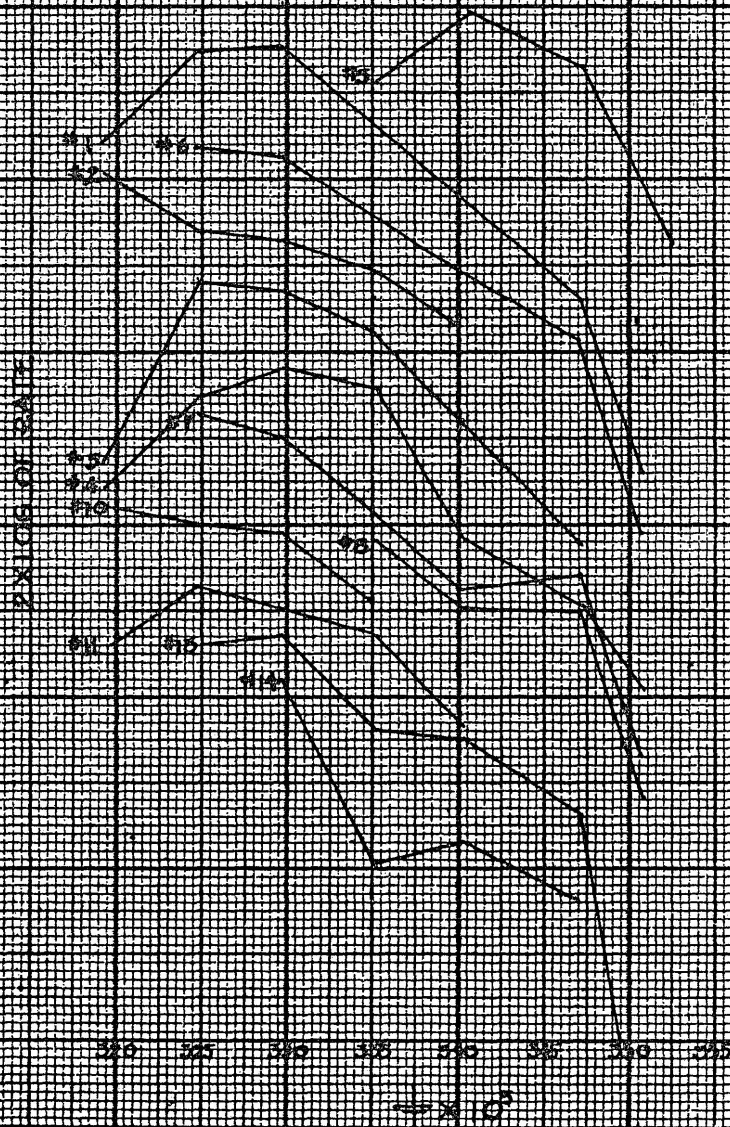


Fig 12

MAYORELLA BIGEMMA
Ave. of 14 Amebas

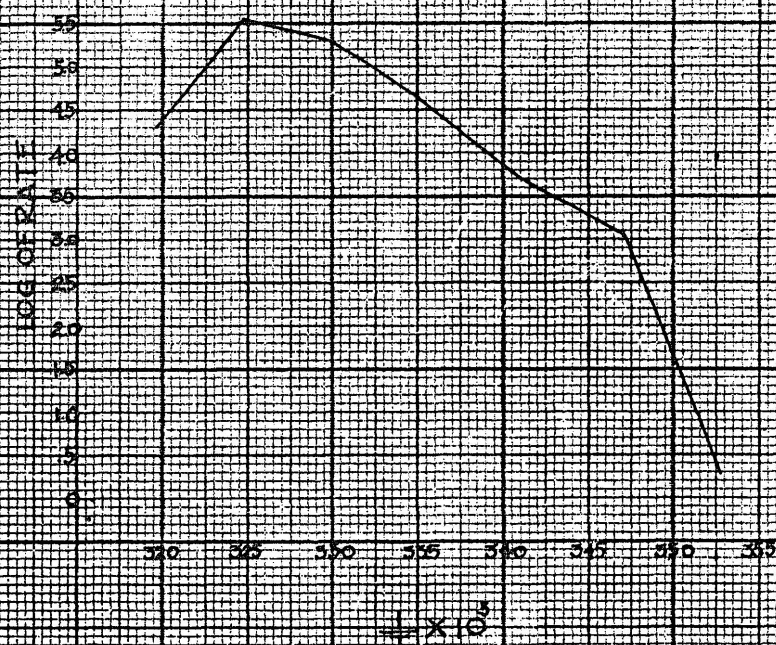


Fig 13

TABLE I
MAYORELLA BIGEMMA

| Ameba No. | Temperature in Degrees Centigrade | | | | | | |
|------------------|--|--------|--------|--------|--------|--------|--------|
| | 40 | 35 | 30 | 25 | 20 | 15 | 10 |
| | Reciprocal of Corresponding Absolute Temperature x 10 ⁵ | | | | | | |
| | 319.4 | 324.6 | 330. | 335.5 | 341.2 | 347.2 | 353.3 |
| 1 | 4.4 | 7.3 | 7.7 | 4.9 | 3.1 | 1.8 | .4 |
| 2 | 16.6 | 15.3 | 11.7 | 9.2 | 7.0 | | |
| 3 | 5 | 14.6 | 14.0 | 10.0 | 6.5 | 3.2 | 1.0 |
| 4 | 7.2 | 12.0 | 14.7 | 12.4 | 5.4 | 3.6 | 1.4 |
| 5 | | | | 10.1 | 5.5 | 4.2 | 1.5 |
| 6 | | 18.5 | 18.0 | 13.1 | 9.1 | 6.4 | 1.6 |
| 7 | | 24.4 | 22.0 | 17.3 | 8.5 | 7.6 | 2.8 |
| 8 | | | | 7.3 | 4.5 | 4.6 | 1.5 |
| 9 | | | | | | 6.8 | .6 |
| 10 | 17.6 | 16.0 | 15.3 | 10.1 | | | |
| 11 | 10.2 | 14.5 | 12.8 | 10.6 | 6.2 | | |
| 12 | | 19.8 | 18.4 | | | 3.5 | 1.5 |
| 13 | 0 | 16.8 | 18.0 | 10.3 | 10.1 | 6.3 | .6 |
| 14 | | | 11.0 | 3.5 | 4.2 | 2.9 | .5 |
| Ave. | 8.71 | 15.92 | 14.87 | 9.9 | 6.37 | 4.63 | 1.21 |
| Log _e | 2.1644 | 2.7665 | 2.6990 | 2.2925 | 1.8516 | 1.5325 | 0.1906 |
| 2 Log | 4.3288 | 5.5330 | 5.3980 | 4.5850 | 3.7032 | 3.0650 | .3812 |

Data relating the rates in mm. per min. of fourteen different amebas to the reciprocals of the absolute temperatures.

Data for Figures 12 and 13.

MAYORELLA CONIPES

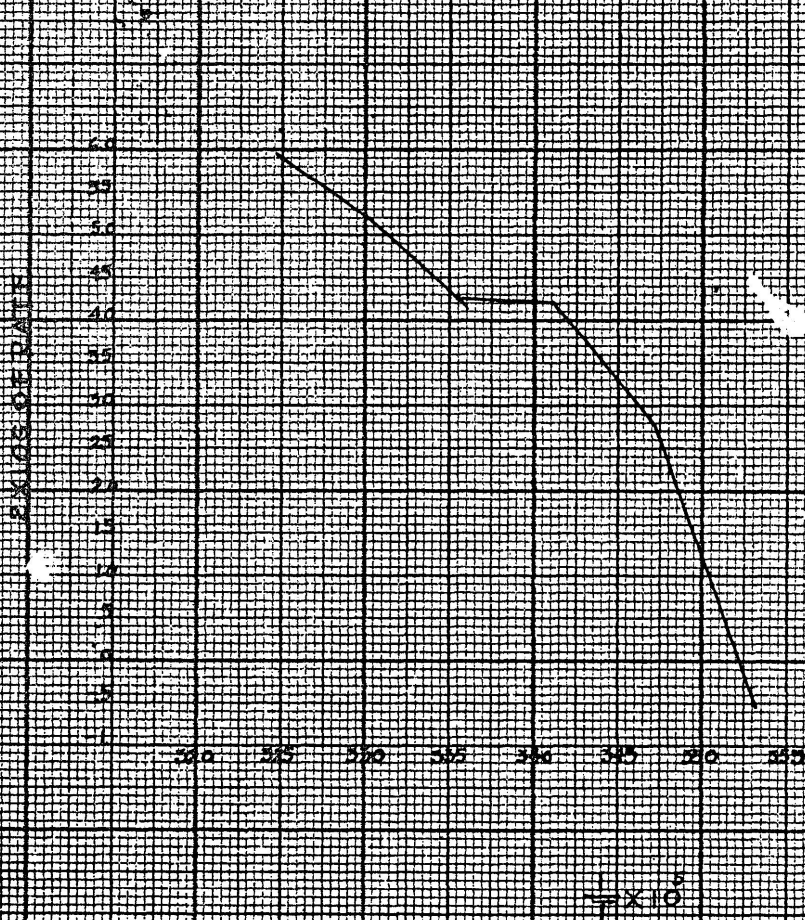


Fig-14

TABLE II

MAYORELLA CONIPES

Average rates in mm. per min. of different groups at different temperatures.

| | Temperature in degrees Centigrade | | | | | |
|------------------|---|-------|--------|--------|--------|-------|
| | 35 | 30 | 25 | 20 | 15 | 10 |
| | Reciprocals of Corresponding Absolute Temperature x 10 ⁵ | | | | | |
| | 324.6 | 330.0 | 335.5 | 341.2 | 347.2 | 353.3 |
| Group | A | B | C | D | E | F |
| Number | 4 | 10 | 9 | 7 | 11 | 11 |
| Rate | 19.46 | 13.5 | 8.38 | 8.37 | 4.13 | .76 |
| Log _e | 2.968 | 2.602 | 2.1258 | 2.1246 | 1.4182 | -.274 |
| 2 x Log | 5.936 | 5.204 | 4.2516 | 4.2492 | 2.8364 | -.548 |

Table II gives the rates of movement for Mayorella conipes. In group A there were four amebas. Their average rate at 35° was 19.46 mm. per minute. In like manner the averages of five other groups are given.

These data are plotted in Figure 14. Each section of the line represents the data for one interval. The slope of the line represents the μ in that interval. If the sections formed a straight line we could conclude a constant rate of increase. Any two sections which are parallel have the same μ .

TABLE III
MATACHAOS DISCOIDES

Rates in mm. per min. of four amebas, measured at various temperatures.

| Degrees C | Rec Abs T $\times 10^5$ | Ameba #1 | 2xLog | Ameba #2 | 2xLog | Ameba #3 | 2xLog | Ameba #4 | 2xLog |
|--------------|----------------------------|-------------|--------|-------------|--------|-------------|--------|-------------|-------|
| 8.8 | 355.2 | | | | | .12 | -4.240 | | |
| 10. | 353.3 | | | .15 | -3.794 | .571 | -1.124 | | |
| 12.5 | 350.2 | .6 | -1.022 | .35 | -2.100 | 3.1 | 2.262 | | |
| 15 | 347.2 | | | 1.6 | .940 | 6.0 | 3.582 | | |
| 15.5 | 346.6 | 5.3 | 3.334 | | | | | | |
| 17.5 | 344.2 | | | | | 15.0 | 5.416 | | |
| 20 | 341.2 | 15.6 | 5.492 | 7.45 | 4.016 | 19.5 | 5.930 | 12.3 | 5.016 |
| 22.5 | 338.4 | | | 10 | 4.604 | | | | |
| 25 | 335.5 | | | 13.5 | 5.352 | | | 13.3 | 5.172 |
| 27 | 333.3 | | | 18.2 | 5.800 | | | | |
| 28.5 | 331.7 | | | 23.4 | 6.304 | | | | |
| 30 | 330.0 | | | | | | | 18.8 | 5.868 |
| 35 | 324.6 | | | | | | | 7.1 | 3.920 |

These data are plotted in Figure 15.

Since we are interested in μ 's within certain intervals, 10-15°C, 15-20°C and so on, Ameba #1 contributes only one μ , that found by using the rates, 5.3 and 15.6. These will give us a μ within the interval 15-20°C. If we use the rates .6 and 5.3, the μ found would not lie in any one of the intervals which we have chosen for comparison, but would overlap the first tow.

METACHAOS DISCOIDES

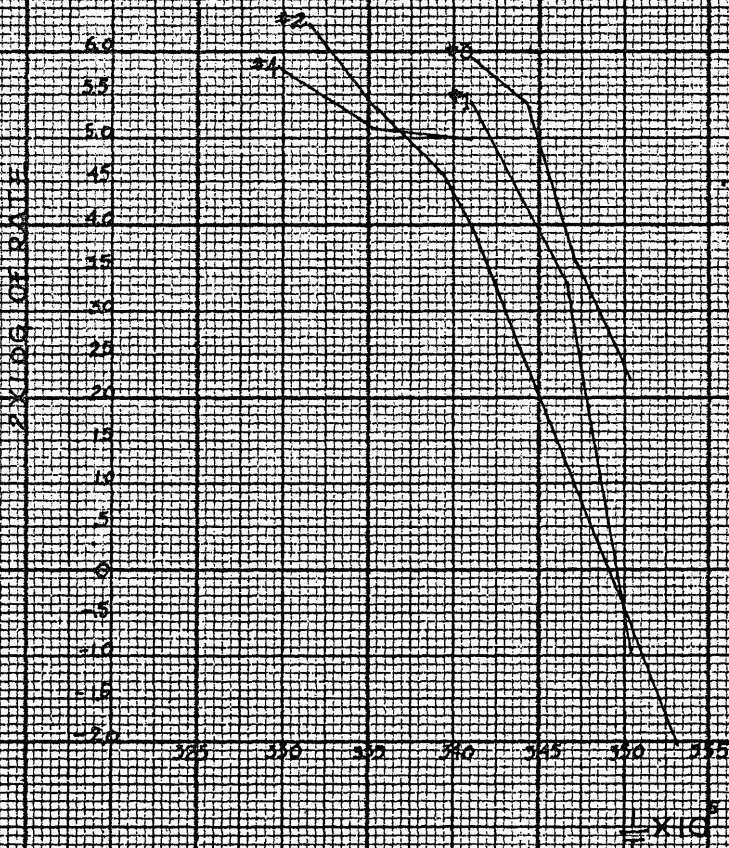


Fig 15

TABLE IV
METACHAOS DISCOIDES

One Ameba

| Degrees C | Rec Abs T x 10 ⁵ | mm. per min. | 2xLog _e |
|--------------|--------------------------------|-----------------|--------------------|
| 26 | 334.4 | 27.7 | 6.642 |
| 27 | 333.3 | 28.6 | 6.704 |
| 27.1 | 333.2 | 30.0 | 6.802 |
| 27.5 | 332.8 | 32.2 | 7.004 |
| 28 | 332.2 | 26 | 6.516 |
| 29 | 331.1 | 33 | 6.992 |
| 30.3 | 329.7 | 32 | 6.930 |
| 31 | 329 | 39 | 7.326 |
| 31.2 | 328.7 | 39 | 7.326 |
| 32.2 | 327.7 | 32 | 6.930 |
| 35 | 324.6 | 32.3 | 7.010 |
| 37 | 322.5 | 0 | |

Table IV gives the data for one ameba which was tracked and measured as the temperature was gradually raised.

TRICHAMOEBIA CLAVA

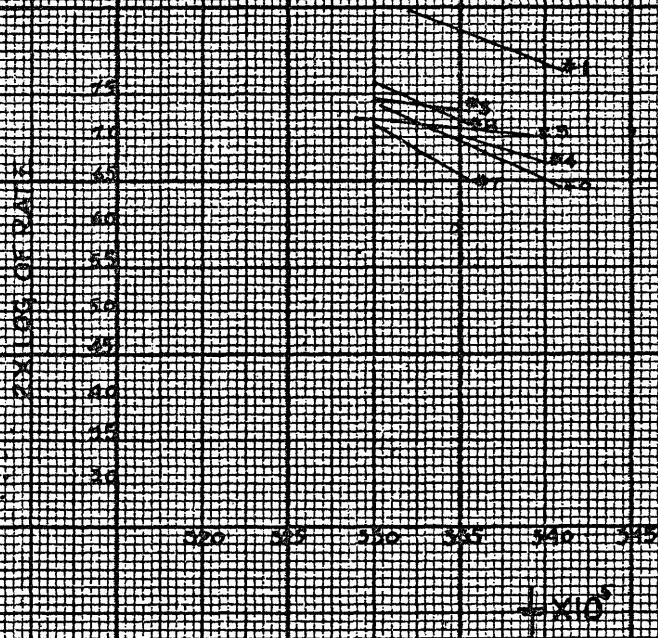


Fig-1b-

TABLE V
TRICHAMOEBIA CLAVA

| No. | Degrees C | Rec Abs T $\times 10^5$ | Rate in mm. per min. | $2 \times \text{Log}_e$ |
|-----|--------------|----------------------------|-------------------------|-------------------------|
| 1 | 20 | 341.2 | 49.1 | 7.786 |
| | 28 | 332.2 | 67.8 | 8.444 |
| | 32 | 327.8 | 61 | 8.22 |
| | 34 | 325.7 | 39.8 | 7.350 |
| | 36 | 323.6 | 27.4 | 6.618 |
| | 38 | 321.6 | 4.8 | 3.136 |
| 2 | 25 | 335.5 | 32.8 | 6.978 |
| | 30 | 330.0 | 30.5 | 6.834 |
| | 35 | 324.6 | 17.6 | 5.734 |
| 3 | 25 | 335.5 | 38.6 | 7.322 |
| | 30 | 330.0 | 41.3 | 7.4400 |
| | 35 | 324.6 | 36.1 | 7.170 |
| 4 | 21 | 340.1 | 29.5 | 6.768 |
| | 25 | 335.5 | 35.8 | 7.128 |
| | 30 | 330.0 | 33.7 | 7.034 |
| 5 | 21.5 | 339.1 | 33.2 | 7.004 |
| | 31 | 331.1 | 38.4 | 7.294 |
| 6 | 25.3 | 341.2 | 25.3 | 6.438 |
| | 30.0 | 330.0 | 42.1 | 7.478 |
| 7 | 25 | 335.5 | 26.9 | 6.588 |
| | 30 | 330.0 | 36.5 | 7.202 |
| | 36 | 323.6 | 38.6 | 7.322 |
| | 37 | 322.5 | 31.5 | 6.898 |
| 8 | 25 | 335.5 | 34.5 | 7.080 |
| | 30 | 330.0 | 46.5 | 7.678 |
| | 35 | 324.6 | 39.5 | 7.356 |
| | 38 | 321.6 | 19.1 | 5.898 |
| | 40 | 319.4 | 1 | 0 |
| 9 | 22 | 338.9 | 45.6 | 7.636 |
| | 25 | 335.5 | 28.3 | 6.684 |
| | 30 | 330.0 | 19.5 | 5.440 |

Table V shows the rates in mm. per min. of nine amebas of *Trichamoeba clava*. The readings were taken at various temperatures for the different amebas.

Table VI shows the readings taken for five amebas of *F. citata* and one ameba of *Tricamoeba Verrucosa*.

The reciprocals of the absolute temperatures and twice the logarithms of the rates are given because these data are used in making the corresponding graphs.

TABLE VI
F. CITATA

| No. | Degrees | Rec Abs T $\times 10^5$ | Rate in mm. per min. | $2 \times \text{Log}_e$ |
|-----------|---------|----------------------------|-------------------------|-------------------------|
| 1 | 20 | 341.2 | 3.7 | 2.616 |
| | 25 | 335.5 | 6.7 | 3.804 |
| | 30 | 330.0 | 9 | 4.394 |
| | 35 | 324.5 | 12.7 | 5.080 |
| | 40 | 318.4 | 16.6 | 5.616 |
| | 42.5 | 317 | 17 | 5.666 |
| 2 | 10 | 353.5 | .1 | -4.606 |
| | 15 | 347.2 | 2.6 | 1.911 |
| | 20 | 341.2 | 5.5 | 3.408 |
| | 25 | 335.5 | 9 | 4.394 |
| | 30 | 330.0 | 13 | 5.120 |
| 3 | 25 | 335.5 | 26.7 | 6.566 |
| | 27 | 333.3 | 27.5 | 6.626 |
| | 30 | 330.0 | 29 | 6.794 |
| | 35 | 324.6 | 17.8 | 5.756 |
| | 36 | 323.6 | .5 | -1.386 |
| | | | | |
| 4 | 15 | 353.5 | 7.4 | 4.002 |
| | 20 | 341.2 | 17.2 | 5.688 |
| | 25 | 335.5 | 21.9 | 6.172 |
| | 30 | 330.0 | 11.3 | 4.798 |
| 5 | 20 | 341.2 | 36.9 | 7.214 |
| | 25 | 335.5 | 39.5 | 7.340 |
| | 30 | 330.0 | 37.3 | 7.236 |
| VERRUCOSA | | | | |
| 1 | 17 | 344.8 | .8 | -.446 |
| | 20 | 341.2 | 4.8 | 3.136 |
| | 25 | 335.5 | 5.6 | 3.444 |
| | 30 | 330.0 | 6.4 | 3.712 |

FLABELLULA CITATA

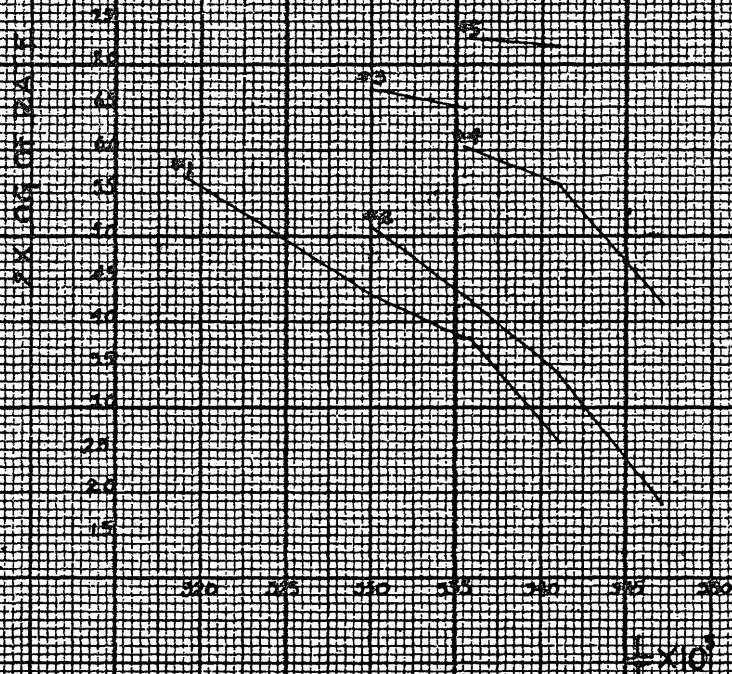


Fig-17

7. VISCOSITY

Throughout this work it was constantly noticed that some amebas move at a much faster rate than other amebas belonging to the same species. Schwitalla (1924) noticed this same behavior and termed such faster moving forms "racers". The interesting thing is that while some amebas moved many times faster than others of the same species, the character of the curve remained the same. Schwitalla in his work omitted an explanation of this seemingly peculiar behavior.

One thing that is very noticeable throughout the data in this experiment is that the larger species of amebas move faster than the smaller species. It was also noticed that the larger amebas usually moved faster than the smaller ones of the same species. In order to prove that this is not a racial difference an ameba was selected and followed (tracked) by camera lucida drawings, at a constant temperature until it divided, then after division both the daughter amebas were followed. After one moved out of the field the remaining one was followed.

When the rate of movement of the amebas was computed before and after division it was found that the rate before division was 13 mm. per minute and that the rate after division was 10.6 mm. per min, in the same protoplasm under the same conditions. (Figure 18). The only other measur-

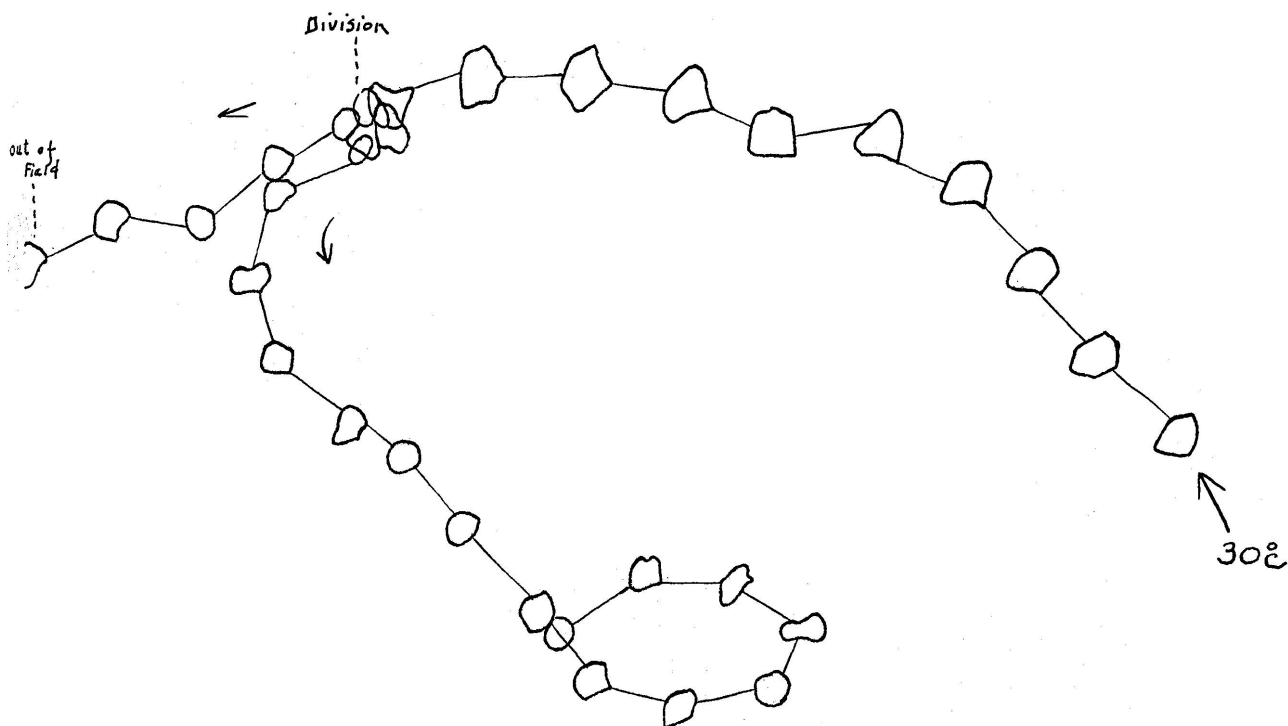


Figure 18

Camer lucida drawing of *Flabellula citata* showing portion of drawing just before and after division. The temperature was 30°C . The rate before division was 13 mm. per min. After division the rate was 10.6 mm. per min. Notice difference in size of ameba before and after division.

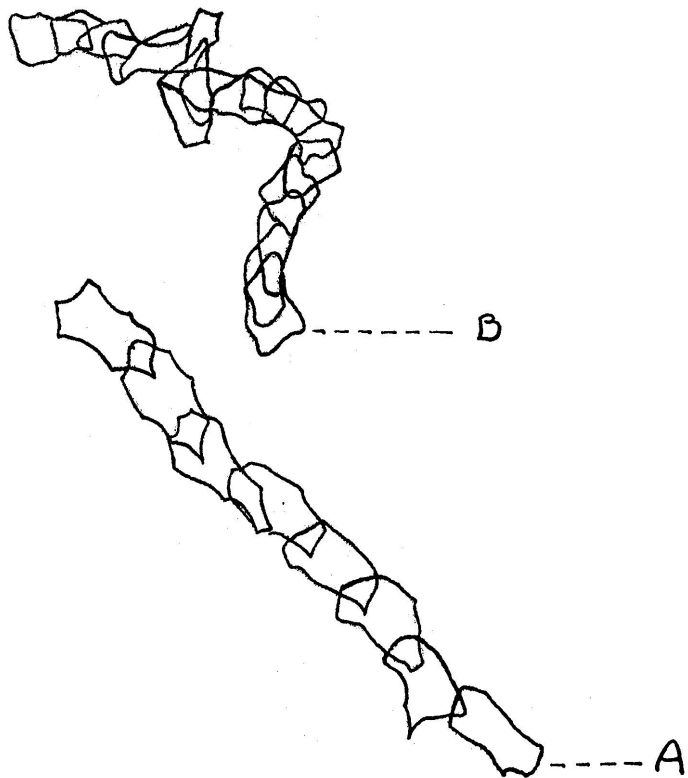


Figure 19

Mayorella conipes at a temperature of 25°C tracked just after division (B) and again five hours later (A). The rate in (A) is 10.5 mm. per min. and in (B) 3.9 mm. per min.

able difference was size, the ameba after division being only about half as big in diameter as it was before division. There is justification for criticism here, that maybe the difference in rate was due to some physiological "upset" during division; but this seems not to be the reason when we observe that an ameba tracked at 25°C moved at a rate of 3.9 mm. per min. just after division, then 5 hours later the same ameba was tracked again, after time had been allowed for it to increase in volume, and it moved at 10.5 mm. per min. (Figure 19)

This seems to show, at least for amebas, that the rate of locomotion depends to some extent on the size, while the other protoplasmic activities may be going on at the same rate.

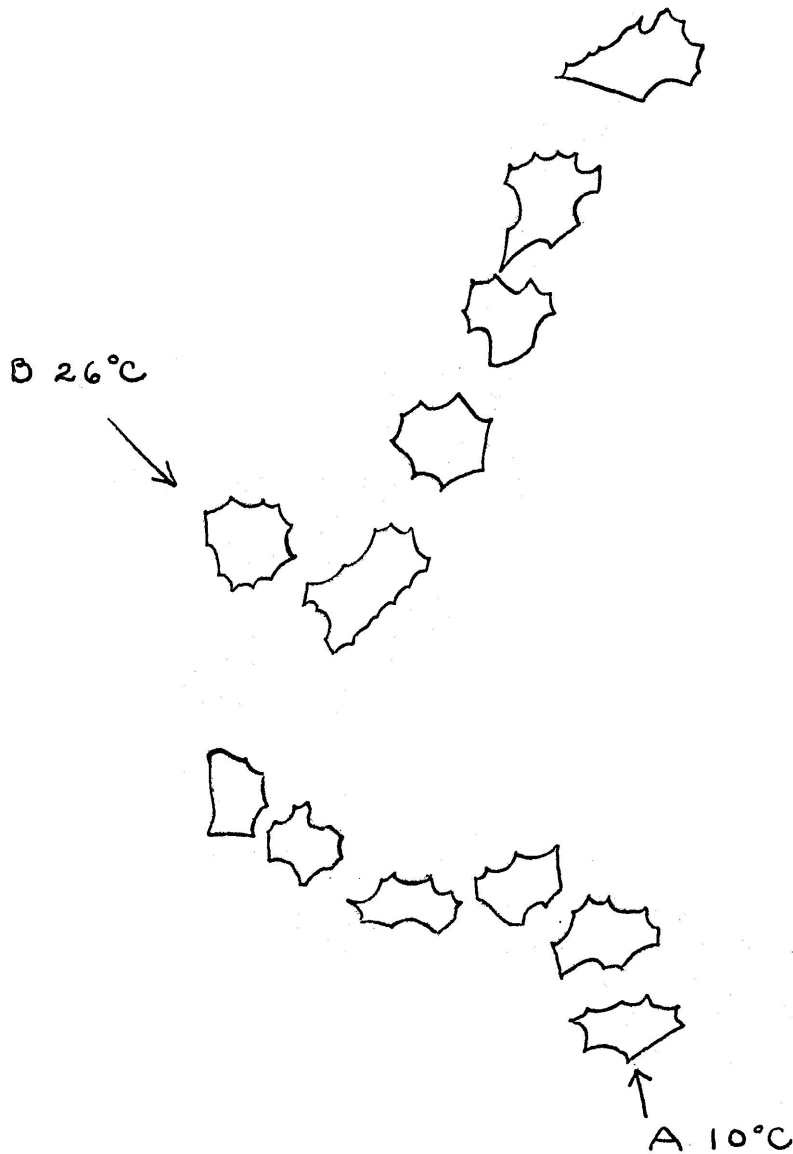


Figure 20

The same amoeba (*Mayorella bigemma*) at two different temperatures. Path A was made at 10°C while path B was made at 26°C. The rate of movement for path A is 6.6 mm. per min. while for B it is 9.9 mm. per min. Notice also that the outlines of the amoeba in path B appear larger than in A.

CONCLUSIONS

1. When Mayorella conipes is kept at a constant low temperature (14°C) for long periods of time (60 to 214 minutes) the change in rate of movement is much less than at high temperatures (35°C). The range at the low temperatures was from 0.5 mm. per minute to 3.25 mm. per minute while in the high temperatures it was from 4.5 mm. per minute to 12.5 mm. per minute. The greatest uniformity in movement was found to be at the low temperatures (14°C); as the temperature was increased the rate of movement became less uniform as the optimum was approached.
2. The optimum temperature for this ameba was found to be between 38°C and 39°C . The optimum for this ameba was found to be actually above the death point of Pantin's limax forms. (Type A, 20°C , Type B, 22° - 25°C).
3. With an increase in temperature, the velocity of movement increased until the optimum was reached (39°C). Beyond this point some substance was destroyed according to Pantin probably a protein, but in all probability an enzyme, since the speed temperature curve of amebas is identical to enzyme action curves. The ameba failed to respond by an accelerated rate of movement to a further increase in temperature. The ameba recovers if the temperature is below 42°C . With regard to recovery, conipes corresponds to other amebas that have been observed.

4. At temperatures near the optimum the ameba sometimes assumes a characteristic crescent shape. This is a response on the part of the ameba to temperatures close to the optimum, and has been interpreted as an attempt to form pseudopods. In the large amebas studied, (*Chaos diffluens*) where large pseudopods normally occur, a similar response is always made to temperatures near the optimum and is always accompanied by the formation of many pseudopods. This seems to be a racial character rather than an individual one. It occurred in every experiment and has been observed in other members of the family Mayorellidae as well as in amebas of other families.

5. The idea that the velocity is greater when approached from a high temperature than when approached from a low one does not always hold for Mayorella conipes. Occasionally there may be a distinct "lag" under the above conditions but that it is not a permanent response on the part of the ameba is shown by the fact that there are just as many cases where the "lag" does not occur where it rightfully should be expected. (Figure 4). The acclimitization period eliminates this lag.

6. These experiments show that the history of the ameba previous to any given experiment must be taken into account and that the rate of movement of the ameba at a given temperature cannot be taken solely as an expression of its

physical state at that temperature, regardless of its previous temperature history.

7. According to the common definitions of rhythm and as we know it in connection with other biological processes, it can be said that the rate of movement of the amebas used in this investigation are not rhythmical. These amebas have periods of acceleration and retardation but these were not found to occur rhythmically and when they did occur were due to the formation of pseudopods in the retarding phases and lack of pseudopods in the accelerating phases. This point was not detected and in fact could not have been detected by Schwitalla since he drew only the posterior ends of the ameba. The reasons for the retarding and accelerating phases are to be found only by knowing the condition of the pseudopods. It seems probable that these phases can be understood completely only by studying the entire outlines of the amebas.

8. The size of an ameba is correlated with its rate of movement. In general it can be said the larger the ameba the faster the rate of locomotion. The "fast and slow" amebas of Pantin and the "racers" of Schwitalla can be explained on this basis. (Figures 5, 18, 19, 20) The difference in rates is due to size rather than a difference in protoplasmic activity.

9. The range of values of critical thermal increments

for the movement of amebas concerns the general theory of ameboid movement in (1) showing that there is not one temperature coefficient for this process--a "temperature characteristic for ameboid movement"--but several; and furthermore (2) in regard to the possible identification of the values found with a variety of specific catalysts found to control the rates of vital processes.

The reactions controlling movement in amebas seems to be a catenary series process in which those values of μ grouped around 11,500 depend upon a reaction catalyzed by hydroxyl ions (Crozier) whereas those between 16,000 - 17,000 involve some oxidation-reduction system and are also common for respiration processes (O_2 consumption and CO_2 production). The higher values of μ (30,000 - 45,000) are due to controlling influences of hydrolytic reactions. The cause of the extremely high values for μ (102,000 - 103,000) are still unexplained.

In amebas the highest increments were found at the lower temperatures ($10^\circ - 15^\circ C$) and the lowest increments were found at the higher temperatures ($30^\circ - 35^\circ C$) which is in accordance with those of other biological processes.

In *Mayorella bigemma* the value of μ between $10^\circ - 15^\circ C$ was found to be 43,748; between $15^\circ - 20^\circ C$, 10,770; between $20^\circ - 25^\circ C$, 15,398; between $25^\circ - 30^\circ C$, 14,681 and between $30^\circ - 35^\circ C$, 2,519.

In *Mayorella conipes* the thermal increment (Mu) between 10°- 15°C was found to be 55,161; 15°- 20°C, 23,843; 25°- 30°C, 17,195; 30°- 35°C, 13,662.

In *Metachaos discoides* at 10°- 15°C Mu was 44,000 and at 26°C to 27.5°C, Mu was 22,625; between 29°- 31°C, 15,900.

In *Trichamoeba clava* between 20 and 25° C, Mu was 7,800; between 25°- 30°C, Mu was 10,800 - 11,100.

In *Verrucosa* the highest Mu was found between 10°- 15°C. Mu was 102,000. Between 20°- 25°C, Mu was 5,400, between 25°- 30°C, Mu was 4,870.

In *F. citata* between 10°-15°C, Mu was 103,400; 15°- 20°C, Mu was 24,950 to 28,100; 20°- 25°C, Mu was 20,840; 25°- 30°C, Mu was 10,700 - 13,200; 30°- 35°C, Mu was 12,700; 35°- 40°C, Mu was 10,300.

The critical thermal increments determined for the amebas studied compare favorably to those determined for other biological processes. By the determination of μ in the Arrhenius equation the basic chemical reactions that supply energy required for the execution of amoeboid movement have been determined.

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