

Observations on Division, Nuclear-Body-Size
Relation and Mitosis of the Common Marine
Ameba, Flabellula Citata.

by

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1. ACKNOWLEDGMENTS
2. INTRODUCTION
3. HISTORICAL
4. MATERIAL
5. METHODS
6. OBSERVATIONS
7. MITOSIS
8. LITERATURE CITED

Introduction

"Because of the prominent part the ameba has played in the development of the basic conceptions of Zoology, it takes its place with the dog and the frog in the great "Zoological Trinity" as outstanding inspirers of basic researchs". 1

The recent work on the nuclei of parasitic amebas has aided greatly in understanding other steps in the process of mitosis. Such discoveries have been made as the origin of the centrosome from the Karyosome (the central mass of deeply staining material in the nucleus); the origin and fate of the centrosomes or intradesmose; (The intradesmose is a structure very similar to the centrosome in the metazoan cell, but apparently more persistent;) and the splitting of the Chromosomes in the metaphase.

The recent contributions of Kofoid and Swezy and their collaborators in establishing a definite number of chromosomes for a number of parasitic amebas is the most definite characteristic for identifying the different species of amebas. This of course is of great importance to the field

1. Schaeffer, A. A. Quartely Review Biol. Vol. I. No. 1. Page 97.

of parasitology.

Since the entire mitotic process occurs within the nuclear membrane, ~~which~~^{this} suggests that the apparent extra-nuclear origin of the spindle of the metazoan cells is a secondary development of the evolution of the mitotic process.

It is of great interest to discover the types of mitosis found in non-parasitic amebas to compare the processes found ~~with~~ⁱⁿ the parasitic forms. With this in view, the writer chose to study the mitosis of a common small marine forms, Flabellula citata.

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Historical

Division of animal cells was first observed by Prévost and Dumas in 1824. Von Mohl in 1827 and several other botanists soon observed this phenomenon in lower plants. Schleiden and Schwann in 1838-39 believed that cells arose by a condensation of matter around the nucleus, while medical men prior to 1858 believed in free cell formation within a matrix of secreted substances.

However, the significance of the discoveries of Prévost and contemporaries was not recognized until after the promulgation of the cell theory by Schleiden and Schuman~~y~~, and later by Virchow and contemporary workers. It has been seventy years since Virchow first stated the principle of genetic continuity of cells by division--"Omnis cellula e cellula."

The mechanism of cell division was not investigated until later when Remak and Koelliker showed that the process involves a division of both the nucleus and cytoplasm. Many observations were begun upon the early cleavages in living animal eggs.

As a result of this work it became evident that nuclear division is of two widely different types which came to be known as direct and indirect as suggested by Flemming in 1879. In 1882 he proposed the names "mitosis" and "amitosis".

Amitosis is the mass division of the nucleus without the formation of spireme, chromosomes, or spindle figures. Remak and his contemporaries regarded amitosis as the typical mode of division. Modern research has shown that it is rare and perhaps a secondary process and is often unaccompanied by division of the cell body. It is especially frequent in highly specialized cells and is often regarded as a kind of degeneration or temporary disturbed mitosis, (Rukert; Ziegler (1891); Van der Stricht (1906). Waldeyer regards amitosis as the original form of nuclear division. Loewitt (1891) regards it as a regenerative as well as a degenerative type of mitosis; while Verson and Frenzel (1891) even thinks that the nuclei that have been formed by amitosis have previously divided mitotically. The case of extreme amitosis is not known in the lower protozoans. According to Nägler, perhaps amitosis has no connection with nuclear division of the inheritance units (Genes) and no case is known where it is exclusively connected with

cell generation as a mode of division. Division by amitosis is faster than division by mitosis and it can be regarded as a means of keeping alive physiologically abnormal cells. Judging from all known cases, amitosis is not regular in cell generation, but rather it seems to be based on the already complicated scheme of nuclear centrosomes where there are double nuclei, while in mitosis no similar cases are known. The discovery of amitosis in amebas was first observed by Butschli, and others in bacteria and related organisms and more recently by Schaudinn, as a result of his work on A. binucleata and ^{by} Dobell ('11).

Lowenthal in 1840 observed transitory forms in A. crystalligera ranging from a direct to an indirect primitive karyokinetic process of mitosis. He observed no chromosomes and no spindle fibers, but the centrosomes remained constantly present. In metazoans certain organs of cells, the centrosomes, dominate the process of division. These may appear in protozoa, but as a rule the achromatic substance suffices to be the means of division. In Euglena viridis the "Nukleocentrosome" has taken the function of the centrosome. The chromosomes collect in the equatorial zone and pull apart, but no spindle fibers are formed.

Mitosis is the usual and typical mode of division in the higher and many of the lower forms of life. Mitosis or indirect nuclear division includes the formation of a spindle, conversion of chromatin into threads or spiremes, and the longitudinal splitting of the threads. Vahlkampf (1904) was the first to discover mitosis in amebas. He names the type which he observed in "limax" amebas promitosis. Prior to this time binary fission of amebas was described as amitotic. In this type of promitosis he observed a karyosome which appears to contain both the chromatin and achromatic material. During nuclear division the interior body or karyosome elongates and the achromatic substance separates from the chromatin. Chromatic masses are formed which flow toward the poles in the elongated karyosome.

The achromatic substance lies as a bridge between the polar bodies and has the form of a barrel. It arranges itself slowly in indistinct parallel threads so that it forms a spindle. All parts of the spindle seem to be furnished by the karyosome. The polar bodies move apart and the spindle becomes longer and thinner.

The equatorial plate usually forms just after the spindle is formed. Chromatin grains have been collecting on the equatorial plate. These grains

arrange and form chromosomes by a heaping up process.

There are three chromosomes which are arranged parallel to the spindle fibers. Each chromosome divides and moves to the polar bodies and are later connected to the polar bodies by spindle fibers. They grow and become club shaped lying with the flattened ends towards the spindle.

In many cases the chromatin grains do not form chromosomes. In some cases there is a collection of very fine chromatin grains which appear on the equatorial plate. They become dense and grow broader and wider in the direction of the two polar bodies without forming definite chromosomes. However, the final result is the same in both cases.

This process is characterized by the formation of polar plates, the division of the karyosome, and the appearance of three chromosomes upon the achromatic filaments which unite the polar plates. The chromosomes divide and move toward the polar plates.

Since the discovery made by Valkamp^h many kinds of cell division have been described. From a general survey of the various types of cell division that are found among the protozoa, the following five groups will be used as a basis for discussion.

1. Mitosis with polar caps.
 - a. Polar caps formed from Karyosome (promitosis (a) ^{and} protomitosis)
 - b. Polar caps formed from peripheral chromatin. (haplomitosis and (b) Crypto haplomitosis).
2. Mitosis with Centioles (Mesomitosis (a) and (b))
3. Mitosis where there are no polar caps and no centrioles (paramitosis (a) and (b) ~~paramitosis~~ (a) and (b)).
4. ~~Meta~~mitosis (complete mitosis)

Promitosis (a) The first type which will be discussed will be the one mentioned in the preceding paragraph, that of promitosis. Strictly speaking, according to Nagler (1909), promitosis may be characterized as follows: (1) The karyosome divides into halves which move toward the nuclear poles in division. (2) The karyosome forms polar bodies there. (3) The peripheral chromatin forms an equatorial plate which may sometimes be partly made up from karyosomic chromatin. (4) The achromatic fibers appear more or less clear and are formed at the expense of the karyoplasmic plastin. Promitosis is found in a few other rhizopods such as Arcella vulgaris. In Arcella the spindle appears to be formed from the ~~Protomitosis~~ ~~Karyosome~~. (Swarcewsky '08) (Plate I. Figs. A-I)

(b) Another variety of promitosis was observed by Alexeieff in 1912, which he called pro-

tomitosis. This variety is characterized by the fact that there is no clear equatorial plate, the peripheral chromatin is found in a diffused ^{condition} way between two polar bodies. An example is Sappinia A. diploidea observed by Nagler in 1909. In it he observed: (1) The two nuclei divide at the same time during this process and division figures lie parallel to each other at first and later cross each other. (2) Each daughter individual receives one-half of each nucleus. (3) The karyosome often shows a large grain in the interior, or the centriole. (4) The outer chromatin grains form in a ring in the granular zone in the middle of the elongated karyosome and later the ring is received into the karyosome. (5) In later stages the karyosome constricts in the middle leaving the ends connected by threads. The karyosomic ends separate and remain as puffed up masses at the two poles. (6) The daughter karyosomes fuse with the polar plates which contain chromatin. (7) The equatorial plate is not visible. (8) The outer chromatin has loosened itself from the karyosome, becomes denser, and lies opposite the karyosome in the nucleus. It now dissolves and surrounds the karyosome in the form of fine grains. Cell division does not always follow and thus there may appear two, four, six, and rarely eight nuclei in one ameba.

In both the cases mentioned above more or less

voluminous polar bodies appear. The polar masses appear to be constituted by karyosomic chromatin and thus the mitosis appears to be quite similiar. On the other hand an equatorial plate is present in the promitosis described by Nagler while it is lacking in the observations of Alexeieff.

(c) Flabellula (Vahlkampfia) patuxent described by Hogue in 1921 might also be classed here. Peripheral chromatin, karyosome, polar masses, chromosome masses, one equatorial plate and a spindle were observed. (Plate III. Figs. 20, 21).

(d) Vahlkampf ('05) in "A. limax" observed a large karyosome, polar bodies, a spindle and an equatorial^{plate} formed at the expense of the polar body. (Plate II. Fig. 13).

(e)ⁱⁿ Mayarella in (A.) verspertilio described by Zwerinzeu the peripheral chromatin grains are found in pairs of four, no centrioles, equatorial plates with spindle fibers and chromosomes, long spindles, polar plates. The karyosome contains a-chromatic substance from which spindle fibers originate.

(f) The division process of "A. mira" described by Glaser is similar to horticola described by Nagler. Peripheral chromatin, spindle fibers, between the daughter plates, eight or more chromosomes were observed. Glaser believes that

all amebas form division balls during division.
(Plate III. Figs. 25, 26, 27).

(g) Pro^házek and Wenyon show the existence of promitosis in Endomaeba buccalis.

(h) Recently Doflein showed the existence of indirect division in Mayarella (A.) vespertilo, but elements of the outer nucleus divide by amitosis.

Haplomitosis. (a) A second type of mitosis designated as hapl^omitosis is exemplified in the work of Dangeard in 1902. The characteristics are:
(1) Elongation of the karyosome in the form of a little stick whose outer ends become very large.
(2) The repartition of the peripheral chromatin which arises at the poles and there ~~it~~ forms polar masses. (3) Division of peripheral chromatin in the form of little caps of linin. These pseudo-monoliformed have been named "chromospines" by Dangeard. They are very characteristic for nuclei of most of the Eugleniens, Coccidies, Hemogregarines, especially Hemogregarina lutzii (Hartmann and Chagas, (1910).
(Plate I. Fig. II.).

Rarely the karyosome in this type of mitosis is fragmented and thus several "little sticks" are formed. This variation appears to be without much importance and is sometimes called fractional haplomitosis. This variation is exemplified by Eugl^{ina} sanguinea (Ehrenberg, Dangeard, 1902), and Hemogregarina stepanour (Reichenow, 1910).

Crypto-haplomitosis (b) A similar form of mitosis was described by Alexeieff in 1911. The chief characteristics of this form are: (1) The chromatin which constitutes the polar pseudobodies does not separate itself from the karyosome before the beginning of the division and do not assume the moniliform shape. Some examples of this type of mitosis are: certain Eugleniens, some Flagellates, some Rhizopods, Chlamydephrys ~~Chlamydephrys~~ stercorea (Schaudinn 1903), Orphryocystis caulleryi (Leger, 1907). In the latter the elongation of the karyosome into a little stick is absent. (Plate I. Fig. III. A-i).

In comparing the two types described the peripheral chromatin appears to form the polar bodies in both forms (a) and (b). In (b) an equatorial plate has not been observed.

Mesomitosis (a) Still another type of mitosis was designated as mesomitosis. The work of Chatton in 1910 shows the distinguishing feature of this type. These features are: (1) The centrioles appear at the two poles of the nucleus. (2) The equatorial plate is formed by a karyosome. (3) The karyosome is massive in the beginning and breaks up into a certain number of chromosomes. The chromosomes may or may not become double and flow toward the two poles where they fuse with the centrioles. Some examples of this type are Pelomyxa palustris

(Bott, 1907) and Trypanosomes.

Rheomitosis (b) Alexeieff in 1912 observed a somewhat similar type of mitosis as a result of his work on *Malpighiella*. He observed the following: (1) At the beginning of division the centrioles appear to originate from the karyosomes. (2) No chromosomes were present. (3) The substance of the karyosome forms an equatorial plate and is gradually spread over the two poles by small grains moving along plastinic formations (centradesmose and sometimes astral rays extend from the center to all sides). (4) The chromatin bodies move toward the poles where they fuse with the centrioles. (Plate I. Fig. IV. A-f)

In these two forms (a) and (b) of mesomitosi centrioles appear and an equatorial plate was observed. However, (a) possessed chromosomes while none were seen in (b).

(c) *A. froschi* as described by Nägler in 1909 appears to possess a karyosome, a centriole, which divides, centradesmose, polar masses connected by a-chromatic threads, an equatorial plate, and the chromosomes migrate and fuse with the polar masses, but are indistinct. (Plate III. Figs. 28, 29, 30).

(d) *A. spinifera* described by Nagler in 1909 is very similar to *A. froschi* except there are no chromosomes and the polar plates are small.

(Plate III. Figs. 31, 32, 33)

(e) In A. diploidea described by Nägler in 1909 there appears a karyosome, peripheral chromatin, centriole and equatorial plate (Plate IV. Figs. 46, 47)

(f) In A. albida described by Nägler in 1909 was observed, much peripheral chromatin, vacuoles, in ^{the} karyosome, centriole, polar plates and but little indication of equatorial plates. (Plates IV. Figs. 43, 44, 45)

(g) In A. lacertae described by Hartmann there appears a karyosome with granules at its margin, a centriole that divides, spindle, centrademes and daughter nuclei. (Plate III. Figs. 34, 35, 36).

(h) In A. lacustris (Nägler) the equatorial plate becomes club shaped, centriole divides, no chromosomes and no daughter nuclei were observed. (Plate IV. Figs. 37, 38, 39)

(i) A. diplomitotica as described by Aragaõ (1909) has an indirect process of division which may occur in two ways. The first is characterized by: (1) The existence of double mitosis of the karyosome and elements of the outer nucleus. The karyosome fragments and (2) there appears a radial arrangement of the elements of the outer nucleus around the nucleus. (3) The chromosomes of the karyosome are oriented by two centrioles.

(4) The chromatin of the outer nucleus moves toward the karyosome. (5) The karyosome forms two polar plates. (6) The centrioles appear. (7) Division occurs. (8) Centradesmose is seen etc., This division is similar to flagella and Polutonella agilis. (Plate II. Figs. 14, 15)

In the second process there is direct division of the karyosome and indirect division of the outer nucleus. The karyosome elongates and the elements of the outer nucleus become grouped around it. The karyosome constricts in the middle and numerous achromatic threads form in this place. As the karyosome divides into two masses an intradesmose (connecting threads) of the centrioles appears. Then begins the division of the chromosome plate of the outer nucleus. The two daughter plates are arranged on achromatic threads and move toward one of the karyosomic masses. They surround the karyosomic mass entirely. This latter process is not necessarily followed by cytoplasmic division.

Both of these processes of A. diplomitica are quite different. The process of double mitosis corresponds to vegetative division and spreads the chromatin of the outer nucleus and karyosome upon the secondary nucleus.

In contrast to this, the second kind of

division is characterized by the irregular spreading of chromatin upon the secondary nucleus. The fragmentation of the cytoplasm may or may not follow.

(j) In A. horticola (Nägler) there are: karyosome, a centriole which divides, about six chromosomes, three spindle fibers, intradesmose, and two daughter nuclei. (Plate IV. Figs. 40, 41, 42)

(k) A similar form of mitosis was described by Wilson in 1916 as a result of work on a soil ameba. Eight subequal chromosomes were observed and there was no evidence of longitudinal splitting, but each became constricted in the middle. The karyosome is located in the center and a centriole may be seen.

Paramitosis. (a) Another type of mitosis was observed by Alexeieff in 1912 in Endamoeba blattaew which he designated as paramitosis. The chief characteristics are: (1) The spindle is pointed at the ends. (2) The achromatic fibers (outer spindle fibers) and chromatic fibers (somewhat similar to centradesmose) appear. (3) More or less constant number of chromosomes were observed. (4) No centrioles appear. This type also occurs in most of the Opalina. (Plate I. Fig. V)

^{geno}
Parateno mitosis (b) Alexeieff describes another type similar to paramitosis which he called

paratenomitosi (meaning mitosis by pulling out).

This form is distinguished from (a) as follows:

(1) The nuclear structures do not undergo as many changes (2) There are no chromosomes (3) The different parts of the karyosome pull out and divide after they have fragmented in an irregular way. (4) The peripheral chromatin grains divide into two daughter nuclei. (5) No centrioles were observed. Alexeieff gives as an example parasitic amebas of vertebrates and Opalina saturnalia.

However, recent discoveries have shown a definite number of chromosomes in six parasitic amebas, which are: E. coli (6) E. dipentheria (histolytica) C. decumani (4) and C. lablauri (8) (Kaffoid and Swezy and collaborators). In both of the types just mentioned there were no polar masses observed and no centrioles. However, in the former case (paramitosis) an equatorial plate was observed while it was not seen in the latter. (Plate I. Fig. VI.)

Panmitosis (a monopanmitosis) Alexeieff further describes another type of mitosis called panmitosis. This mitosis is distinguished by the following; (1) It has only one fusorial fiber. (a) fiber connecting the two daughter nuclei). (2) All of the chromatic material (peripheral as well as karyoplasmic chromatin) is used in the formation of chromosomes. (3) The stage of the

equatorial plate is not clear. (4) There is not any need of centrioles nor polar masses. (5) In the anaphase, one fusorial fiber stretches out between the daughter nuclei. This type is exemplified by many flagellates, in particular the Diplozoan Flagellates. (Plate I. Fig. VII.)

~~Eurifpan~~mitosis (b) Alexeieff still further describes another mode of division which he places under panmitosis type but he adds that this division is quite artificial and is only done for convenience. This mitosis is distinguished from the foregoing by the following: (1) The division is much stretched out. (2) A clear equatorial plate is seen. (3) Chromosomes are in the form of a grain or little stick. (4) For each chromosome in the form of a grain there exists one chromatic fusorial fiber of linin. (5) No centrioles were present (Hertwig). Some examples are: Trichosphaerium Sieboldi (Schaudinn), Actinopshaerium Eichorni (Hertwig), Cochliopodium bibimbosum and some other amebas, also Chilomonas paramecium, Plasmodium vivax (Schaudinn). It is not without interest to observe that this type of mitosis where the centrioles are lacking is often found in the mitosis of maturation of the oocytes of metazoa. (Plate I. Fig. VIII)

Quite a different type of cell division has

been described by M. Taylor as a result^{of} other work on Chaos diffluens. In this type peripheral chromatin grains appear to divide and separate with the karyosome dividing into two plates simultaneously. The outer layer of chromatin grains sloughs off while one of the daughter karyosomes passes into it. The other karyosome remains within the inner layer. Thus, the two nuclei are formed.

Metamitosis (complete mitosis) Some of the later workers have shown to us in an almost picturesque manner true mitosis in parasitic amebas. This work has been done largely by Kofoid, Swezy and collaborators. In Endamoeba dysenteriae was observed: (1) Spherical karyosome in subcentral position; (2) Centriole at the ends of the paradesmose; (3) and spoke radii which pass from the peripheral chromatin to the karyosome. They appear to be about twelve in number while at the prophase they appear as six in number, but often split distally. (4) Chromosomes split lengthwise in the prophase. (5) Six chromosomes divide longitudinally. (6) Spindle fibers and equatorial plate and (7) nuclear membrane persists throughout the entire process of division. (Plate II. Figs. 7, 8, 9).

Similar types of mitosis have been observed by Kofoid, Swezy and Co-workers such as: Councilmania lafleuri (eight chromosomes). (Plate II.

Figs. 1, 2). Endamoeba coli (six chromosomes) (Plate II. Figs. 3, 4) C. decumani (four chromosomes (Plate IV. Fig. 50) and C muris (6 chromosomes) (Plate III. Figs. 17, 18) Kary-amoebaⁿ falcata (about 20 chromosomes). (Plate III. Fig. 16).

In concluding, it may be stated that it is quite evident that mitosis (indirect) is more common and the more normal process, among many protozoa and especially among the parasitic forms. In the case of Endamoeba dysenteriae the material was selected from both cysts and from motile amebas. It is quite difficult to classify the various kinds of mitosis because there are just as important differences between the various kinds of mitosis as between the direct division in metazoans and metaphytes. Alexeieff suggests that mitosis or indirect cell division should not be called "primitive". He believes that the name is too inclusive because there are so many different types. ^{Investigators} ~~Scientists~~ have tried to establish several types of cell division under the general term "primitive" mitosis, ^using the type occurring most as the basis for all the remaining types.

In the cytoplasmic division the cytoplasm may become elongated in the direction of the long axis of the spindle in the early anaphase and always in the late anaphase. It begins to

constrict and when very slender the daughter amebas put out pseudopodia and pull apart. The cytoplasm immediately rounds off. The remains of nuclear connection persists for some time in some cases. More shall be stated later concerning the relation of the body size to nuclear size.

The definite chromosome number has been established by Kofoid and Swezy and Kessel. In the following amebas: (1) E. dysenteriae--6 (Plate II. Fig. 8); (2) C. Lafleuri--8 (Plate II. Figs. 1, 2); (3) E. coli--6 (Plate II. Figs. 3, 4); (4) C. decumani--4 (Plate IV. Fig. 50); (Kessel); and (5) C. ^mMuris--6 (Plate III. Figs. 17, 18) (Kessel). Alexeieff "thinks" that chromosomes having definite form and number have not appeared suddenly in phylogenetic evolution of the nucleus, but have originated by a gradual process. He further states that he believes the mitosis of the Opalina group furnish some of the intermediate stages. (Most of the Opalina mitosis are placed under Paramitosis (a) (See Page 7).

The source of the chromatin material is very much disputed. Alexeieff ('11) believes that the chromosomes arise from both peripheral chromatin and karyosomic chromatin. His evidence for supporting this view is based on: (1) The character and behavior of the peripheral chromatin during mitosis. The peripheral Chromatin migrates and unites with

the Karyosome. (2) Character of spindle fibers and staining of the equatorial plate. The spindle filers are only slightly stained and darker thickenings appear on them in the equatorial plate region at the time when the peripheral chromatin appears to be migrating. These darker stained granules become the chromosomes. (3) Occasional occurrence of large granules with variable staining capacity contained within the karyosome, and (4) The extrusion of a chromatin net in reorganization of nuclei.

Doflein (from work on Mayarella A. versperitilio) states that strongly stainable grains come from the peripheral chromatin and are pressed in the karyosome, which perhaps is the chromosome substance. Valkampf ('04) in his work on "A. limax" is of the opinion that the chromosomes are formed at the expense of the polar masses due to their decrease in size during division. In A. diplomitotica (Plate II. Fig. 14) observed by Aragao, the chromosomes appear to come from both the peripheral chromatin and the karyosomic chromatin. As a result of work on a soil ameba Wilson ('16) is of the opinion that the chromosomes come from both the karyosome and the peripheral chromatin. Hogue ('21) observed in Valkampfia patuxent that it came from mainly peripheral chromatin. Kofoid and Swezy from their observations on E. dysenteriae state that the chromosomes emerge from the peripheral

chromatin due to the following facts: (1) During the splitting and thickening of the chromatin threads they seem to lie on or near the inner surface of the membrane and (2) that the peripheral chromatin grows less evident during this process. However, it is very difficult to estimate the amount of chromatin in the transforming karyosome because of its changes in shape and its division.

It might be mentioned here that Dobell ('19) and Wenyon ('21) in their work believed that there was no definite chromosome number for *E. coli* and seem to reject the recent work of Kofoed and Swezy in which they have clearly shown six chromosomes. However, Dobell gives no indication of chromosome formation and his figures contain only two pro-phases, a single metaphase, two anaphases, and four telophases. His single metaphase shows some evidence of the emerging and assembling chromosomes. Their number is uncertain since there are a number of grayish structures in addition to about six intact or parting darker masses which are presumably chromosomes.

The question of the centriole is also much disputed. Nägler, Hartmann, Chatton, Kofoed and Swezy hold that the centriole is common in the nuclei of protozoa. Other workers (Dangeard, Alexeieff, Glaser) regard its occurrence as extremely

rare. "Glaser even quite recently has criticized without much foundation the affirmations of the centriolists". (Alexeieff). Nagler found centrioles in A. albida, A. diploidea, A. froschi and A. spinifera. Kofoid and Swezy have observed centrioles in E. dysenteriae and other parasitic amebas (two instances in Karyamoeba falcata in an unquestionable manner with the intradesmose extending between them. Similar observations were made by Kessel on C. muris where he observed a centriole. Dobell ('14) observed no centriole in A. lacertae. Hartmann ('13) found a centriole in A. lacertae and in A. hyalina (no polar caps). Hogue ('14) found "a structure resembling the centriole".

Alexeieff is of the opinion that the polar masses in promitosis may be homologous to the centrioles or centrosomes. In this case the centrioles would be much reduced polar masses and the complete centriole (centriole and attraction sphere formed by plastin) would represent a kind of intermediate stage between the voluminous polar bodies and their extremely reduced homologous centrioles. Only in comparatively few cases are centrioles observed. Kessel also suggests that the polar masses form the centrosome in C. decumani (1924). Kofoid and Swezy observed polar caps to be situated in the morphological position of the centrosome.

The intradesmose is formed as the pairs of split chromosomes shorten up. It persists into the stage of nuclear constriction and so far has not been observed in the daughter nuclei. In its completed form it is a slender uniform thread and often having a sigmoid curvature in its course. The intradesmose resembles the centradesmose in the metazoan cells except that it appears to be more persistent. In a few instances the intradesmose appears to be spread out as it merges into the proximal margins of the polar caps.

In two instances, both in amebas from the cat, a polar granule rather sharply defined has been observed. It is situated at the ends of the intradesmose. The intradesmose crosses the chromatin masses and the centrioles at its ends lie in a halo-like area of the polar mass of chromatin.

Encystment in some amebas regularly occurs after a period of rapid division. Encysting individuals are spheroidal, lack food vacuoles, may have an enlarged contractile vacuole, and may have from two to sixteen nuclei. Mitosis may occur the same as in free motile forms or may ^{be} ~~be~~ of a different type as in Chaos diffluens.

In some species the cysts form a chromophile ridge from which the cytoplasm emerges through a pore in the cyst wall as in Councilmania lafleuri

A nucleus migrates into the bud which detaches itself. This process is repeated until the cyst is empty of nuclei. Encystment often follows a period of rapid division.

Plate I.

- I. Promitosis--A--I. *Diamastigameeba*
Gruber (*Schardinger-ameba punctata*
Dangeard) After Alexeieff (1913)
- III. (A-I) Crypto-haplomitosis
Scytomonas pusilla Stein (*Capromonas*
subtilis Dobell) After Alexeieff (1913)
- IV. (A--F) Rheomitosis
Malpighiella after Alexeieff
- II. Haplomitosis after Dangeard
Anisonema grande
- V. Para-Mitosis
Opalina intestinalis
Ehrbg. After Alexeieff (1913)
- VI. Paratenomitosis
After Alexeieff (1913)
- VII. Monopanmitosis *Cryptolia Dahli* (Mebuis)
After Alexeieff (1913)
- VIII. Panmitosis (eurypanmitosis) *Chilodlon dentatus*
in conjugation. Ehrbg. (After Alexeieff '13)

I



III



V



IV



VIII



II



VI

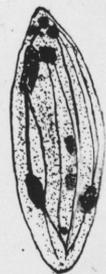


Plate II

- Figure 1- Nucleus of binucleated cyst of *Clafleuri*. Kofoid and Swezy after original (1925 Pl. A. fig. 1)
- Figure 2- *Clafleuri* (1925 Pl. 18. fig. 4)
- Figure 3- *Endamoeba coli* (Loesch) Swezy (1922 Pl. 29 fig. 9)
- Figure 4- " " (") " (" Pl. 29 fig. 1)
- Figure 5- *Endamoeba legeri* Mathis. After Mathis and mercer (1917 fig. 2 d.)
- Figure 6- *Hartmannella aquarium*. Jollos. After Jollos (1917 Pl. 16 fig. 70)
- Figure 7- *Endamoeba dysenteriae* (Councilman and Lafleur) [Craig after Kofoid and Swezy 1925 fig. A. 6)
- Figure 8- *End. deperenteriae*, Kofoid and Swezy-Early prophase showing beginning of migration of Karyosome spoke radii show accumulating chromatin. After Kofoid and Swezy (1925 Pl. 31 fig 2)
- Figure 9- *End. deperenteriae*, Kofoid and Swezy showing intrad-esmose. After Kofoid and Swezy (1925 Pl. 31 fig.11)
- Figure 10-^U*Valkampfia diplomitotica* Aragaõ. After Aragaõ (1909 Pl. 2 fig. 18)
- Figure 11-A diplogena Bêlar. After Bêlar (1915 Pl. 2 fig. 26)
- Figure 12-*Valkampfia limax* (Dujardin) After Valkampf (1905 Pl. 6 fig. 5)
- Figure 13-*V. limax* (Dujardin) After Valkampf (1905 Pl. 6 fig 11)

Figure 14- *V. dipomitotica*. Aragaõ. After Aragaõ (1909 Pl.
2 fig. 18)

Figure 15- *V. dipomitotica*. Aragaõ. After Aragaõ (1909 Pl.
2 fig 1)

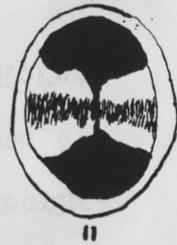
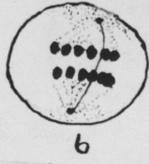
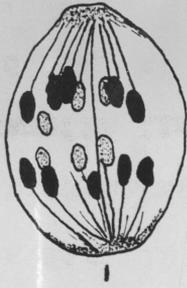


Plate III

- Figure 16- *Karyomoeba falcata*. Koifod and Swezy. After
Koifod and Swezy. (1924 plate 23 fig. 9)
- Figure 17- *Counalmanis nuris Grassi*. After Kessel (1924
Pl. 44 fig. 17)
- Figure 18- *C. muris (grassi)* After Kessel (1924 pl. 44 fig.17)
- Figure 19- *V. lacertae* (Hartmann. After Jollos) (1917 pl. 5
fig. 52)
- Figure 20- *V. patuxent* after Hague (1921 Plate 3 fig. 18)
- Figure 21- " " " " (1921 Plate 3 fig. 13)
- Figure 22- *Naegleri grueberi* (Schardinger) After Wilson (16
Pl. 18 fig. 4)
- Figure 23- *Naegleri grueberi* (Schardinger) After Wilson (16
Pl. 20 fig. 48)
- Figure 24- *Naegleri* (Schardinger) After Wilson (16 Pl.20 fig.
47)
- Figure 25- *A. mira* Glaser. (1912 Pl. 7 fig. 7)
- Figure 26- " " " (1912 Pl. 7 fig. 11)
- Figure 27- " " " (1912 Pl. 8 fig. 27)
- Figure 28- *A. frosche* Hartmann After Nägler (1909 Pl. 2 fig 33)
- Figure 29- *frosche* After Nägler (1909 Pl. 2 fig.26)
- Figure 30₂ *frosche* After Nägler (1909 Pl. 0 fig.36)
- Figure 31- *A. Spinifera* Hartmann after Nagler (1909 Pl. 2 fig
40.)
- Figure 32- *A. Spinifera* Hartmann after Nagler (1909 pl.2
fig. 42)

Figure 33- *A. Spinifera* Hartmann after Nägler (1909 pl 2
fig. 43)

Figure 34- *A. lacertae* Hartmann after Nägler (1909 pl 2 fig.
45)

Figure 35- *A. lacertae* Hartmann after Nägler (1909 pl 2 fig.
49)

Figure 36- *A. lacertae* Hartmann after Nägler (1909 Pl 2 fig.
35)

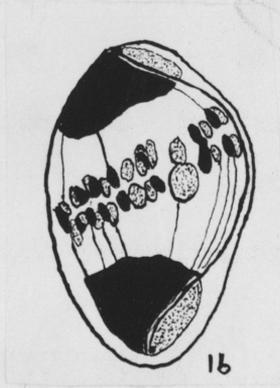
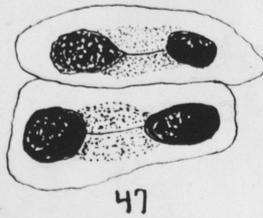
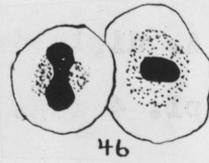


Plate IV

- Figure 37- *A. lacustris* Hartmann after Nägler (1909 pl 2 fig. 56)
- Figure 38- *A. Lacustris* Hartmann after Nägler (1909 pl 2 fig. 57)
- Figure 39- *A. lacustris* Hartmann after Nägler (1909 pl 2 fig. 58)
- Figure 40- *A. Harticola* After Nägler (1909 pl 3 fig. 65)
- Figure 41- *A.* " " " (1909 pl 3 fig. 68)
- Figure 42- *A.* " " " (1909 pl 3 fig. 71)
- Figure 43- *A. albida* after Nägler (1909 pl 3 fig. 65)
- Figure 44- *A.* " " " (1909 pl 3 fig. 74)
- Figure 45- *A.* " " " (1909 pl 3 fig. 77)
- Figure 46- *A. diploedeia* Hartmann and Nägler after Nägler (1909 pl. 4 fig. 108)
- Figure 47- *A. diploedeia* Hartmann and Nägler after Nägler (1909 pl. 4 fig. 3)
- Figure 48- *A. proteus* after Daflen (1918 fig. 1b)
- Figure 49- *A.* " " " (1918 Fig. 6 C.)
- Figure 50- *C. decumani* Rudovsky After Kessel (1924 pl 47 fig. 45)



IV Material

All of my slides were made from a common marine form. Flabellula citata, first described May 25, 1919 by Dr. A. A. Schaeffer at Tortugas Is. Florida. The maximum size of this ameba is about 7.5 μ . In younger cultures the ameba appear larger and more uniform in size. Often one ~~two~~ contractile vacuoles are seen in the posterior region. Numerous large vacuoles appear while in fresh water which regularly become contractile but when the ameba are transferred to salt water the vacuoles slowly disappear. The endoplasm located in the posterior region is densely granular and is set off from the clear ectoplasm by a sharp line.

The nucleus is always found in the granular end during locomotion. It is spherical and a denser greenish-blue than the rest of the ameba. Peripheral chromatin and a distinct nuclear membrane were found. The karyosome is a compact spherical chromatin mass about 2 μ . in diameter.

During locomotion F. citata is oval with the broad side oval advancing having determinate pseudopods. In changing directions the ameba flows to one side at right angles to its original directions. It moves four-six times its own length in the space of one minute. Uroidial connections were found on the posterior part of the ameba during active locomotion.

From a six day culture grown at 13 $1/2^{\circ}$, a single

ameba was picked by means of a finely drawn sterile pipetter and placed in a hanging drop of culture fluid and allowed to stand in a moist chamber for three days. The amebas were counted every five hours. As a result of this experiment which was tried only in a few cases, it appeared that F. citata divided once every two hours. After a few hours the bacteria, which serves largely for food for the ameba, appeared in clumps. In all cases the ameba were located near the bacteria. When the bacteria were no longer present, the amebas disappeared also.

In the majority of the ameba cultures used in my work on Flabellula citata, sea weed (Fucus Vesiculosus - "Rock weed") was used which was obtained from Cold Spring Harbor. A small handful of sea weed was placed in sterile Stender dishes filled about three fourths full of sea water. The cultures grown at 28° C. usually reached their optimum in about three days after incubation. A grayish scum, usually spread quite uniformly over the top, often appeared. As the culture became older ciliates appeared. The cultures grown at 13.5° C. appeared in great numbers in about ten days.

In 0.5% solution of agar and sea water large clumps of amebas appeared in two days on cultures grown at 13.5° C. In four or five days the amebas became scattered out over the agar. They lived in this medium for two weeks. This material was sterilized in an auto clave and placed in sterilized petri dishes.

V. Methods.

A drop of scum with amebas was placed on the slide with several drops of clear culture fluid. The amebas spread out on the slide in a few minutes, and because of their being close to the surface of the slide, no fixation methods were necessary. The slides were dropped face down in hot Schaudinn's solution with the result that practically all the amebas were properly fixed, adhering to the slide, while the scum and debris of the culture floated off.

The following fixing solutions were used: Bouins, Picro-formal and Schaudinn's solution. The last mentioned proved to be the most satisfactory. It was used both hot and cold.

The stains were Iron-haematoxylin and Mann's Stain. Iron-haematoxylin proved to be the most satisfactory. It stained the nuclear structures more clearly and differentiation could be carried further.

A complete history of each slide was recorded. This included (1) The age and temperature of the original culture from which each slide was made. (2) The length of time that each slide was allowed to stand in a moist chamber at 28°C. and at 13.5°C. (3) The length of time each slide was allowed to stand in the fixing solution and the temperature of the solution was noted. (4) Throughout the remainder of the

process the exact length of time which the slide was allowed to stand in the alchols, Iron alum, Haematoxylin was tabulated. The temperature of the mordant and stain was also noted.

VI. OBSERVATIONS.

In culturing *Flabellula citata* many questions began to arise involving the relation of the nuclear to the body size and other factors which might be involved. Since this ameba is a small marine form it became quite necessary to discover the effect of temperature, age and other factors upon the size of the body and upon the size of the nucleus. Quantitative relations of different cell constituents at various phases of activity, is of significance in determining some of the inner activities and in the study of many fundamental problems of growth, differentiation, and cellular physiology. Many modern workers have emphasized the importance of the underlying processes of metabolism and the resulting accumulation in size of the cell and its components. Some work which has been done might properly be mentioned here.

The maintenance of a normal quantitative relation between the nuclear mass and the cytoplasmic mass is well illustrated in the cleavage of the ovum of such forms as annelids or gastropods where very marked inequalities of division is exactly equal so that all the cells receive equal amounts of chromatin. However, after the nuclei are

reformed, they grow to a size that is roughly proportional to the size of the cytoplasm. A wide variation exists in this relation. (conklin, '12) In cases of this type the size of the nucleus is obviously related to that of the cytoplasm.

Conclusions similar in principle have been found as a result of experiments on dwarf larvae of sea urchins arising from egg fragments (Morgan '05, '01, '03 ; Dreisch, '93 '00; Boveri '05). In dwarf larvae arising from egg fragments of different or of the same size the cells are nearly or the same size but different in number.

The larvae from a fragment of about one half the normal size contained about one-half the normal number of cells, and the fragment of about one-fourth the normal size contained about one-fourth of the normal number of cells characteristic of an entire egg at the corresponding stages. From a giant larva produced from two fused eggs the number of cells is double the normal number. Dreisch ('00) and Morgan reached the conclusion that the cleavage is so regular as to produce a fixed or typical cell size at a given stage

rather than a fixed number of cells. This was also confirmed by Boveri. In the above cases the volume of the nucleus varies primarily with that of the cytoplasm.

In other cases the nuclear mass appears to be the primary factor. Gerasimoff's experiments on *Spirogyra* prove that the artificial increase in the size of the nucleus leads to a corresponding increase in the size of the cytoplasm. In the tetraploid giant forms the nuclear volume is doubled as a result of a doubling of the number of chromosomes. Here the normal "nucleoplasmic" ratio (ratio between the nucleus and cytoplasm) is restored by a corresponding growth of the cytoplasm to twice its former size.

Boveri in his work on embryos of the sea urchin Paracentrotus clearly shows that the size of the nuclei at any given stage is directly proportional to the number of chromosomes that they contain. The nuclei are the smallest in the haploid larvae, largest in the tetraploid and of varying size in dispermic larvae.

In this case the primary factor is the number of chromosomes which leads to corresponding variation in the size of the nuclei and of the cytoplasm, while in the case of Dreisch and Morgan on egg fragments the primary variable factor was the total size of the embryo. However, in both cases the normal "nucleoplasmic" ratio was maintained by the process of cleavage. In Boveri's words, "The constant, which we must accept as something given and not at present further analyzable is the fixed proportion between the nuclear volume and the cytoplasmic volume, namely the "Nucleoplasmic ratio". ('05, p. 68)

Some forms of the "Nucleoplasmic ratio" is subject to wide variation. Conklin ('12) in the cleavage stages of *Crepidula* shows that the large cells usually have larger nuclei than the small but the ratio varies widely with the length of interkinesis and the amount of yolk material. In *C. plana* the ratio of cytoplasmic to nuclear volume in protoplasmic cells free from yolk were measured. At their maximum size the ratio varies from 14.5 - 8.7 microns or 35.7 - 7. microns when

measured from their mean size.

Conklin also shows that the nuclear volume varies not with the total volume of the cytoplasm but only with that of its active protoplasm. As an example: - In the eggs centrifuged during cleavage, a sharp separation takes place between the heavier yolk and the lighter protoplasm so that their volume may readily be determined. In such eggs, during cleavage the largest nuclei appear in the blastomeres that contain the largest amount of active protoplasm irrespective of their total size. Thus some of the largest nuclei are situated in the smaller cells. Thus we see that although the "Karyoplasmic ratio" is an important cell constant it results from a complex of factors often difficult to analyze.

Not only the nuclear size but also that of the formed components of both nucleus and cytoplasm often varies proportionally to that of the cytoplasmic mass. This is seen in the mitotic figures of dividing blastomeres and the centrosomes (centrospheres) of the interphase Conklin ('12).

Structures such as centrioles,

chondriosomes, acroblasts, and chromatoid bodies show a proportional variation to the dytoplasm and to the nucleus in nearly 20 species and genera of (Heteroptera Rentatomidae) of Bowen ('22).

Chloroplasts and pollen grains also vary proportionally to the cytoplasmic mass and to the nucleus as observed by Winkler('16) in the tetraploid Gigas mutants of tomato (Solanum.) These differences evidently result from the increased size of the cytoplasm, however caused, for they appear in the poly-megalous spermatocytes of insects which are diploid as well as in the tetraploid cells of Gigas forms.

Montgomery ('10) in his work on Euschistus states that the size of the plasmosomes varies with the cytoplasm and nucleus.

In some cases of chromosomes during cleavage the size of chromosomes varies proportionally to the size of the nucleus and to the size of the cytoplasm. The chromosome ratio was observed by Erdman ('08), Baltzer ('08), and Conklin ('12).

In general then one might say that a small nucleus gives rise to small chromosomes. The size of the spindle, centrosome, and sphere in any cell is not definitely fixed but may be modified by altering the quantity of cytoplasm, the larger the quantity of cytoplasm in a cell the larger are the structures named according to Jennings ('12, page 77). The size of the nucleus depends upon at least three factors, (1) the initial quantity of chromatin (Boveri), (2) volume of cytoplasm, and (3) the length of the resting period.

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370
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220
210
200
190
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170
160
150
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130
120
110
100
90
80
70
60
50
40
30
20
10

Figure 1
Flabellula citata
 Temperature-13° C (red)
 Temperature-28° C (white)
 Grand total- Red-3000
 - White 3017

25 24½ 24 23½ 23 22½ 22 21½ 21 20½ 20 19½ 19 18½ 18 17½ 17 16½ 16 15½ 15 14½ 14 13½ 13 12½ 12 11½ 11 10½ 10 9½ 9 8½ 8 7½ 7 6½ 6 5½ 5 4½ 4 3½ 3 2½ 2 1½ 1

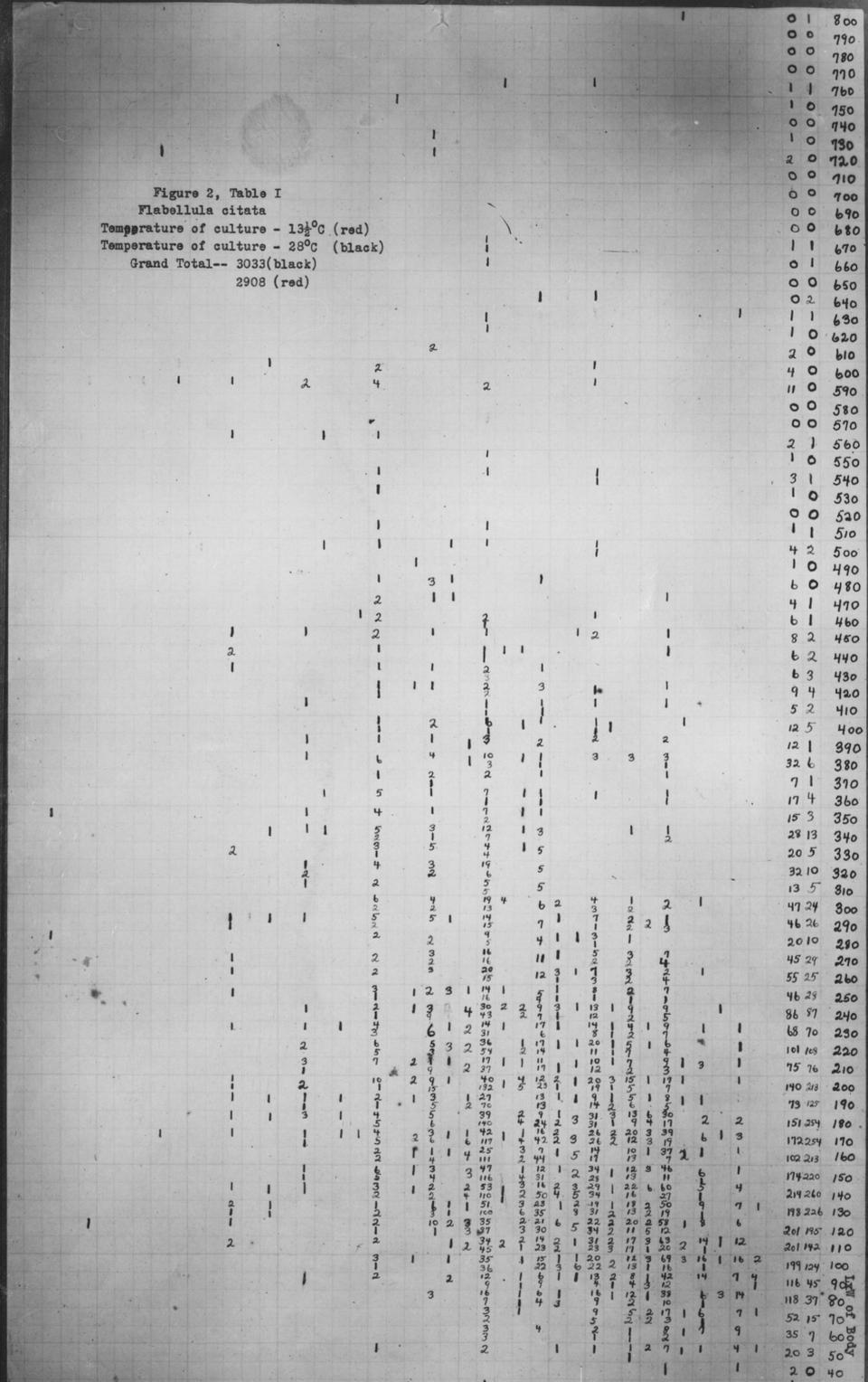
Length x width of nucleus

In figure 1, the 6,017 amebas are represented by dots, each dot representing an ameba. The 3,000 red dots represent the amebas grown at 13.5°C. and the 3,017 black dots represent the amebas grown at 28°C. The product of the length of the body X the width of the body was used as the body size in order to obtain as nearly as possible the size of the body regardless of its shape and to retain the proportion of the body size to nuclear size. For instance in a very much elongated but narrow ameba the product of the length X width would secure both of the above mentioned results. Therefore thruout all of my measurements the product of the L x W has been used.

In culturing Flabellula Citata the question arose whether or not temperature was a factor in determining the size of the nucleus and the body size. In order to determine the importance of this factor cultures were grown at 13.5°C. and at 28°C. Slides were made from these cultures. The amebas were fixed in Schaudinn's solution heated to 60°C. and stained in Iron - Haematoxylin. A complete history of each slide was recorded.

Amebas from these slides made from both cultures were measured. All the measurements

Figure 2, Table I
Flabellula citata
 Temperature of culture - 13½°C (red)
 Temperature of culture - 28°C (black)
 Grand Total-- 3033(black)
 2908 (red)



800
790
780
770
760
750
740
730
720
710
700
690
680
670
660
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630
620
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580
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560
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490
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470
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20
10

21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
 21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
 L x W of Nucleus

Black
Red

were made with a micrometer disc in a 10 x eye piece under oil immersion lens. In all cases only amebas during locomotion were measured. The criteria used to determine locomotion were: (1) in most cases clear ectoplasm can be seen at the anterior region if in locomotion, (2) the nucleus is located in the posterior half of the body, (3) and the general form of the ameba during locomotion is fan-shaped as its name suggests. There are doubtless some non motile amebas included in the measurements but the great majority are in locomotion when stained and killed.

The total number of amebas measured were 6,017. The measurements were made according to the following method: (1) the longest antero-postero axis exclusive of slender pseudopods or uroidal projections was measured and this was designated as the length of the body of the ameba (2) the longest trans diameter or greatest width was measured and this was called the width of the body.

Similar measurements were made of the nucleus, the longer diameter being called the length and the smaller diameter the width. Since it is impossible to measure a fraction below one-fourth with any degree of accuracy, this method of measuring is not

entirely satisfactory but it seems better than any other simple method. The body size ranges from 10 units to 1,000 units (ordinates) (One unit = 1.75 microns). The product of the length X the width of the nucleus was used in the following figures as the nuclear size. The nuclear size ranges from 1 unit (on the scale selected) to 23 units (abscissa) (one unit = 1.75 microns).

In figure 2, table 11 represents figure 1 in tabular form. It shows the distribution of amebas with respect to body size and nuclear size. The black numbers indicate the amebas grown at 13.5°C. and the red dots represent those grown at 28°C. exactly as in figure 1. The totals for each nuclear size and each body size are shown at the appropriate places. The grand total of amebas represented on this table is 2,908 amebas grown at 13.5°C. (red), and 3,033 (black) grown at 28°C. Due to their extreme large size, a few amebas represented in figure 1 were not included in this table for convenience of presentation. Since it is impossible to measure, with any degree of accuracy, a fraction below one-fourth of a unit or less there appear in this table some irregularities of distribution of nuclear sizes.

In figure 3, a correlation curve shows the relation between the nuclear size and the body size of amebas grown at 13.5°C . and at 28°C . The black dots with figures represent the number of amebas grown at 28°C . and the red dots with figures represents the number of amebas grown at 13.5°C . The 21 classes of nuclear size were combined into 7 columns for convenience of presentation.

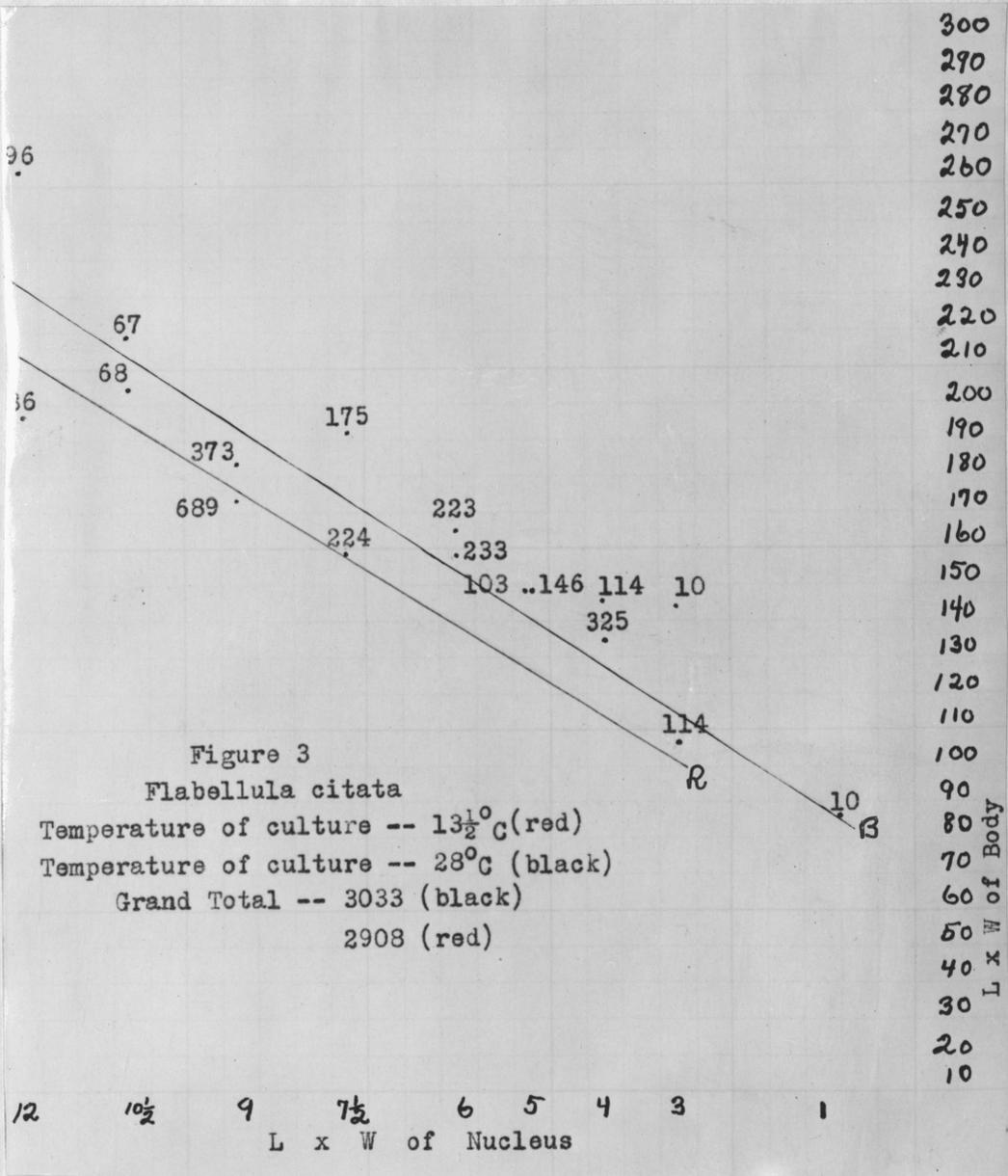
According to the scale selected the correlation curve shows that:

(1) The amebas grown at 13.5°C . were more uniform in size of body and in nuclear size. Nearly one-fourth of the 2,938 amebas at 13.5°C . had a body size of 160 units and a nuclear size of 9 units.

(2) The amebas grown at 13.5°C . and at 28°C . increase in body size in proportion to nuclear size.

(3) The nucleus increases at almost the same rate as the body in both cultures.

Note: There appears to be no marked difference in cell size and in the nuclear size of these amebas grown at different temperatures due to the fact that some of the amebas represented were grown in young cultures and some in old



cultures. However there are nearly twice as many amebas from the 13.5° C. culture which have a nucleus and body size of 17 units and 9 units respectively. Thus there is a slight preponderance in favor of the amebas grown at 13.5° C.

In order to discover the effect of age of the culture on the size of the body and on the size of the nucleus a comparison was made between two cultures that were exposed to identical conditions except that they were of difference ages. Amebas from both cultures were allowed to set 30 minutes on the slide in a moist chamber. They were then fixed in Schaudinn's solution heated to 60° C. and stained with Iron Haematoxylin. Measurements were made as mentioned for amebas in the preceding groups and only amebas during locomotion were elected.

In figure 4 each dot represents an ameba. The 683 black dots represent the amebas from a three day culture grown at 28° C. and the 466 red dots represent the amebas from a seven day culture at 28° C. The units indicate the body size ranging from 10 units to 500 units, (Ordinate) (1 unit = 1.75 microns) The units showing the nuclear size range from 1 unit to 12 units (abscissa).

Figure 4

Flabellula citata

Age of culture ----- 3 days (white)
 Age of culture ----- 7 days (red)
 Set on slide ----- 30 minutes
 Temperature ----- 28°C
 Grand Total ----- 683 (white)
 466 (red)

490
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470
460
450
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420
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340
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320
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290
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270
260
250
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17½ 17 16½ 16 15½ 15 14½ 14 13½ 13 12½ 12 11½ 11 10½ 10 9½ 9 8½ 8 7½ 7 6½ 6 5½ 5 4½ 4 3½ 3 2½ 2 1½ 1

In figure 5, table II, represents figure 4 in tabular form. It shows the distribution of amebas with respect to body size and nuclear size of a three day (black) and a seven day (red) culture. The totals for each nuclear size and each body size are shown in appropriate places. The grand total of amebas represented is 681 (black) amebas grown in a three day culture at 28°C. and 465 (red) amebas from a seven day culture at 28° C.

In figure 6 the relation between the nuclear size and the body size of three day ameba culture is shown. The black dots with figures represent the number of amebas grown in a three day culture and the red dots with figures represent the number of amebas grown in a seven day culture. The 12 classes of nuclear size were combined into seven columns (3 day) and six columns (7 day) for convenience of presentation in this graph.

According to scale selected the correlation curve shows: (1) In both the seven day cultures (red) and the three day cultures (black) the nuclear size increases in direct proportion as the body size increases. (2) The increase in nuclear size to the body size is at a much slower rate in the seven day culture (red) than in the three day culture. (by inspection)

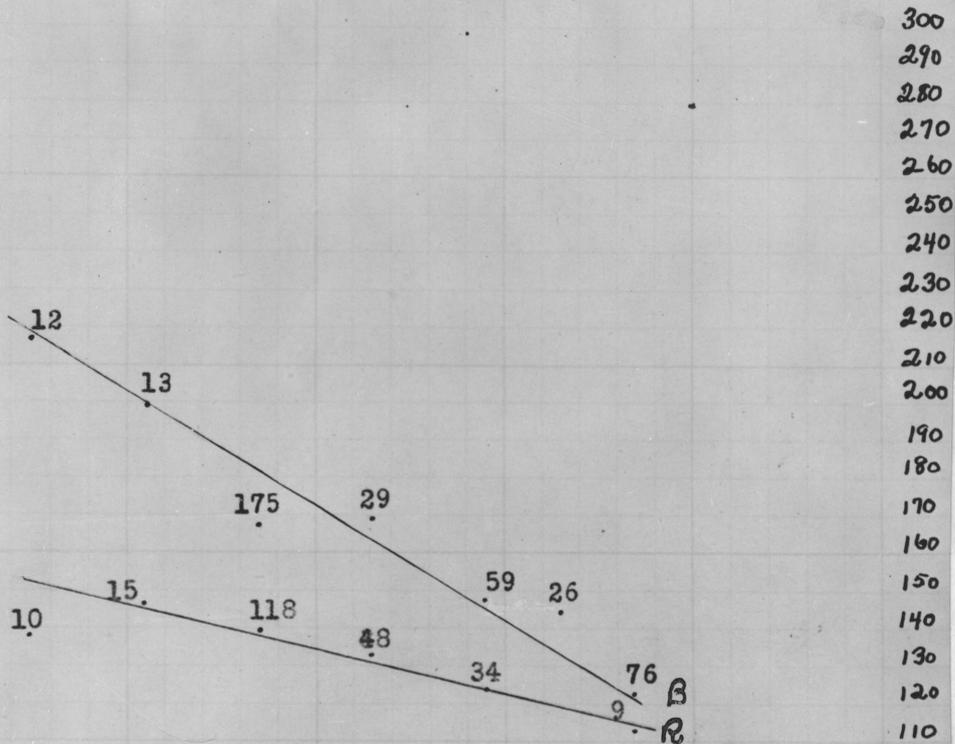


Figure 6
Flabellula citata

Age of culture ---- 3 days (black)

Age of culture ---- 7 days (red)

Set on slide ---- 30 minutes

Temperature of cultures - 28°C

Grand Total -- 681 (black)

465 (red)

12 10½ 9 8 7 6 5 4 3 2

L x W of Nucleus

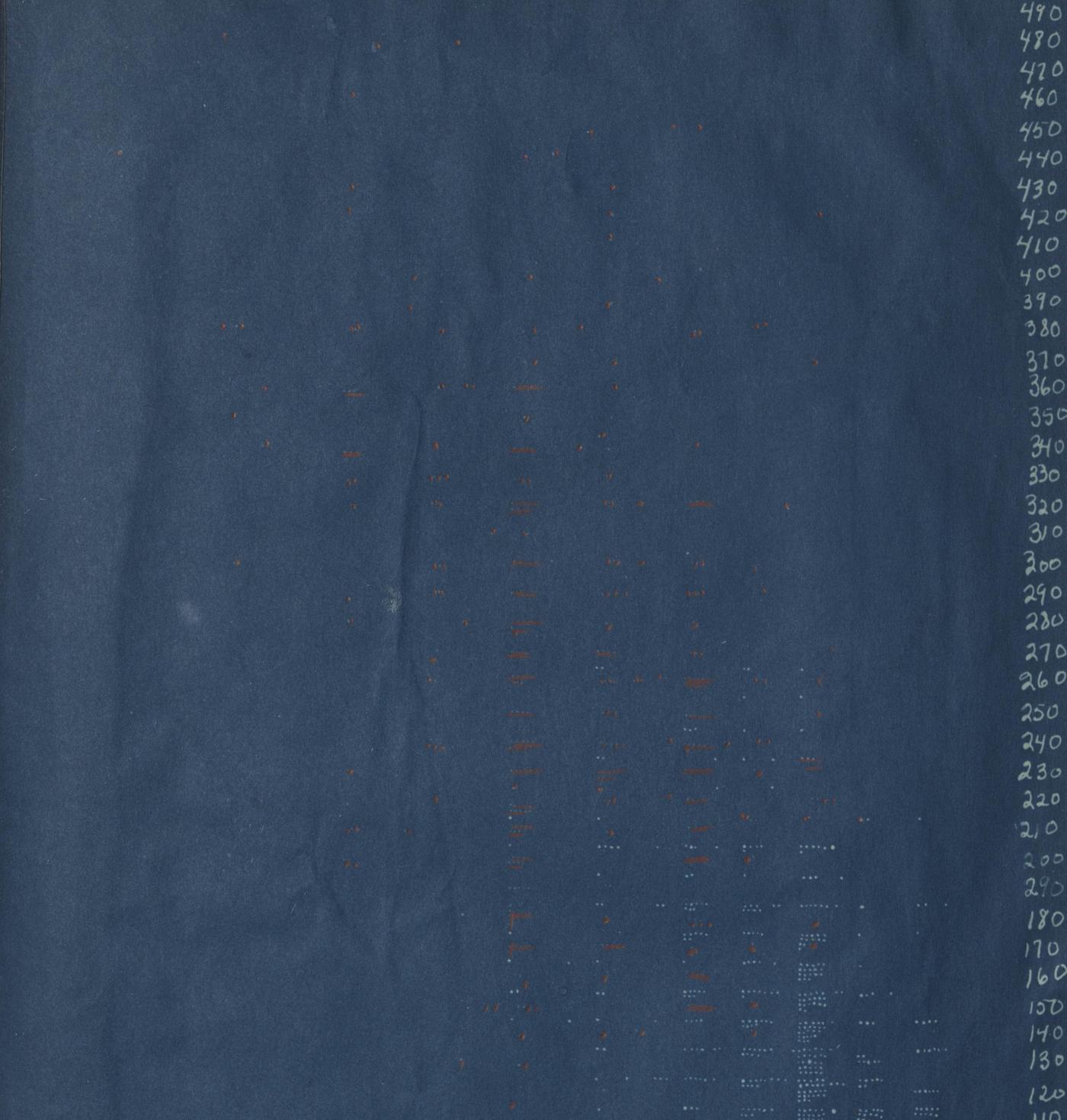
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L x W of Body

In order to discover the effect of age on the size of the body of the ameba a very young culture (2 day) and an old culture (15 day) were used. Both cultures were grown at 28°C. The amebas from a 15 day culture were allowed to set on a slide $2\frac{1}{2}$ hours in a moist chamber and those from a 2 day culture were allowed to set 2 hours in a moist chamber. Slides were fixed in Schaudinn's heated to 60°C. and stained in Iron Haematoxylin. Measurements were made exactly as mentioned previously and only amebas during locomotion were chosen.

In figure 7 the 652 black dots each represent an ameba from a 15 day culture. The 346 amebas from the 2 day culture are each represented by a red dot. The units referring to the body size are arranged along the ordinate ranging from 10 to 500 units and the units referring to the nuclear size along the abscissa range from 1 to 12 units. (one unit = 1.75 microns).

In figure 8, table III represents figure 7 in tabular form. This table shows the distribution of amebas with respect to nuclear size and the body size of three day and seven day cultures. The black numbers indicate the amebas from a 15 day culture and the red those from a two day culture. The totals for each nuclear size and the body size



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40
30
20
10

Figure 7

Flabellula citata

Age of culture -- 15 days (white)
 Age of culture -- 2 days (red)
 Set on slide -- 2½ hrs. (white)
 Set on slide -- 2 hrs. (red)
 Temperature of cultures - 28°C
 Grand Total-- 652 (white)
 -- 346 (red)

17½ 17 16½ 16 15½ 15 14½ 14 13½ 13 12½ 12 11½ 11 10½ 10 9½ 9 8½ 8 7½ 7 6½ 6 5½ 5 4½ 4 3½ 3 2½ 2 1½ 1
 L x W of Nucleus

are shown in appropriate places. The grand total of 15 day culture amebas is 651 (black) and of the two day culture amebas is 346 (red).

In figure 9, the correlation between the nuclear size and the body size of a 15 day cultures is shown. The black dots with figures represent the number of amebas grown in a two day culture. The 12 classes of nuclear size were combined into seven columns for convenience of presentation.

According to the scale selected the correlation curve shows:- (1) Amebas from a two day culture are 50 - 75% larger in body size than in a 15 day culture. (Comparison made by inspection). (2) The nuclear size is 250% larger in amebas grown in two day culture at 28°C. than amebas grown in a 15 day culture at 28° C. (Comparison made by inspection.) (3) The nuclear size increases in direct proportion to the body size in the amebas from both cultures. (4) The rate of increase of the nuclear size is much slower in the 15 day culture than in the two day culture (by inspection).

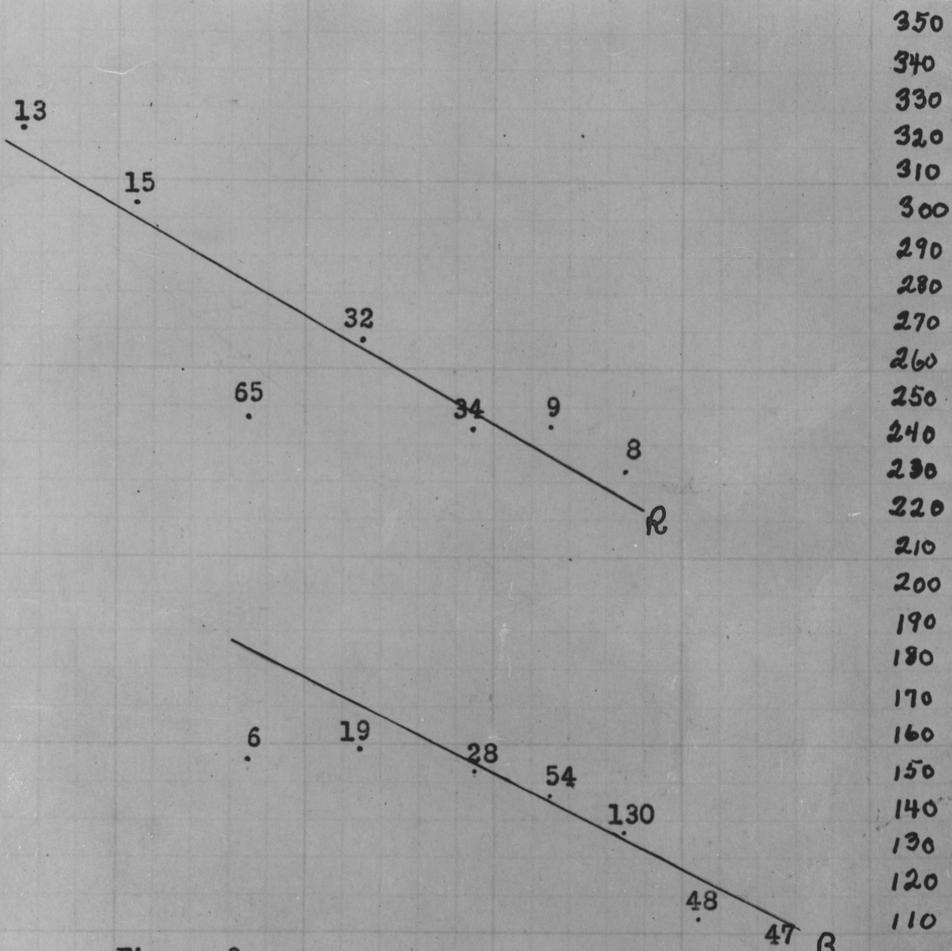


Figure 9

Flabellula citata

- Age of culture --- 15 days (black)
- Age of culture --- 2 days (red)
- Set on slide --- 2½ hours (black)
- Set on slide --- 2 hours (red)
- Temperature of cultures -- 28°C
- Grand Total --- 651 (black)
- 346 (red)

12 10½ 9 7½ 6 5 4 3 2
 L x W of Nucleus

350
 340
 330
 320
 310
 300
 290
 280
 270
 260
 250
 240
 230
 220
 210
 200
 190
 180
 170
 160
 150
 140
 130
 120
 110
 100
 90
 80
 70 Body
 60 W
 50 of
 40
 30 x
 20 L
 10

To determine the effect of age on the size of the body and on the size of the nucleus, amebas of a three day culture and 11 day culture were used. Both cultures were grown at 28° C. and were allowed to stand seven minutes and five minutes respectively in a moist chamber. Slides were prepared as in preceeding cultures and only motile amebas were selected for measurements.

In figure 10 the black dots represent amebas from three day cultures and the red dots represent the amebas from 11 day cultures. The units indicating the body size range from 10 to 500bunits (ordinates). (One unit = 1.75 microns) The units showing the nuclear size range from 1 to 12 units (abcissas). (one unit = 1.75 microns).

In figure 11, table IV represents figure 10 in tabular form. The distribution of amebas with respect to body size and nuclear size is shown. The numbers in black indicate the three day culture and the red numbers the 11 day culture. The total number of amebas of the same body size and of the same nuclear size is indicated at the proper places. The grand total of three day culture amebas (black) is 529 and of the seven day culture is 405 amebas (red).

In figure 12 the correlation curve shows the relation between the nuclear size and body

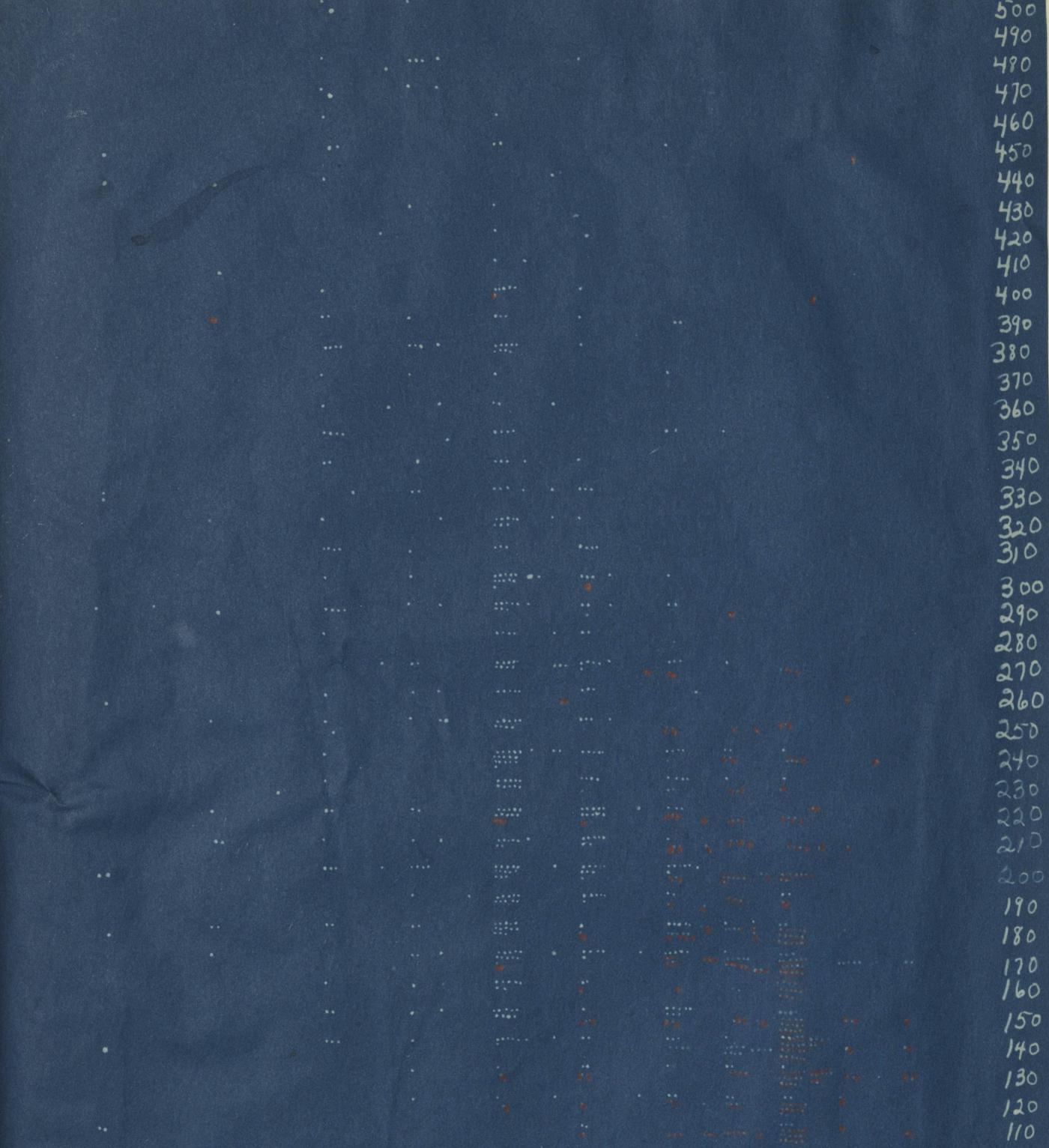


Figure 10

Flabellula citata

Age of culture --- 11 days (red)
 Age of culture --- 3 days (white)
 Set on slide --- 5 minutes
 Temperature of cultures --- 28°C
 Grand Total -- 541 (white)
 -- 407 (red.)

500
490
480
470
460
450
440
430
420
410
400
390
380
370
360
350
340
330
320
310
300
290
280
270
260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

17½ 17 16½ 16 15½ 15 14½ 14 13½ 13 12½ 12 11½ 11 10½ 10 9½ 9 8½ 8 7½ 7 6½ 6 5½ 5 4½ 4 3½ 3 2½ 2 1½ 1

L. X. V. of Nucleus

Number of Rows

size of the three day (black) and 11 day (red) cultures. The black dots with figures represent the number of amebas from a three day culture and the red dots with figures represent the number of amebas from a 11 day culture. The 12 classes of nuclear size were combined into seven and six columns respectively for convenience of presentation.

According to the scale selected the correlation curve shows:- (1) The amebas from a three day culture are 100% larger in nuclear size than amebas from a 11 day culture. (By inspection). (2) Amebas from a three day culture are 80% larger in body size than amebas grown in a 11 day culture (comparison by inspection). (3) In both the 3 day and 11 day cultures the amebas increase in nuclear size in direct proportion to their body size. (4) A more direct correlation exists between the nuclear size and the body size in the 3 day culture than in the 7 day culture.

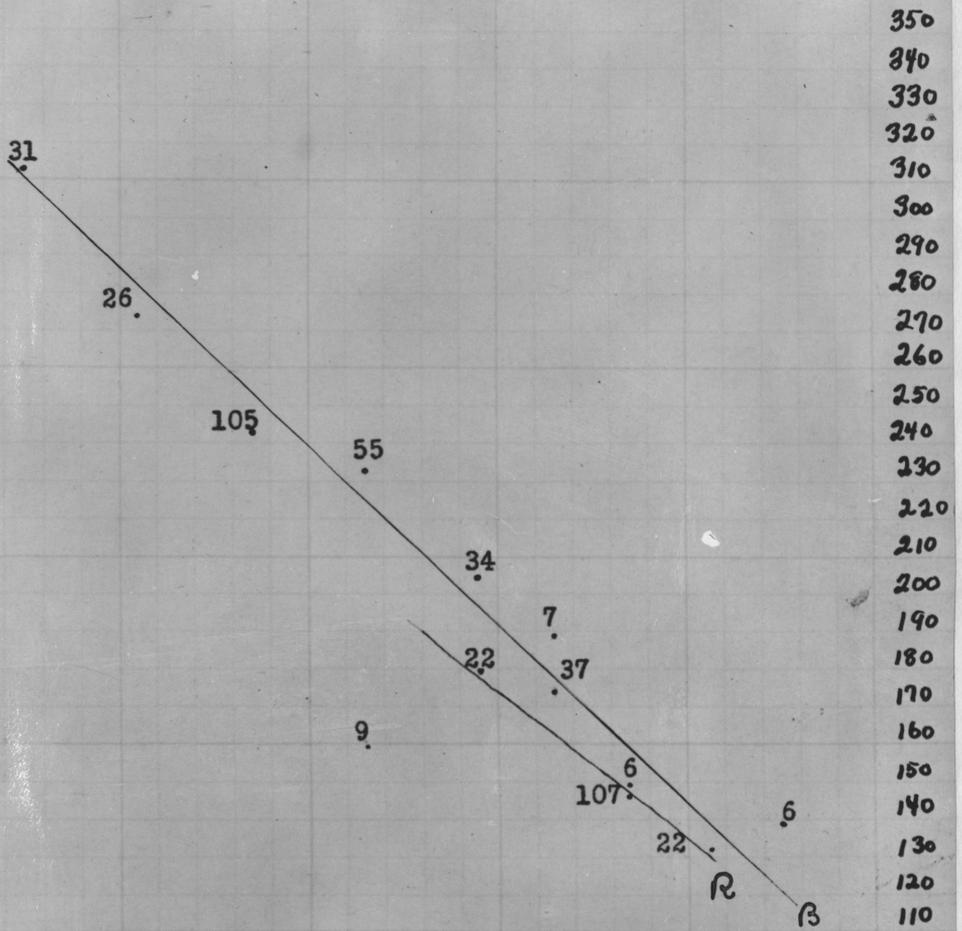


Figure 12

Flabellula citata

Age of culture --- 3 days (black)

Age of culture --- 11 days (red)

Set on slide --- 5 minutes

Temperature of cultures -- 28°C

Grand Total -- 529 (black)

405 (red)

12 10½ 9 7½ 6 5 4 3 2

L x W of Nucleus

350
340
330
320
310
300
290
280
270
260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90 Body
80
70 of
60
50 W
40 x
30 L
20
10

In order to determine the effect of allowing the amebas to set on a slide in a moist chamber amebas was used from a 3 day and a 2 day culture and allowed to set on slides in a moist chamber 30 minutes and 2 hours respectively at 28° C.

In figure 13 the black dots represent amebas from the three day culture which were allowed to set on a slide 30' and the red dots represent those from the two day culture which were allowed to set on slide two hours. The body size ranges from 10 to 500 units (ordinates), (one unit = 1.75 microns) The units showing the nuclear size range from 1 to 12 units (abscissas). (one unit = 1.75 microns)

In figure 14, table V represents figure 13 in tabular form. The distribution of amebas with respect to body and to nuclear size is shown. The black numbers indicate the culture that stood 30' and the red numbers indicated the culture which was allowed to stand 2 hours in moist chamber. The total number of amebas of the same body size and of the same nuclear size is indicated at the proper places. The grand total of the 3 day culture which set 30'

on the slide is 58 (black) and of the 2 day culture which set two hours is 346 (red).

In figure 15 the correlation curve shows the relation between the nuclear size and body size of the three day culture which set 30 minutes (black) and the two day culture which set two days (red). The black dots with figures represent the number of amebas from a culture which set 30 minutes on slide (3 day) and the red dots with figures represent the number of amebas which set two hours on slide (2 days). The 12 classes of nuclear size were combined into seven columns for convenience for presentation.

According to the scale selected the correlation curve shows:- (1) The nuclear size is approximately the same in both cultures (by inspection). (2) The amebas which were allowed to stand two hours were larger in body size due probably to the tendency of these amebas to spread out on the slide, since the nuclear size relations remains constant for both cultures.

The number of amebas measured is small and derived from only a few slides. Owing to this the conclusion should be regarded more or less as provisional in this particular case.

500
490
480
470
460
450
440
430
420
410
400
390
380
370
360
350
340
330
320
310
300
290
280
270
260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

Figure 13 Table V
Flabellula citata
 Age of culture-3 da. (white)
 Age of culture-2 da. (red)
 Set on slide - 30 min. (white).
 Set on slide - 3 hr. (red)
 Temperature of cultures - 28°C
 Grand Total -- 579 (white)
 -- 345 (red)

17½ 17 16½ 16 15½ 15 14½ 14 13½ 13 12½ 12 11½ 11 10½ 10 9½ 9 8½ 8 7½ 7 6½ 6 5½ 5 4½ 4 3½ 3 2½ 2 1½ 1

L x W of Nucleus

Figure 14, Table V
 Flabellula citata
 Age of culture-3 da. (bl)
 Age of culture-2 da. (red)
 Set on slide -30 min. (bl)
 Set on slide -2 hr. (red)
 Temperature of cultures - 28°C
 Grand Total--581 (black)
 346 (red)

0	0	500
0	0	470
0	1	460
0	0	470
2	0	463
0	2	450
0	2	440
1	2	430
2	3	420
0	1	410
5	3	400
2	2	390
7	9	380
0	3	370
3	12	360
3	2	350
4	13	340
1	8	330
1	11	320
6	2	310
2	14	300
6	15	290
3	10	280
8	12	270
13	23	260
4	12	250
13	21	240
8	21	230
20	21	220
14	18	210
20	14	200
18	8	190
29	15	180
32	23	170
26	6	160
26	9	150
34	5	140
40	2	130
40	2	120
45	2	110
36	0	100
30	1	90
37	0	80
16	0	70
7	0	60
1	0	50
2	0	40
1	0	30

Left of Body

Black: Totals = 11 1 3 11 4 3 11 3 10 4 7 5 11 6 4 4 4 1 0 0 2
 Red: Totals = 23 0 1 20 8 1 20 2 2 5 4 5 4 6 1 16 0 15 0 0 0 0
 12 11 11 10 10 9 8 8 7 6 6 5 5 4 4 3 3 2 1 1
 L x W of Nucleus

Actual

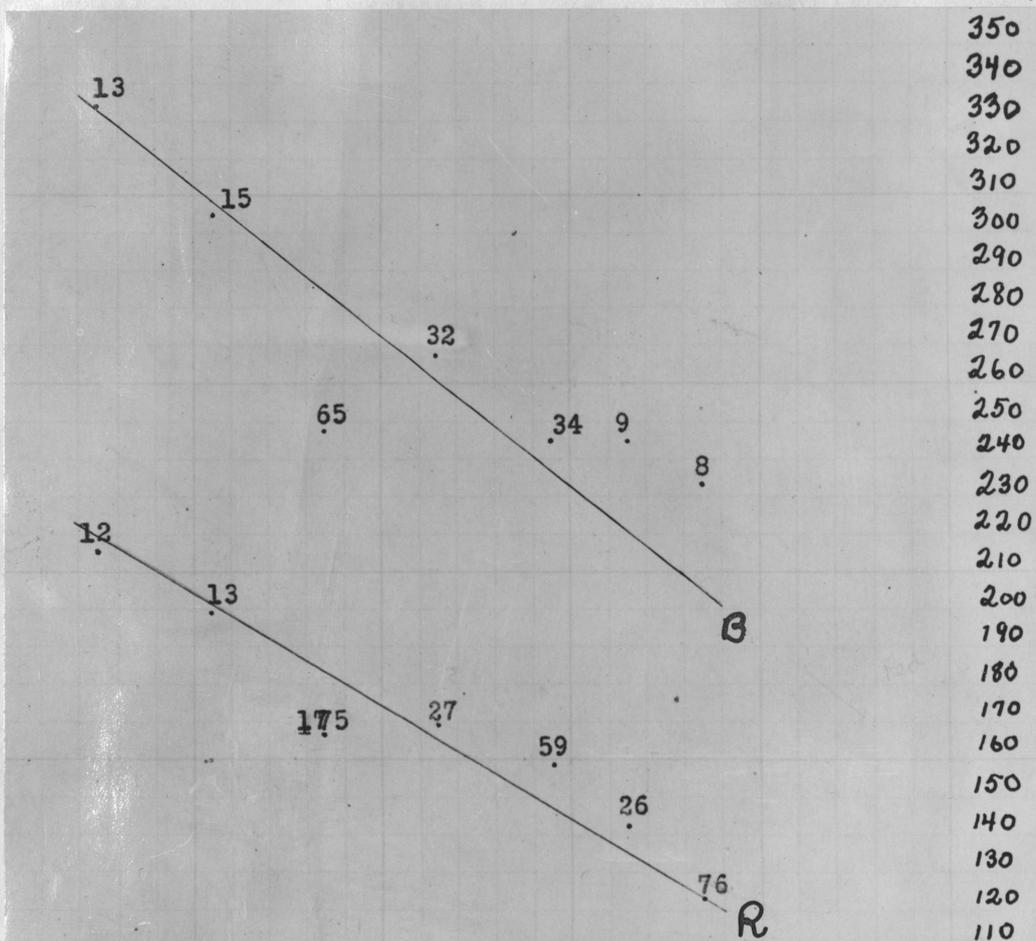


Figure 15

Flabellula citata

Age of culture --- 3 days (black)
 Age of culture --- 2 days (red)
 Set on slide --- 30 min. (black)
 Set on slide --- 2 hrs. (red)
 Temperature of cultures -- 28°C
 Grand Total -- 581 (black)
 346 (red)

350
340
330
320
310
300
290
280
270
260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

12 10½ 9 7½ 6 5 4
 L x W of Nucleus

In order to discover the effect of age on the size of the body and on the size of the nucleus, amebas from seven and 21 day cultures grown at 13.5°C. were used. The amebas from the seven day culture were allowed to set on the slide 12 hours and those from 21 day culture were allowed to set 10 minutes.

In figure 16 the black dots represent the amebas (921) from a seven day culture, each dot representing an ameba. The amebas (653) from the 21 day culture are each represented by a red dot. The units referring to body size range from 10 to 500 units (ordinates, one = 1.75 microns) The units referring to the nuclear size range from 1-12 units along the abscissa. (one unit = 1.75 microns).

In figure 17, table VI represents figure 16 in tabular form. This table shows the distribution of amebas with respect to nuclear size and body size of seven day and 21 day cultures. The totals for each nuclear size and each body size are shown in appropriate places. The grand total of the seven day culture is 920 (black) and from the 21 day cultures in 653 (red).

500
490
480
470
460
450
440
430
420
410
400
390
380
370
360
350
340
330
320
310
300
290
280
270
260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

Figure 16

Flabellula citata

Age of culture ----- 7 days (white)
 Age of culture ----- 31 days (red)
 Set on slide ----- 12 hr. (white)
 Set on slide ----- 10 min. (red)
 Temperature of cultures - 13½°C
 Grand Total ----- 921 (white)
 653 (red)



17½ 17 16½ 16 15½ 15 14½ 14 13½ 13 12½ 12 11½ " 10½ 10 9½ 9 8½ 8 7½ 7 6½ 6 5½ 5 4½ 4 3½ 3 2½ 2 1½ 1

L x W of Nucleus

In Fig. 18, the correlation between the nuclear size and the body size of the seven and 21 day cultures is shown. The black dots with figures represent the number of amebas grown in a seven day culture and the red dots with figures, the amebas grown in a 21 day culture. The 12 classes of nuclear size were combined into six columns for convenience of presentation.

According to the scale selected the correlation curve shows:--

- (1) Amebas are about the same size in the seven and 21 day cultures.
- (2) There is not the same amount of "drop" in the old cultures grown at 13.5°C . as in the old cultures grown at 28°C .
- (3) The proportion of nuclear size to cell size is nearly the same (by inspection).
- (4) The correlation of nuclear size to body size is definite in both cultures.
- (5) The nuclear size of amebas from both cultures increases in direct proportion as the body size.

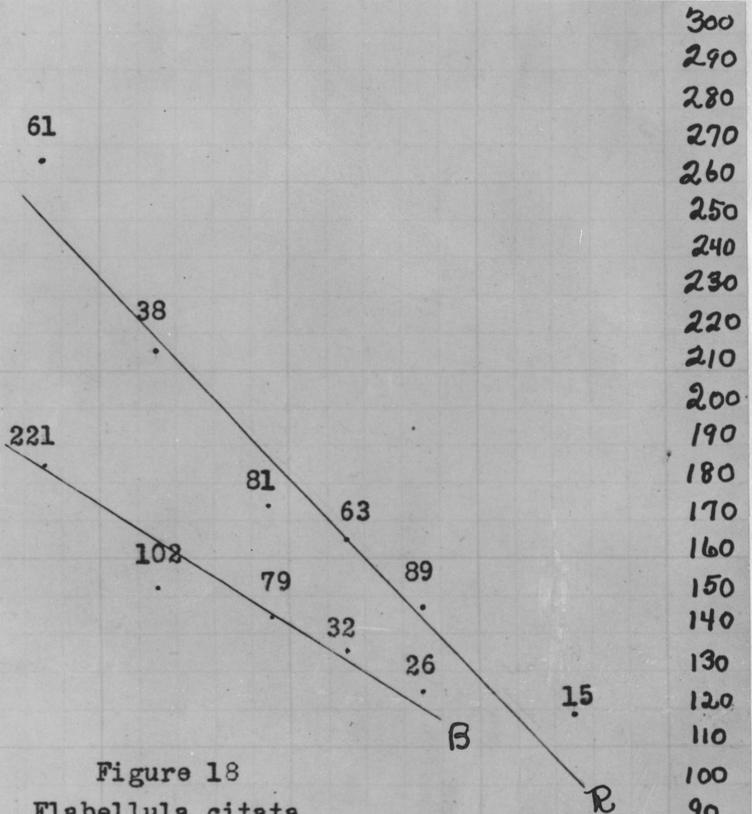


Figure 18

Flabellula citata

Age of culture ----- 7 days (black)
 Age of culture ----- 21 days (red)
 Set on slide ----- 12 hrs. (black)
 Set on slide ----- 10 min. (red)
 Temperature of cultures - $13\frac{1}{2}^{\circ}\text{C}$
 Grand Total --- 920 (black)
 653 (red)

10 9 7 6 5 4 2
 L x W of Nucleus

300
290
280
270
260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
L x W of Body

Mitosis

Mitotic Stages of Flabellula Citata

The division process of Flabellula Citata is promitotic. It possesses a karyosome which forms polar masses. A spindle is formed between these masses and the entire process occurs within the nuclear membrane. The amebas appear to move about during most of the stages of the mitotic process.

Interphase--The karyosome is a spherical deeply stained body which lies usually in the center of the nucleus with a clear area surrounding it. The Peripheral Chromatin is composed of small granules and lies quite evenly distributed, on the inner surface of the nuclear membrane.

Prophases. The chromatin is broken up into small granules which are evenly distributed over the karyosome (Pl. V. Fog. 1.). The first indication of division is the elongation of the karyosome and with it the stretching out of the nuclear membrane (Plate V. Fog. 2.). As the karyosome was in the process of elongating there were about twenty granules visible in several cases. (Plate V. Figs. 4,5). Between these karyosomic masses, there appears a much lighter stained area which later formed the spindle fibers. This is achromatic material. (Plate VI. Fig. 15). Simultaneous with the above process, the peripheral chromatin migrates in from

the nuclear membrane toward the Karyosome. It forms, in part, perhaps the chromosomes which soon become gathered on the spindle fibers at the equatorial plate. (Plate VI. Fig. 10).

Metaphase--In the metaphase, an equatorial plate is observed with darker chromatin masses arranged on it. (Plate VI. Fig. 10 and 11). There are about twenty chromosome masses arranged on it. The chromosomes are of uniform size which do not appear to divide synchronously.

Anaphases--The chromosomes gradually move toward the Karyosomic masses. In Plate VI. Fig. 11 is shown an early anaphase while later stages are shown in (Plate V. Fig. 6 and in Plate Vi. Figs. 9, 11, and 13.)

Telophase--The chromosomes fuse with the polar masses. The spindle constricts until it is finally divided into two parts. The nuclear wall divides and the two daughter nuclei are gradually drawn apart. Reconstruction begins at once after constriction of the spindle. (Plate Vi. Fig. 16)

Cytoplasmic division--The division of the nucleus is normally complete before the ameba begins to divide into two new individuals, however, at this time reconstruction may not be complete. During division the ameba moves about freely. When this ameba is dividing it is often in the form of a

"fan shape" with the clear ectoplasm visible as a zone along the anterior margin. As the daughter amebas move apart a long, slender filament was observed between them. When this breaks the amebas are free individuals. (Plate V. Fig. 7 is an individual which has perhaps just become separated and the process of reconstruction is not complete). Cytoplasmic division appears to follow soon after nuclear division.

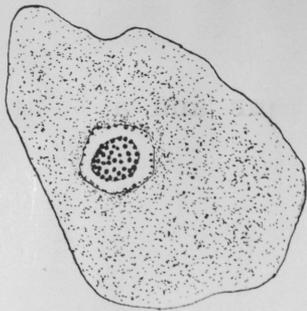
These observations on mitotic stages of Flabellula citata are not to be regarded as complete, but only as a preliminary study.

Explanation of Plates

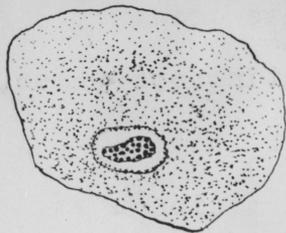
All the figures of Flabellula Citata were drawn with a camera lucida, 10 x compensating eye piece, ap~~h~~romatic lens and a tube length 160. All material was fixed with Schaudinn's solution and stained with iron haematoxylin.

Plate I.

- Figure 1--Resting stage. Early prophase showing Karyosomic granules, clear area, and peripheral chromatin.
- Figure 2--Early p~~e~~r^ophase with karyosome and nuclear wall beginning to elongate. Peripheral chromatin still can be observed.
- Figure 3--Early prophase showing karyosome masses stretching apart. This slide was not differentiated very far.
- Figure 4--Telophase--Reconstruction not complete.
- Figure 5--Prophase showing elongating of karyosome and nuclear wall.
- Figure 6--Anaphase showing spindle fibers on which the chromosomes are migrating toward the polar masses.



1



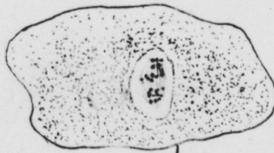
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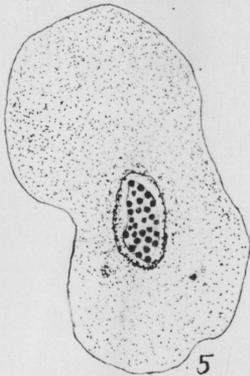
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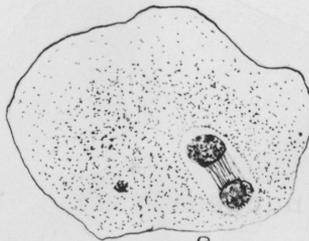
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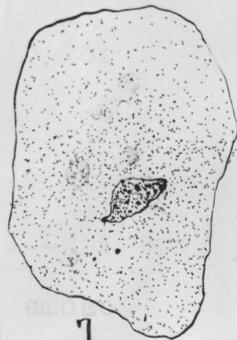
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5



8



7

Figure 7--Telophase--perhaps just divided and
reconstruction incomplete.

Figure 8--Late anaphase--showing the daughter
nuclei and spindle fibers remaining.

Figure 9--Early anaphase showing the chromosomes moving toward the polar masses.

Figure 10--Metaphase with equatorial plate and chromosomes massed together. Chromosomes are slightly elongated as if they were splitting lengthwise.

Figure 11--Late metaphase with polar masses and spindle fibers, and few chromosomes beginning to move toward poles.

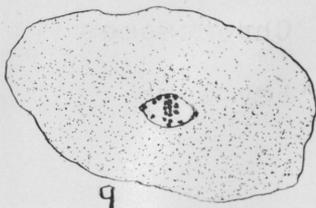
Figure 12--Metaphase side view showing spindle fibers and polar masses.

Figure 13--Prophase with chromosomes beginning to form an equatorial plate.

Figure 14--Prophase Karyosome elongating to form polar masses.

Figure 15--Prophase with spindle fibers (achromatic) beginning to form between karyosomic masses.

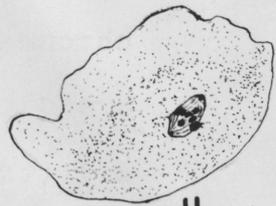
Figure 16--Telophase showing daughter nuclei with reconstruction incomplete.



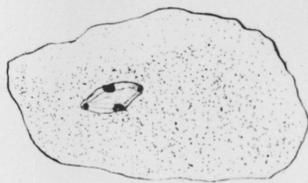
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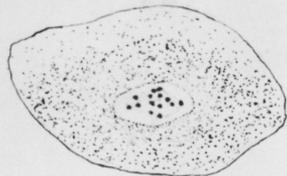
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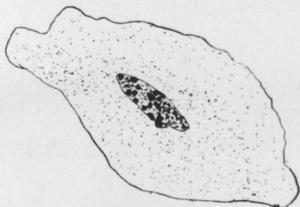
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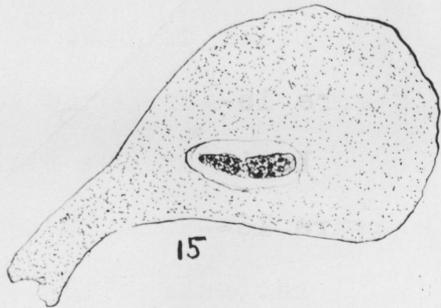
12



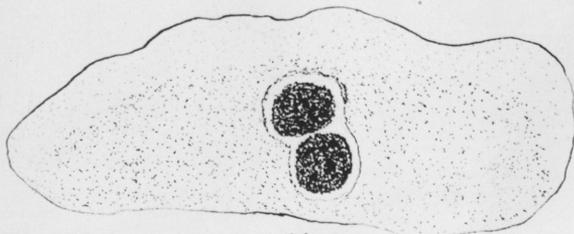
13



14



15



16

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