· THE ·
REGULATION
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THE REGULATION OF SIZE
AS ILLUSTRATED
IN UNICELLULAR ORGANISMS

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WITH 66 FIGURES AND 15 TABLES

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PREFACE

Small beings may present large problems. Thus far the micro-organisms exhibit attributes more worthy of note than do the scientists who are and should be studying them. The recognition and description of the problems constitute, however, the first step in their analysis and solution.

The problem of body size dates from the days when giants were thought to walk the earth. There are two main reasons for beginning the study of size regulation upon unicellular organisms. First, their patterns and functions are most readily modified by changes of environment, so that influential factors may be quantitatively identified in isolation. Second, uniparental inheritance is the rule, with the result that, in any species, genetically uniform individuals may be obtained for study. This is an advantage which has received too little attention from physiologists.

To measure and control the environments of organisms the biologist has had to place his pets under laboratory conditions which are often stigmatised as highly artificial. But who can elucidate cause and effect, factor and regulation, among *Bacilli coli* while living in their usual environments? On the other hand, it is a common failure not to remember that the environments determine what properties organisms shall exhibit. In many biological studies, just as much natural history is manifested in one set of conditions as in another.

It is hoped that the data which are analysed and synthesized in the following pages have been arranged and weighed as objectively as a live science will permit.

E. F. ADOLPH

Rochester, New York,
March 24, 1930.

*It is the capacity of organic form
to gather and store experience which
demarks it from the inorganic.*

—N. S. SHALEK

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THE REGULATION OF SIZE
AS ILLUSTRATED
IN UNICELLULAR ORGANISMS

CHAPTER I

BODY SIZE AS STRUCTURE AND AS FUNCTION

EVERY KIND of living organism is distinguished by its size. Size is one of the chief of its properties, and the bodies of individuals belonging to one kind are alike in bulk. Even a giant or a pigmy is not so different from the average individual as is usually the average individual of another species. Among men, for instance, all adults fit the same doorways and the same beds; Procrustes had to be excessively particular to find any cause for mutilating his victims. A hasty comparison with all men of all the inorganic objects of some one kind, such as all crystals of a single kind of salt, all granite rocks, or all dolomite mountains, reveals the extent to which size characterizes men.

1. SIZE A REGULATED CHARACTERISTIC

Metabolism and size. Uniform size of body and of its parts is not, however, peculiar to living things. Drops of water and grains of sand are each similar to one another in volume as well as in shape. But a living body does not keep its component materials as does the grain of sand, nor depend upon isolation for its integrity as does the drop of water.

The organism is, in fact, continually exchanging its constituents for new ones. Thus, its water is constantly being thrown away, only to be replaced by other molecules which are the same in every respect. The living body is like a river; its content is ever changing but its shape and its size remain unmodified to any large extent. A river is contained in a bed which limits it; but the mold in which the individual

organism is confined is invisible, for its boundaries and its skeleton are also changing. It is independent of any particular structural constituent and maintains its individuality in a medium composed of the same substances as itself. Driesch ('98) suggested that the accuracy with which size is controlled by metabolizing organisms proved the presence of a sort of regulation which could not be performed in the absence of special vital properties.

Regulation. The concrete resultant of the various processes which produce the constancy of size in an organism, and among organisms, is not unique. Every characteristic of a living being represents the manifestation of similar regulations. Each specific structure, each mode of physiological response, each activity, is a product of accurately measured functions. But the regulation of size is certainly as universal as, and perhaps more universal than, any other function that has yet been measured in living organisms.

The characteristic called size has not been ignored by students of life; there have been numerous discourses upon the theoretical importance of it, and upon the physical forces probably involved in its control. But experimental work has been infrequent and of an isolated character. Nevertheless, for many kinds of organisms there exist data which, though having been recorded chiefly for other purposes, yet deserve to be correlated with one another, toward the end of finding what factors are directly concerned in the regulation of size. The cultivation of this field of science-in-the-making will undoubtedly result in a yield that will be significant in proportion to the intensity of the cultivation.

In the present review it is proposed to discover what conclusions can be drawn regarding the everyday methods by which organisms regulate their sizes, from

an examination of the data, heretofore scattered, that have been obtained by measuring unicellular organisms. While the data to be actually analysed are limited to one class of organisms, it is fairly certain that conclusions reached will be applicable to many or all kinds of organisms or their constituent tissues and cells. Moreover, some problems of size, such as the fundamental one of the cessation of the increase in size at maturity, can best be studied upon organisms such as unicellular ones whose inhibitors of growth are chiefly outside their bodies.

The problem in protista. Among most species of unicellular organisms the question of "how big" is presented in an extraordinarily simple way. A typical individual spends definite amounts of time and of activity in gaining to itself more body substance; it grows. Then it divides into two individuals. How is the point of time set at which the individual has gained substance enough to become two individuals? Or, in other species, an individual of adult size forms a protuberance or bud as it accumulates living material in excess of its own natural size, until the material in the bud is equal in bulk to the parent, when separation into two adults occurs. What keeps the size of the parent from increasing within itself; and why does separation occur upon the attainment of the specific size?

It must first be ascertained just how precisely the adult size is controlled. An estimate of this precision is to be obtained by measuring under the microscope (with an ocular micrometer) a considerable number of individuals. In a certain sample of trypanosomes, the uniformity of body length shown by the frequency curve of figure 1 was found. In this case the individual trypanosomes were automatically selected as adults, for Taliaferro ('23) observed that, as these trypanosomes were cultured in the body blood of a rat, their

reproduction ceased after some days of infection, and a maximum growth was then attained by each parasite. The lengths of the individuals in this sample are so similar, that three-fourths of the individuals differ from the mean length by only 2.5 per cent of the mean

Figure 1. Distribution of total body lengths in 100 individuals of *Trypanosoma lewisi*. The standard deviation is 2.68 per cent of the mean length, which is the lowest coefficient of variation reported for any measurement on any unicellular organisms. The trypanosomes were a clone in the blood of a single rat on the 49th day of the infection. (Redrawn from Taliaferro, '23).

length. This value for the tendency to deviate from the mean is the coefficient of variation. The uniformity among this group of a hundred parasites of one race is much greater than could be obtained among a hundred sticks if an attempt were made to cut them to a uniform length without any means of measurement. It is much greater than the uniformity of the heights of the men or of the women in any human population.

It is the purpose of these chapters to attempt to identify some of the means which an individual organism, such as one trypanosome, has of measuring itself so that growth shall proceed or stop, or fission shall intervene, in the way that it naturally does.

The cell theory. Unicellular organisms are characterized by their universally microscopic size. They are often designated as micro-organisms, though there are some multicellular organisms which fall within their range of sizes. The fact that living structures cannot attain to the largest sizes without organization into more than one cell, has usually been taken to mean that some barrier of size limits the efficiency of single cells.

The cell theory, the very principle which gave a basis for generalization with respect both to structure and to function among organisms, has always recognized that cells are minute, hence limited in size. But the meaning of this simple fact is still obscure, even though much discussion has flowed concerning surface-mass ratios, surface tension-viscosity ratios, and many other possibly important quantities. Actually, within the general range of sizes among cells, the diversities of bulk are so enormous that none but specific populations can be profitably discussed.

How unicellular individuals are to be compared with multicellular tissues, has never received a general answer. In some respects and in some instances the analogy of the protistan individual to a single metazoan cell is fairly complete. In other instances, as in multinucleate types, protista are better regarded as non-cellular in structure. Certainly cytoplasmic material separated from all nuclear material can carry on neither growth nor reproduction. Size is therefore typically regulated only in living units consisting of both nucleus and cytoplasm.

Further, differentiations of other sorts, both structural and functional, are, it seems, inseparably connected with the living state. It is apparent, therefore, that the mechanisms of size regulation can never be studied in homogeneous units. The "inclusions" are integral parts of any protoplasm.

A minute study. Those unicellular organisms that are most complicated in visible structure are of course the larger species. It is the larger types, such as ciliated protozoa, that have inevitably been most studied with respect to size. Yet the individuals even of these kinds are extremely small; it takes a million of the largest race of *Paramecium* to fill a volume of one cubic centimeter. All the unicellular individuals that have ever been measured, and that are likely to be measured for some years to come, would not occupy a volume equal to these pages.

2. METABOLIC TURN-OVER

When the dimensions of a unicellular organism are ascertained, there are lumped together in one measurement a large variety not only of structures, but as well of chemical substances. Unicellular organisms, like larger organisms, and probably to a greater extent than larger organisms, are very variable in chemical composition.

Water. As of nearly all organisms, water is their chief constituent. In *Paramecium caudatum*, water is said, by Grobicka and Wasilewska ('25), to constitute 87.4 to 91.6 per cent of the body substance. Among yeasts and bacteria, where many analyses have been made, the values range from 68 to 91 per cent. (Buchanan and Fulmer, '28). A. I. Dawson ('19) found that the water content of a certain strain of *Bacillus coli*, cultivated on an agar medium, changed tremendously with the age of the culture, as is shown in figure

2. These values mean that the measurement of an individual *Bacillus coli* on the tenth day of culture represents nine times the amount of solid matter that it represents on the second day of culture.

In general, the size as measured represents very closely the absolute amount of water incorporated in

Figure 2. Progression in percentage water content of *Bacillus coli* with age of culture, on an agar medium at 37°C. (Data of A. I. Dawson, '19).

the individual, but only very approximately the amount of any other chemical entity. Structurally we cannot tell the difference between an organism which is nearly all water and one which is in large proportion other substances. In any case the substance measured can only be obscurely termed "protoplasm."

Food vacuoles. But the visible "inclusions" of a protoplasm are as variable as its water content. The combined volumes of all the food vacuoles have been determined by Unger ('26) in a large number of individual *Paramecia*. The modal volume of the vacuoles in a race of *Paramecium aurelia* turns out to be one-sixth of the total body volume of modal individuals of the same race as measured by Erdmann ('20). A few estimates of food vacuole volumes made by Eisenberg ('26) in *Paramecium caudatum* indicated that the vacuoles occupy 7 per cent of the body volume. A *Paramecium* carrying on active digestion, therefore, has reason to be larger than a starved one by at least this much.

J. A. Dawson ('19) showed that *Oxytrichæ* may ingest their cousins of the same species until they become truly giant individuals. In one instance fifteen victims had been ingested, and the giant had thus made itself at least eight times as large as its non-cannibalistic relatives. Most of the extra size consisted at first of unincorporated food, and could in no wise be considered as part of the body substance of the giant. The giant was large not in its own right but because of foreign puffing. Similar instances were described by Mast ('09); a *Didinium* may ingest a *Paramecium* of ten times its own volume, and then pass through three or four generations on the strength of the food and the bulk thus obtained.

Contractile vacuoles. Contractile or excretory vacuoles do not ordinarily constitute so large a portion of the body mass as do food vacuoles. In an average *Ameba* the contractile vacuole was one-hundredth of the body volume, and in one extreme case was as much as one-twentieth (Adolph, '26).

For the one substance water, calculations can be made as to how fast the material is turned over or re-

placed in the body of a unicellular individual. They have been made for several species where the body produces contractile vacuoles, by assuming that all the

Figure 3. Progression of the volumes of contractile vacuoles in each of three individuals of *Ameba proteus*. The data were obtained by measuring the diameters of the vacuoles at successive times. (From Adolph, '26).

water which passes out of the body does so through the vacuoles. It is necessary then only to measure the maximal volumes and the frequency of contractions

of such vacuoles. It is characteristic of individual protozoa, though not of clones or of species, that in the same medium the final sizes of successive vacuoles are relatively uniform. This is indicated for *Ameba* in figure 3.

In table 1 are presented the data obtained by various observers for the rates of water output through vacuoles in numerous species. Unfortunately most of the

TABLE 1.
RATES OF TURN-OVER OF WATER THROUGH THE CONTRACTILE VACUOLES OF VARIOUS CILIATES AND
OF AMEBA

Species	Temp. °C	Duration of diastole seconds	Body volume μ^3	Time required for excretion of one body volume minutes	Data of
<i>Cryptochilum nigricans</i>	28	..	..	2.0	Maupas ('83)
<i>Lembus pusillus</i>	26	..	..	2.5	"
<i>Euplotes patella</i>	25	..	..	14.3	"
<i>Stylonychia pustulata</i>	24	..	..	20.5	"
<i>Stylonychia mytilus</i>	18	..	..	45.	"
<i>Leucophrys patula</i>	21	10	468,000	44.	Maupas ('88)
<i>Glaucoma colpidium</i>	18	14	..	35.	Degen ('05)
<i>Gastrostyla steinii</i>	18	10	..	43.	Herfs ('22)
<i>Nyctotherus cordiformis</i>	25	72	..	245.	"
<i>Urotricha globosa</i>	..	30	220	12.1	Metzner ('27)
<i>Cyclidium glaucoma</i>	..	8	1,660	6.6	"
<i>Halteria grandinella</i>	..	7	14,100	7.4	"
<i>Urocentrum turbo</i>	..	15	36,900	13.2	"
<i>Vorticella convallaria</i>	..	11	98,600	47.3	"
<i>Colpidium colpoda</i>	..	20	229,000	53.	"
<i>Frontonia leucas</i>	..	10	2,211,000	143.	"
<i>Spirostomum ambiguum</i>	..	780	13,100,000	261.	"
<i>Paramecium aurelia</i>	27	..	..	46.	Maupas ('83)
<i>Paramecium caudatum</i>	22	..	194,000	23.	Hance ('17)
" "	22	11.0	284,000	35.	"
" "	22	8.2	398,000	49.	"
" "	22	6.2	..	14.7	Herfs ('22)
" "	26	10.4	350,000	38.	Adolph ('26)
" "	20	8.6	493,000	20.9	Eisenberg ('26)
" "	..	20.0	653,000	42.2	Metzner ('27)
<i>Ameba proteus</i>	23	390	3,200,000	606.	Adolph ('26)
" "	26	224	4,000,000 (?)	480.	Botsford ('26)
" "	..	480	14,100	98.	Metzner ('27)

data are extremely approximate, for only the frequencies of emptying were measured on living individuals, whereas the vacuolar sizes, which may not be maximal or may be super-maximal, were taken upon fixed specimens. But for *Ameba* (Adolph, '26; Botsford, '26; Howland and Pollack, '27) the same vacuoles which were timed were also measured at the time of bursting.

Setting aside the question of the essential functions

of contractile vacuoles, it is evident that in most protozoa tremendous volumes of water are taken into the body and excreted again. The fastest turn-over belongs to *Cryptochilum* (Maupas, '83); while *Paramecium caudatum* puts out its own volume of water in 15 to 50 minutes. The slowest turn-over recorded is for an *Ameba* which required 31.5 hours to put out its own volume (Adolph, '26).

Surface permeabilities. Intimately related to the question of vacuole formation are the permeabilities of the superficial boundaries of unicellular organisms. The distributions of materials which they maintain are to a small extent known, but practically nothing is certain with respect to their means of regulating the passage of substances. That the body's boundaries are important in this regulation is illustrated by the older observations of Brandt ('95) upon the specific gravity of the large marine colonial radiolarian *Thalassicola*. He observed that the specific gravity of the body or its vacuoles was much less than that of the sea-water, and so the salt content of its fluids must have differed from that of sea-water. More extensive observations of Harvey ('17) on *Noctiluca*¹ show that the specific gravity may readily change in the body, and in a direction opposite to that which would be attained if the salts became equally distributed between body and medium. Changes of permeability, presumably at the surface, have been demonstrated in a great many species of unicellular organisms.

It must be fairly evident that all sorts of properties of the surfaces and of the internal structures of unicellular organisms are directly concerned with the regulation of the passage of those substances that constitute the body. But at present almost no correlations among these properties can be drawn. The passage of sub-

¹ Also of Ludwig ('28c).

stances, in turn, has to do with the amount of each present at any moment. And the amount present is a component of the total body substance present; namely, the body size. The problem of size regulation is obviously in an initial stage of study; body sizes may be compared, though in complete ignorance of the roll of the components, with the exception of slight information concerning the one component, water.

3. SOME THEORETICAL POINTS

At the present time the consideration of various simple physical and geometrical factors is found to be singularly futile in solving the problem of the regulation of size, for in no case has the role of any one fundamental factor been evaluated. It is worth while, however, to mention a few of the likely factors in the hope that their application may become, in course, justified.

Similitude. The principle of similitude states that similarly shaped and proportioned objects which differ in size have significantly different properties. This principle, and its application to biological matters, was probably first fully recognized by Galileo (1638). He stated, "In a diminishing solid the weight grows less in proportion to the volume, and since the volume always diminishes more rapidly than the surface, when the same shape is maintained, the weight must diminish more rapidly than the surface". The significance of this relation depends upon what processes are limited by the surface and what properties depend on the volume. Spencer ('66) believed that inertia and gravity are the essential correlatives of volume, while cohesion and absorption are limited by surface. Expenditure in work increases as the cube of the linear dimension; supply or absorbing surface as the square.

The sizes of micro-organisms must be chiefly dependent upon a very different set of factors from the sizes of large organisms. Intermolecular and surface forces

are in the former highly important, convection of molecules and gravity are probably never limiting circumstances (Thompson, '17). Thus, the surface-electrical charge upon a bacterium may be of great consequence for this individual, but can play no role of life and death in a whale. Again, forces with which aquatic organisms are concerned are far other from those with which terrestrial organisms cope. Viscosity of the medium and convection of its constituents assume different roles. For two *Paramecia* of different lengths to swim at the same velocity, the force expended in resistance to the medium is as the square of the length, and the force required to accelerate by the same amount is as the cube. But that any of these is a limiting factor in controlling the size of *Paramecium* itself, has not been discovered.

The consequences of subdividing *ad infinitum* a sphere or a cube have frequently been pictured, as by Ryder ('93a). The acceleration of the increase in surface-mass ratio as subdivision proceeds serves to focus attention upon certain classes of geometrical factors; but no real progress has been made until some of these possible factors have been tested to find whether they are actually effective in modifying size.

Lower limits to size. As is well known, there are theoretical lower limits to the size of organized beings. One of the obvious limits, as was pointed out by Maxwell ('75), is the size of one molecule itself. Errera ('03) further developed this line of argument. All living matter that is known contains proteins. A spherical micro-organism less than 0.01 micron in diameter, and having 14 per cent of proteins, could contain only about ten molecules of proteins. If it be assumed that the proteins present contain sulfur in about the proportion usual in living matter, such an organism would contain not more than three atoms of sulfur.

Figure 4. Diagrammatic representation of several large species of unicellular organisms, all drawn to the same scale. Nuclei are stippled, vacuoles are outlined. 1. *Spirostomum ambiguum* (posterior half only). 2. *Actinosphaerium eichhorni* (pseudopodia amputated). 3. *Difflugia corona*. 4. *Frontonia leucas*. 5. *Colpoda cucullus*. 6. *Colpidium colpoda*. 7. *Arcella dentata*. 8. *Ameba proteus*. 9. *Spirogyra bellis*. 10. *Stylonychia pustulata* (side view). 11. *Paramecium caudatum*. 12. *Actinophrys sol*. Linear magnification $\times 175$.

Figure 5. Several small species of unicellular organisms, all drawn to ten times the linear scale of the preceding figure. 12. *Actinophrys sol* (pseudopodia amputated). 13. *Polytoma uvella*. 14. *Saccharomyces cerevisiae*. 15. *Chilomonas paramecium*. 16. *Pleurococcus vulgaris*. 17. *Trypanosoma lewisi*. 18. *Micrococcus progrediens*. 19. *Bacillus coli*. 20. *Bacillus ramosus*.

The limit of vision through a microscope, due to the length of light waves, is about 0.20 micron. It seems as though the varieties of ultra-visible organisms could not be very numerous. Some of the smallest organisms which have been seen by direct vision are compared in table 2. It is not wholly certain that there exist organisms which cannot be seen.

TABLE 2.
SOME SMALLEST VISIBLE AND SOME LARGEST KNOWN SPECIES OF UNICELLULAR ORGANISMS. VOLUMES ARE CALCULATED AS $l \times b \times t \times \pi/6$.

Species	Length μ	Breadth μ	Thick- ness μ	Volume cubic μ	Reference
<i>Pseudomonas indigofera</i>	0.18	0.06	0.06	0.0003	Errera ('03)
<i>Micrococcus progrediens</i>	0.15	0.15	0.15	0.0018	"
<i>Bacillus pneumosintes</i>	0.30	0.15	0.15	0.0053	Tanner ('28)
<i>Frontonia leucas</i>	280	150	130	2,900,000	Popoff ('08)
<i>Actinosphaerium eichhorni</i>	228	228	228	6,170,000	author
<i>Spirostomum ambiguum</i>	1,600	125	125	13,100,000	Metzner ('27)
<i>Opalina ranarum</i>	750	430	230	38,800,000	Doflein ('11)

Relative sizes. In table 2 are given, for comparison with the two species of minute bacteria, the smallest known species of protozoan (*Pseudomonas*) and three of the largest species of unicellular organisms, which however are not the very largest. The body of one *Spirostomum* then contains 4×10^{10} times as much substance as the body of a *Pseudomonas*. Even in length it is 9×10^4 times. The hugeness of the diversity in volumes may be made clear by stating that an average man has only 5×10^6 times the volume of a *Spirostomum*. On the whole, there is as much diversity in size among unicellular organisms, as there is between unicellular organisms and any other group of organisms. Their unicellular structure is clearly no explanation of their size.

The diversity of sizes within the group of unicellular species is not generally appreciated, for the simple reason that in practice the magnification of the microscope or the scale of the illustration is suited to convenience. To the investigator, as well as to the textbook reader, there appears to be little difference of size between an average flagellate and a bacterium. Even upon a single page one usually views a heterogeneous selection of species without reference to their relative sizes. To bring to mind just what it means to compare the bulks of various species, representatives of a number are rendered to scale in figures 4 and 5. To get a small bacterium delineated as the shortest possible line it was necessary to increase the linear scale ten times, therefore the volume scale a thousand times, between figure 4 and figure 5.

Size factors. The number of factors which may possibly limit the size attained by a particular organism is very large. The number of combinations of these factors is still larger. While some of the factors may be recognized as physical forces or chemical concentrations, others can at present be described only as physiological complexes. While equations could be fitted to many correlations between body size and quantity of environmental influence, the meanings of the equations would at present remain unidentified. The working out of these correlations and the quantitative comparison of the various influences is the task of the following chapters.

CHAPTER II

RATES OF GROWTH OF INDIVIDUALS

IT IS DIFFICULT to compare with one another the sizes of different individuals, until the changes of size which a single individual undergoes have been recognized. There is a progressive increase of mass in the normal life of each individual; this is properly termed growth. Ordinarily between one fission and the next, in the course of vegetative generations, materials are assimilated and size is increased. The rate at which this increase occurs has been studied most extensively in *Paramecium*.

1. *PARAMECIUM*

The first attempt to find how fast single individuals grow was made by Simpson ('02), who measured with an ocular micrometer the length and the breadth of living *Paramecium caudatum*. He isolated grown individuals which showed signs of constricting into two; and timed exactly when fission became complete. Each member of the daughter pairs thus produced was measured twice; first a few hours after fission and again many hours after fission. In all of the scores of individuals, some increase in length and in breadth occurred. But it is the mass or volume of the body rather than any one dimension which best measures growth, and therefore it is necessary to attempt, however questionably, to estimate volume from the length and breadth which were the actual data. To derive anything far-reaching from the measurements of protista, we are forced to make free with them to the extent of calculating volumes in ways which are not rigorously justified. For *Paramecium* it is best to consider the volume of the body as proportional to length $\times$ breadth

× breadth. From the data of Simpson one arrives at the result that the growth, in length, in breadth, and in volume, was greater in the period from 14 to 33 hours than in the period from 6 to 26 hours after fission. As we shall see, larger numbers of measurements and more constant environmental conditions are necessary adequately to estimate growth.

Jennings's data. Jennings ('08b) studied the growth of *Paramecium caudatum*, using very large numbers of individuals. To obtain accurate measurements he killed and fixed the individuals at known times after fission; he demonstrated that the specimens fixed by his method did not change in size from the living. The length and breadth of each was recorded, from which frequency distributions and averages were obtained at each of several periods of time. Altogether, adequate data for the dimensions of the body were obtained at ten periods during the growth cycle. All the individuals measured were originally derived from a single parent, and thus belonged to one clone; but it was advisable to correct, because variations occurred in the medium furnished, in order to compare different cultures and different days. The corrected results so obtained, based on 769 individuals, are represented in figure 6.

In these *Paramecia*, the breadth changed very little throughout the generation cycle, so that the length and volume were roughly parallel. But just before fission there was a sudden shortening, and just after fission an equally sudden lengthening of the body. It is obvious, therefore, that it would be unjustified either to consider the volume as always proportional to length or to consider the volume as always proportional to the cube of length.

Estabrook ('10), working in Jennings's laboratory, also studied the growth of individual *Paramecium cau-*

datum, using the same methods as in the above work. He did not put together the results at various ages, because he studied primarily the modifications in rate of growth produced by certain chemical substances; but it seems a legitimate procedure to average his data

Figure 6. Growth in length and in volume during the life of an individual *Paramecium caudatum*. For each point plotted, 24 to 188 individuals, derived from one clone, were killed and measured in length and in breadth. The relative volumes were calculated as average breadth squared, multiplied by average length, or $l \times b^2$. (Data of Jennings, '08).

for all *Paramecia* grown under control or standard conditions during his experiments. When this is done, the curves shown in figure 7 for length and for volume are obtained. Again the gain in length is rapid at first and later almost nil. But the gain in volume is exceed-

ingly slow at first and only towards maturity does it become rapid. It is difficult to find any innate inferiority of these data as compared with those of Jennings; but the result is far different with respect to breadth and to volume.

Figure 7. Growth in length and in volume (calculated as $l \times b^2$) in a clone of *Paramecium caudatum*. Each point represents the average of measurements made upon at least 90 fixed individuals. (Data of Estabrook, '10).

Other observations. A few individuals of *Paramecium caudatum* were measured by Popoff ('09). At all ages the number measured was only fifteen, and it is surprising that the results are at all regular, as figure 8 shows them to be. But each of the fifteen was compared in dimensions with its own sister which was

killed immediately after fission; and three dimensions were always measured. These individuals all showed greater growth in breadth than in length, in this respect being more extreme than those measured by Estabrook.

Figure 8. Growth in length and in volume in *Paramecium caudatum* at 25°C. Each point represents a single individual which was fixed, and measured in length, in breadth, and in thickness. Relative volumes were calculated as $l \times b \times t$. (Data of Popoff, '09).

Upon *Paramecium aurelia*, Erdmann ('20) found much the same progression in body length (figure 9) as did Jennings. Judging by the large number of individuals measured, these results should be significant. But body breadth was not studied.

Schmalhausen and Syngajewskaja ('25) used a different technique, measuring body length upon living individuals. In calculating the mean percentage increase of length, each individual was treated separately (figure 10). Large variations of temperature and of

Figure 9. Growth in length in *Paramaecium aurelia*. Two clones were used, line IE being identical with line I, but cultivated on a different medium. For each point 120 individuals were fixed and measured. (Data of Erdmann, '20).

culture medium account for the fact that the duration of a single generation fluctuated between 18 and 60 hours. Contrary to the opinion of the investigators, it is improbable that the lengths represent the relative volumes of the individuals, for at the modal time of fission (or duration of a single generation), which was

24 hours, the length had reached only sixty percent. Either the conditions were not proper for growth, or growth in breadth occurred as well as growth in length.

Figure 10. Growth in length in *Paramecium caudatum* at 15.3° to 18.7°C. Forty-four living individuals were measured at three-hour intervals, and the percentage lengths were averaged. (Data of Schmalhausen and Syngajewskaja, '25).

Mizuno ('27) measured not only length and breadth but also shadow-area of fixed individuals of *Paramecium caudatum*. His data are equally extensive with those of Jennings, but the results shown in figure 11 are different in several important respects. In passing, it may be noted that the product of length and

breadth gives very nearly the same relative values as do the actually measured areas. The volume appeared to increase to at least double the original volume during growth; but the length hardly changed after the first thirtieth of the duration of growth. The breadth

Figure 11. Growth in length, in sagittal area, and in volume, in a clone of *Paramecium caudatum* at 24° to 26°C. At each of the ages studied volume was calculated as area $\times$ breadth; it agrees closely at every point with volume calculated as length $\times$ breadth squared. (Data of Mizuno, 1926).

and the volume showed significant decreases just after fission.

Another method. Another method in addition to that of timing particular individuals might be used to measure the rates of growth of protista, utilizing data

which can be obtained much more easily. In a mass culture of a clone in which growth and reproduction are proceeding at a steady rate, the frequency with which each size occurs in the culture, at some one time,

Figure 12. Growth in length and in volume in *Paramecium caudatum*, as calculated from the frequency of occurrence of each length and each volume in an actively multiplying culture. One hundred and ninety-five individuals were fixed and measured in length and breadth. Volumes were calculated as $l \times b^2$. In the lengths the one longest individual was discarded; in the volumes the 44 smallest and 31 largest individuals were discarded, in order to leave a range in size of only 100 per cent. (Data utilized are from Jennings, '08, p. 412).

will be, roughly, inversely proportional to the length of time required to grow through that size. To make use of this method properly, not only is an old popula-

tion essential, in order that the fissions among its members will not be in step with one another, but also such cultural conditions are necessary as will not change significantly for several days. At present no data of the kind are available; the nearest approach seems to be a certain *Paramecium* culture of Jennings ('08b). This is analyzed in figure 12 in terms of relative lengths and volumes, the latter being taken to be proportional to the length multiplied by the square of the breadth. Lengths can hardly be used without correction in the case of *Paramecium*, since Jennings found (figure 6) that the same length may occur twice in one growth cycle, due to the shortening just before fission. By this method the general shape of the curve for growth in length is confirmed, while for growth in volume we already have several types of curve.

Summary. What shall be considered the normal progression in which body mass is increased in *Paramecium*? With respect to length all the data agree that the increase is very rapid at first, and then is slow (with possibly a decrease just before fission). But with respect to volume we have two diverse pictures from two sets of equally well-founded data. Jennings's results show that *Paramecium* accumulates materials rapidly at first, and then more slowly or not at all; Mizuno's results show that *Paramecium* loses body substance at first, and then gains it at a uniform rate throughout the rest of its individual existence. So far as is now known, both pictures may be characteristic; whether the diversity is due to difference of race or difference of environment cannot yet be decided.

2. OTHER SPECIES

Data showing the rates of volume increase of individuals are available for the ciliate *Frontonia*, the rhizopod *Hartmanella*, and various bacteria.

Figure 13. Growth in volume in *Frontonia leucas* at 25°C. and at 14°C. Each point represents a single individual which was fixed and measured in length, in breadth, and in thickness. Volumes were calculated as $l \times b \times t$. (Data of Popoff, '08).

Frontonia. In *Frontonia*, Popoff ('08) measured the increase of size at two different temperatures, 14° and 25° . At the lower temperature a period six times as long was required to double the volume and to bring on fission, as at the higher temperature. The results are shown in figure 13. Each point represents only one individual measured, and the dashed curves are merely the simplest ones that fit the data. So far as the shapes of the curves are justified by the individual data, it would appear that only at 14° was there faster growth at first and slower growth later. At 25° , where the data were more numerous, growth in volume proceeded, as far as any conclusion can be drawn, at a uniform rate throughout. A single clone was not used for these measurements, but each growing individual was compared in volume with its own sister individual killed immediately after completing fission.

Ameba. In the soil rhizopod *Hartmanella hyalina*, a few measurements of growth rate were made by Cutler and Crump ('27). They traced the shadow-areas of the amebæ at successive periods, the area at each period being based upon ten rapidly-made drawings. They assumed that the mean thicknesses of the amebæ were uniform, and the body volumes therefore proportional to the shadow-areas. In the fed individual of figure 14, the percentage increment of volume was approximately uniform over the entire period of 25 hours. In three other protocols it seems that growth was relatively slow over the first part of the observations; but the observations did not start at the time of fission of the amebæ.

Plasmodium. The growth of a parasitic species was studied by Hartman ('27). It was discovered by Taliaferro ('25) that when *Plasmodium cathemerium* was cultivated in the blood of a living canary-bird, asexual sporulation occurred once every 24 hours, and at

nearly the same time for all individuals. Hartman drew samples of blood every hour and measured the

Figure 14. Progression of total body volume in the rhizopod *Hartmanella hyalina*, and the effect of starvation and refeeding. Each of the two curves represents the body volumes of one individual and all its progeny, kept at 21°C. (Data of Cutler and Crump, '27).

sizes of twenty parasites in each of them. The logarithms of the mean shadow areas were approximately proportional to the time. It must be noted, however,

that in 24 hours the volume increased about 35 times, while at each sporulation only 16 young organisms are usually produced. It is possible that the matrix of the spores constitutes over half of the parent's bulk; or it

Figure 15. Growth in length of two chains of *Bacillus ramosus* in a hanging drop of broth kept in darkness. Length, which is proportional to volume in this form, is plotted logarithmically. A, temperature 17.0° to 18.2° ; B, temperature 16.0° to 16.5° . (Data of Ward, '95, pp. 405-407).

is possible that water is discarded and the other materials are condensed in the process of spore formation. The rate of growth and the length of one generation could be retarded by the peculiarities of the host (Hartman, '27), the temperature of cultivation in vitro

(Taliaferro, '28), and the duration of the daylight cycle to which the host birds were subjected (Boyd, '29).

Bacillus ramosus. Among bacteria, very careful measurements of growth were made by Marshall Ward ('95) in *Bacillus ramosus*. This organism forms

Figure 16. Growth in length of a chain of *Bacillus ramosus* in a hanging drop of broth. At each of the arrows which point downward, septa between daughter cells first became visible, and growth was evidently proceeding at its maximal rate. At each of the arrows which point upward, fission of the cells was occurring, and growth was evidently proceeding at its minimal rate. (Redrawn from Ward, '95, p. 300, with the permission of the Royal Society.)

chains of rod-like individuals. The thicknesses of the cylinders were said to be uniform, however long the individuals and the chains; so that growth in volume was proportional to length. Under the conditions of

growth employed the cylinders were 1.75 micra in diameter.

Figure 15 shows that under good cultural conditions growth in length was uniform in rate, regardless of the number of cells present in one filament; for in this figure the lengths are plotted logarithmically. But when measured more frequently it was found (figure 16) that growth was retarded each time fissions occurred. For a single cell generation the volume curve would then tend to resemble the curves for growth in length of a *Paramecium*.

It is obvious that the true rate of growth for a single individual could be described only from observations made at intervals of one or two minutes.

Some other bacteria. Similar measurements have been made occasionally upon other bacteria. One series was obtained by Schmalhausen and Bordzilovskaja ('26) upon numerous living individuals of three species, *Proteus vulgaris*, *Bacillus coli*, and *Bacillus dysenteriae* Shiga-Kruse. They believed that the volumes were proportional to the lengths in these three species. In all three the averaged lengths increased linearly throughout the ten hours required for one generation.

Growth in bulk of populations. Some data of Henrici ('23) may be utilized to tell something about the growth in bulk of bacterial masses. Henrici measured the average sizes of *Bacillus coli* at various times after inoculating a fluid culture, and also counted their relative numbers (table 3). Within the first three hours the volume per individual appears to have increased eight times, and the total bulk of bacterial body-substance in the culture increased eighty times. The table also shows that no further increase of total bulk occurred within the next nine hours. From this it may be inferred that within this nine-hour period the accumu-

TABLE 3.

RELATIVE PROGRESSIONS OF BODY VOLUME, NUMBER OF INDIVIDUALS, AND TOTAL PROTOPLASMIC BULK IN A BROTH CULTURE OF *BACILLUS COLI*. (DATA OF HENRICI, '23). SEE ALSO FIGURE 46

Time in hours	Body volume	Number of individuals	Total bulk
0	53	0.94	0.50
3	408	10.1	41.2
9	86	47.0	40.4
12	57	70.7	40.3
18	82	89.3	73.2
30	98	93.4	91.6
48	100	100.0	100.0

lation in the organisms of any substances taken from the medium could only be accomplished by relative dehydration of the body substance.

These data for *B. coli* are altogether comparable with the data for *B. ramosus* cited above; the only difference lies in the unessential fact that in *B. ramosus* the individuals remained in filaments instead of separating. But the results differ greatly.

Studies of increase of bulk in yeast populations were made by Balls and Brown ('25) and Richards ('28a). Only with respect to bulk, and not with respect to mere numbers of individuals was the progression logarithmic.

Some results upon the increase of bulk in the green organism *Scenedesmus costulatus*, obtained by Roach ('26), deserve special mention. These algæ increase in size for two or three days, at 24.4°, and then suddenly divide into four or eight progeny. In the cultures studied, enough of the individuals divided synchronously to cause the average body volume to fluctuate in three-day rhythms. When the concentration of the cells or the number in the culture was estimated, there were converse fluctuations in the rate of multiplication. But when average volume was multiplied by number of cells present, then the increase (of pro-

toplasmic bulk) was logarithmic; in other words new bulk was added to the entire population in proportion to the bulk already present at any one time.

Under conditions either of size modification with age of culture or of slow multiplication, therefore, the increase of total bulk must be the criterion of accumulation of living substance, because accumulation is no longer sensibly proportionate to the increase in number of individuals.

Comparison of results. It is far from certain that the growth curves for individual unicellular organisms are similar in all species. It is doubtful whether the curves are the same for all races or under all conditions which allow of growth, even in the single species *Paramecium caudatum*. Whether the increase in size is uniform in rate or not, is important for ascertaining the nature of the regulation of growth. Upon the basis of Jennings's curve for *Paramecium* it might be said that increase of size was cyclic, and that the cycle of size-increments coincided, perhaps causally, with the cycle of mitoses. Upon the basis of most of the other studies reviewed above, it would be said that the increase of size was nearly uniform; that fission was a subsidiary occurrence, coming at whatever time the body volume was doubled; and that its occurrence did not essentially influence the processes of volume increase.

3. VARIOUS INFLUENCES UPON GROWTH IN SIZE

The relative effects of diverse circumstances should help in the analysis of the processes concerned in volume increase, however meager the data may be.

Nutrition. That food is necessary for increase in size might be thought to be axiomatic. The evidence, however, is, in part, to the contrary. Most of the data upon *Paramecium*, especially those of Jennings ('08b),

showed that increase of volume was most rapid just after fission. But Estabrook ('10) found that nothing was ingested by *Paramecium* for the first thirty minutes of its individual life. Estabrook went on to

Figure 17. The influence of starvation upon growth in length and in volume (calculated at $l \times b^2$) in individuals of a clone of *Paramecium caudatum*. Time plotted on a logarithmic scale. Each point represents the average of measurements made upon 10 to 35 fixed individuals. (Data of Estabrook, '10).

compare individuals which were denied food through being placed either in sterile tap-water or in Chamberland-filtered hay-infusion, with controls which were fed. He found (figure 17) that during at least the

first sixty minutes of the growth cycle the starved individuals underwent the same changes of size as the controls. But in these cases the controls also exhibited increases only of length, and no increases of volume. At 5 hours and at 24 hours the starved organisms were far behind the controls with respect to volume; yet they too had gained. As far as the data are dependable, it must be true that volume may increase for at least 24 hours in the absence of food. But it is more likely that small amounts of food were available. Otherwise volume must have increased by the incorporation of water; possibly accompanied by the gain, through processes other than alimentary, of certain dissolved substances.

That growth of individuals may occur by mere changes of relative water content is supported by the evidence regarding *Bacillus coli* already discussed in connection with figure 2 and with table 3. Perhaps most striking of all the changes of water content are the decreases of volume just before fission shown by the data of Jennings ('08b) in figure 6. Whereas it is possible that this shrinkage was due to some sort of defecation preparatory to fission, there were no observations to support the possibility.

Is the rate of growth limited only by nutritive acquirement? This question has not been asked frequently enough; probably because no case is possible in which rapid and continued growth can occur without assimilation of something. But in many organisms, as in an adult man, it may be seen clearly that assimilation can proceed without growth resulting. Upon unicellular organisms the data are insufficient to decide; but the shape of the growth curve of Jennings seems to indicate that increase of size is not proportional to food acquirement, even allowing for the possibly greater speed of digestion and of ejection of fluid

from food vacuoles in the adults. It is usually assumed that a *Paramecium* ingests its food at a fairly uniform rate throughout the hours of its individual existence. If so, the food certainly has not a uniform effect upon the mass of the body. Thus far, at least, growth appears to depend not wholly upon the food supply.

But there is evidence that food is not ingested at a uniform rate throughout one generation's life. Lund ('14) studied the ingestion of food particles in individuals of *Bursaria* which had been previously starved for 24 hours. He found that those which were allowed to feed for only five minutes ingested almost as many particles as those of the same culture which were left with the same food for one hour. Again, in *Hartmannella* starvation was found by Cutler and Crump ('27) to result in a gradual decrease of body volume, as shown in figure 14. But when food was again supplied, the effect was tremendous as a result of the previous 24 hours of starvation; the gain in bulk was six times as great as in the control organisms fed continuously with the same food. Part of this might be due to the inflation of true body-substance by food vacuoles; but not nearly all of it could be such. If, therefore, previous starvation modifies the rate of ingestion, it is misleading to leave out of account the influences of age and other physiological stages upon this rate. Fission might be considered as equivalent to previous starvation, for it can be followed, though perhaps not immediately (Estabrook), by a period of very rapid assimilation.

Chemical influences. The effects of chemical conditions upon growth of individuals were studied by Estabrook ('10) in *Paramecium*. He placed individuals just after fission in solutions of various substances, chiefly alkaloids and sodium chloride. The effect of the alkaloids was not marked except in those con-

centrations which were nearly lethal; then there was often swelling of the body which was probably independent of growth processes. The effects of pure sod-

Figure 18. The influence of various concentrations of sodium chloride made up in hay infusion, upon growth in length in individuals of a clone of *Paramecium caudatum*. Time is plotted on a logarithmic scale. Each point represents the average of measurements upon at least 9 fixed individuals. Additional data for M/30 and M/50 solutions were at all points very near the points which represent the controls in hay-infusion. (Data of Estabrook, '10).

ium chloride solutions were similarly complicated by the use of tin-condensed distilled water which was lethal. But a series of measurements was made upon individuals in sodium chloride solutions containing hay-infusion. Figure 18 shows that in these media increase in body length occurred just as in the controls. But after some time, namely, 40 minutes in 0.10 M and 90 minutes in 0.05 M, sudden decreases occurred, which were followed shortly by the death of some of the individuals. Weaker solutions, though increasing the osmotic pressure several fold, had no significant effects.

Temperature. Temperature, of course, affects markedly the rate of growth. The data of Popoff ('08) show this influence very clearly (figure 13), but he made measurements at only two temperatures.

A large series of different temperatures was studied by Ward ('95) in relation to the growth of *Bacillus ramosus*. In these filaments of bacteria he ignored the partitioning into cells and simply found how long it took for a given chain to double its length, and therefore its volume. The rhythms of increase in length, shown in figure 16, were ironed out by the considerable periods of time which elapsed between measurements; and it has already been seen (figure 15) that the increment is proportional to the time interval, regardless of the absolute length of the chain of bacilli.

Ward's results, shown in figure 19, cover almost the entire range of temperatures at which the bacilli live, for above 39°C. they are killed. The relationship between temperature and rate of increase in volume is not a logarithmic one, as can be supposed to be true in the case of some temperature effects.

If the volume of growing micro-organisms be taken only once for each generation, then, of course, the rate of growth is the same as the rate of fission, assuming

that the adult individuals at the close of each generation may be expected to be uniform in size. The effect of various factors upon the rate of multiplication will be mentioned later.

Figure 19. Effect of temperature upon the growth of *Bacillus ramosus*. Spores were germinated at 22° five to eight hours before the observations began. The bacilli were kept in the dark in a hanging drop of gelatin-broth medium. (Redrawn from Ward, '95, p. 458, with the permission of the Royal Society.)

One of the points most desirable to know is whether the shape of the curve of growth in volume is changed by diversity of temperatures. The data of Popoff (figure 13 above) might be taken to indicate that it is, but the difference of shape is within the range of error in the data.

CHAPTER III

VARIABILITY OF BODY SIZE

THE DIMENSIONS of two individuals, in unicellular species as in man, are almost never exactly alike. The only way in which the role of various influences can be studied, therefore, is by seeing whether groups made up of considerable numbers of individuals differ from one another. Evaluations of quantitative differences require not only the construction of significant averages, but also the comparison of the degrees to which the individuals composing each average are themselves diverse.

1. VARIABILITY AND ITS MEASUREMENT

Modifications of size are induced by many sorts of changes in environmental conditions. But before these are compared, it is necessary to find how much variability occurs when conditions are so far as possible kept constant. Those variations which occur under what appear to be constant conditions will always carry the possibility that they are none the less due to environmental differences, either past or present. Until they are shown to be such, they may legitimately be considered as physiological variations; that is, variations due to internal factors. Ultimately, of course, every variation is a resultant of many factors, both internal and external. Some of the variations can probably not be controlled or equated completely by any possible technique. In the case of unicellular organisms it must be realized that the environments of two individuals that are in the same body of culture medium may in reality differ markedly.

TABLE 4.
VARIABILITY OF LINEAR DIMENSIONS IN *PARAMECIUM CAUDATUM*

Dimension Measured	Mean in micra	Coefficient of variation in per cent	Number of individuals measured	Conditions	Date of
Length Breadth	229.1 68.1	8.36 13.44	} 200	100 pairs of sisters isolated in hay infusion from wild cultures	Simpson ('02, p. 403)
Length	221.8	8.77	500	one clone, one culture	Pearl and Dunbar ('05, p. 82, Expt. 1)
Length	198.4	8.17	218	one wild culture	McClendon ('09, culture <i>a</i>)
Length Breadth	165.2 50.7	5.32 6.77	} 40	one clone, one culture, one age = beginning fission	Jennings ('08b, p. 430, row 30)
Length	193.3	4.94	184	one clone, one culture, in one state = conjugating	Jennings and Lashley, ('13b, p. 195)
Length	163.8	4.95	390	one clone, one culture	Jollos ('21, p. 128, line <i>a</i>)

To define how much the individuals of a group differ among themselves, it is convenient to use coefficients of variation. A coefficient of variation is the percentage of the mean or average which is formed by the mean squared or standard deviation.

Paramecium. In table 4 are represented data which were obtained by various surveyors of *Paramecia*. A number of points are brought to light in this table. There is a general improvement in the degree of similarity among the individuals of one group as more and more factors are taken under control. The factor of age after fission has been evaluated in the previous chapter. Homogeneity of hereditary factors, which is obtained by limiting the group to one clone (which consists of descendants of a single individual) is seen to be important from the fact that the various groups measured differ tremendously in mean dimensions. Homogeneity of cultural conditions over a considerable period of time before measurements were taken, will be shown below to be a prime factor.

The factor of growth or age was first demonstrated to be of significance by Simpson ('02). Sister individuals showed similarity in length such that a correlation coefficient of $+0.70$ was found ($+1.00$ being complete similarity). It also turned out that as growth proceeded, sister individuals became more and more diverse; and this requires that it be recognized that growth in mass was partially independent of age.

Inheritance was shown by Jennings ('08b) to influence size within a unicellular species. It seems necessary to remark, however, that cultures which do not consist of a clone are nevertheless often homogeneous in race, but of course not certainly so, because of the fact that all the parent individuals in the culture may have come eventually from some common ancestor; and because under particular conditions some one

clone usually flourishes, to the almost complete exclusion of other clones. On the other hand, Jennings ('08b) showed that some wild cultures were very heterogeneous in size of individuals.

It is significant that breadth is more variable than length, at least in *Paramecium*, quite apart from the larger percentage error involved in its measurement.

The relatively low variabilities recorded in the last two rows of table 4 indicate that not only homogeneity of the above factors, but also homogeneity of physiological condition prevailed. Particularly among conjugating individuals have low variabilities of body size been found (Pearl, '07; Jennings and Lashley, '13b). But even so, some controllable factors have undoubtedly escaped attention, for it may be recalled that when trypanosomes lived in the "milieu interieure" of a rat their variability in length (figure 1) was often only half of the lowest variabilities ever observed in *Paramecia*.

The variability of body volume is, of course, much greater than that of any linear dimension. In general it is something greater than the sum of the coefficients for each of three dimensions; though of course it would be possible for individuals to vary so that shortness tended to be compensated by fatness. This possibility has not been found in any clone of protista which has been measured.

Some other species. Many other species of unicellular organisms than *Paramecium* have been studied with respect to variability of size. Some representative data are given in table 5. Nearly all, but not all (figure 20) of the distributions are for linear dimensions, and for the most part the coefficients of variation are high. This is, obviously, partly due to the heterogeneity of the material. Particularly have the cultural conditions been neglected. In a few cases, as

TABLE 5.
VARIABILITY OF SIZE IN VARIOUS UNICELLULAR SPECIES

Species	Dimension measured	Mean in micra or cubic micra	Coefficient of variation	Number of individuals measured	Conditions	Date of
<i>Chilodon uncinatus</i>	length	31.22	10.31	920	one clone, one culture	Enriques ('08, p. 241)
<i>Stylonychia pustulata</i>	length	111.01	7.90	120	one clone, one culture	Enriques ('09, p. 120, ser. 3)
<i>Colpoda steinii</i>	length	38.52	12.14	100	one clone, one culture	Enriques ('09, p. 132, ser. 1)
<i>Colpoda cucullus</i>	{ diameter of di- vision cysts volume of di- vision cysts }	132.1 1,219,000	5.03 16.11	} 110	one clone, 26.5°	Adolph ('29, clone A)
<i>Chilomonas paramecium</i>	{ length breadth }	24.66 10.81	10.32 10.87	} 175	one culture during many days	Pearl ('06, p. 61)
<i>Trypanosoma simiae</i>	length	17.87	9.27	500	one host at one time	Bruce <i>et al.</i> ('12)
<i>Trypanosoma caprac</i>	length	25.51	8.58	500	one host at one time	Bruce <i>et al.</i> ('13)
<i>Trypanosoma lewisi</i>	{ length breadth }	31.49 1.58	3.02 14.86	} 100	one clone, one host at one time	Taliaferro ('23, p. 144, 30th day)

TABLE 5 (Continued)

Actinosphaerium eichhorni	{ diameter { volume	{ 45.61 { 38.62 6,170,000	13.61 4.56 31.77	96 22 100	all those having between 125 and 175 nuclei clone, first day of star- vation 26.5°	} Smith ('03, p. 243) author
Actinophrys sol	{ diameter of con- jugation cysts { volume	30.74	11.22	500	one clone, one culture	Belar ('24, p. 387)
Acanthocystis aculeata	diameter	61,100	23.60	50	same clone of Belar, 26.5°	author
	diameter	30.00	11.43	100	one clone, one culture	Stern ('24, p. 440, race 1)
Arcella vulgaris	diameter of shell	55.79	10.27	504	one culture during three weeks	Pearl and Dunbar ('03, p. 327)
Arcella dentata	diameter of shell	116.6	3.64	222	one clone during two weeks	Hegner ('19, p. 127)
Diffugia corona	diameter of shell	169.4	3.74	482	one clone during several months	Jennings ('16, p. 448, line 303)
Bacillus coli	length	2.05	26.8	250	ninth hour on medium	Henrici ('28, table 18)
Bacillus megatherium	length	4.1	21.9	300	fourteenth hour on medium	Henrici ('28, table 7)

in *Actinosphaerium* there are evident internal heterogeneities; in this species the heterogeneity is in the number of nuclei.

Figure 20. Distribution of adult body volumes at 26.5° in *Colpoda*, feeding on *Chlorogonium*. CI Nov. 1-3; CII Jan. 3-5; CIII Jan. 30, four generations after hatching from four permanent cysts. The vertical lines indicate the means for the groups. (From Adolph, '29).

In general the lowest variabilities were found among

the shelled rhizopods. Whether or not this was entirely due to the automatic elimination of the growth factor through measurements of the shell only, is uncertain.

Only in one group of unicellular organisms, the trypanosomes, has size been used as a taxonomic character in more than a general way (Bruce, '11). The identification of species of trypanosomes at first depended almost entirely upon the mean sizes and the size-distributions of a sample of the individuals found in a given drop of the host's blood. But the coefficients of variation (table 5) were always quite high, and it is likely that they were high because the trypanosomes were not of one race or possibly species; in other words, multiple infection had usually occurred. Moreover, it is uncertain that exactly the same variability would be found for the same clone in different hosts, particularly hosts of different species. For, it has been shown that different host-species modify greatly the body sizes of trypanosomes (Wendelstadt and Fellmer, '10; Noller, '13). Some of the points concerning variability were demonstrated by Pearson ('14), utilizing the very numerous data of Bruce ('11, '12, '13).

If the variabilities of size which occur under controlled conditions have once been investigated, it is then possible to use variability as a criterion of some particular condition. Thus Jennings ('08b) and Jollos ('13, '21) used it as a test of genetic homogeneity in *Paramecium*. Taliaferro ('23, '24) used variability as a criterion of the relative numbers of young and growing organisms in clonal infections of trypanosomes. And it may be concluded, almost without discussion, that constancy of environmental conditions is to be judged by the smallness to which the residuum of variability can be reduced through various means of control.

2. CORRELATIVES OF BODY VOLUME

Some of the factors which have proven to influence variability of size among individuals have already been mentioned; such as heredity, age, reproductive rate, and many environmental factors. But granted the constancy of all the identified conditions, there remains the residuum of "normal" variability, which, moreover, in itself leads to important consequences.

Figure 21. Frequency curve of diameters (solid line), and correlation of diameters with specific gravities (broken line), in the green alga *Scenedesmus*. (Redrawn from Allison, '24, permission of *Annals of Applied Biology*).

Some of the causes and the consequences of being larger than the mean or smaller than the mean, can be realized from the consideration of some of the functions and properties which are bound up with size.

Specific gravity. Specific gravity or density was demonstrated by Allison ('24) to be correlated with size. He studied several species of spherical organisms, determining their specific gravity by means of the time required to fall through normal culture fluid. From the diameters and Stokes's law the relative densities were then calculated. It turned out (figures 21

and 22) for each of three species studied, *Scenedesmus*, *Gonostomum*, and *Colpoda*, that the smaller individuals had the higher specific gravities. If anyone had set out to predict such a difference he would probably have anticipated that smaller individuals had higher specific gravities, because then the tendency to sink in water would be approximately alike in all individuals. For, the rate of sinking depends on the ratio of the

Figure 22. Frequency curve of diameters (solid lines), and correlation of diameters with specific gravities (broken line), in the cysts of *Gonostomum* sp. at 12.4°C. (Redrawn from Allison, '24, with the permission of the editors of the *Annals of Applied Biology*).

specific gravity difference to the body surface. But the change of specific gravity with size is in no case sufficiently large to compensate mechanically for the increase of surface.

Probably the same factor of density has been demonstrated in another form by Fortner ('25) in *Paramecium*. He measured how much the bodies lost in volume upon being put into a sucrose solution, and found (figure 23) that less shrinkage occurred in the small

individuals. During this shrinkage it is probable that water alone was lost, whence the water content may be regarded as relatively less in the small individuals. If, instead, the shrinkage had been limited by the surface-mass ratio of the body, then it would have been less in the large animals.

Figure 23. Effect of solutions of sucrose upon the mean body volume of *Paramecium caudatum*. Triangles, sucrose of 9 atmospheres osmotic pressure; circles, sucrose of 10 atmospheres osmotic pressure. Broken line, average linear correlation. The *Paramecia* measured were classified into groups of 7 to 34 individuals according to their original body lengths (abscissae) and the mean body volume after shrinkage is given in per cent of the mean original volume of each group (ordinates). (Data of Fortner, '25).

The relation of locomotion to size also involves the relation of rate of sinking to size. As an example, locomotion by cilia is limited by the number of cilia that can operate on the surface, while the mass to be moved is relatively larger than the surface in large individuals. Ludwig ('28a) estimated the limitation of size of *Paramecium* upon this basis. But there is

no evidence that speed of locomotion has any survival or other value within the range of sizes found in a single clonal culture. This and similar consequences of surface will be reconsidered in Chapter X.

Toxicity. Various quantities, such as toxicity, have been shown to be correlated with age of individuals. Whether or not age, then, is important because of its size correlative, is by no means clear. Estabrook ('10) found that certain alkaloids killed *Paramecium* most readily, soon after fission; but one alkaloid, at least, killed old individuals more readily. It is obvious (from figure 23) that any agent whose action depended upon the concentration of water or of some solute within the body would have a different effect upon large and small individuals. It was shown in the previous chapter that the concentration of water probably changed during the growth of individuals.

Summary. It is clear that definite chemical and physical differences exist among the individuals of a clonal culture which depend solely upon the fact that the individuals are diverse in size. These differences have not helped to distinguish what produced the diversity in size; yet it is fairly probable that a change in specific gravity or in the ability to buffer toxic reagents would of itself induce modification of the size, or select among sizes, or both. It has not been demonstrated directly that the small diversities of size which prevail among individuals of one age, one clone, and one culture, are accompanied by physical and chemical differences that matter, but it is likely to be so. This may help to understand the variability which exists among such individuals, but it does not help to describe how the variability arose. This residuum of variation can at present only be recognized and measured.

CHAPTER IV

INHERITANCE OF SIZE

SIZE HAS ALWAYS been recognized as sufficiently characteristic of unicellular organisms that approximate dimensions were quite properly included in taxonomic descriptions of species. But with the demonstration of variability within a species it became necessary not only to define the probable limits of variability, but also to find out whether or not all members of a species inherited size alike.

1. PARAMECIUM

The close similarity of size among numerous adult individuals would indicate that in general the individuals of one generation are of the same size as the individuals of the preceding generation. In *Paramecium* there is no direct proof that this is so; it is perfectly conceivable that there is some sort of alternation of generations; that large and small generations are mixed. This similarity has, however, proved actually to exist in *Colpoda* and in several species of shelled rhizopods. Over periods of at least two or three generations which are living in equilibrium with a constant environment, adults of successive generations are alike in size. But there are obvious exceptions in those cases where some sort of differentiation of generations occurs, as for example in the formation of cysts, spores, or gametes.

Racial constancy. The problem of inheritance of size was studied in great detail in *Paramecium* by Jennings ('08b). He noticed that length or breadth was often much more variable among the individuals of wild cultures than among the individuals belonging

to one clone. After isolating individuals from various sources, the descendants of each were found to be not only closely similar in size among themselves, but also highly diverse from the other clones. Moreover, as changes of environment resulted from variations in

Figure 24. Inheritance of body length in various clones of *Paramecium*. The individuals which were fixed and measured grew in mass cultures. Each point represents the mean length for many individuals killed at one time. The synchronous upward and downward trends are due to variations in the environmental conditions. Clones L^2 and D were *Paramecium caudatum*; clones g and i were *Paramecium aurelia*. (Data of Jennings, '08b; and Jennings and Hargitt, '10).

culture fluid and temperature, the various clones shifted their mean sizes in parallel fashions, as indicated in figure 24. Jennings and Hargitt ('10) observed the persistence of the size characteristics for more than three years, and Erdmann ('20) demonstrated the

constancy of mean body length in one clone for more than six years.

Selection. The next step was to see if large or small individuals could be selected, and possibly give rise to lines with new size characters. Jennings ('08b) showed that whereas selection in a wild culture might isolate distinct size biotypes, selection within a clone had no effect upon the sizes of the descendants. The variability found within a pure line could not be further reduced or segregated. Within those few generations after selection had ceased which were necessary for securing enough individuals upon which to make measurements of significant frequencies, the *Paramecia* had reverted exactly to the original size distribution. This fact was confirmed by Jollos ('13, '21) and Ackert ('16).

Interposed influences. Further purposely interposed environmental effects did not interfere with the transmission of size constancy throughout the generations of *Paramecium*. Jennings ('08b) showed that starvation, old culture fluid, rich culture fluid, deep culture fluid, all caused size variations. But restoration to basal circumstances resulted in restoration of size within the time required for the breeding of enough individuals to measure. Rautmann ('09) and Jollos ('13, '21) found that temperature changes modified the dimensions of *Paramecium*. But the latter investigator showed that in some clones either holding at the new temperature for some weeks or restoring to the original temperature resulted in full return to the original characteristic size. DeGaris ('27) found that gross bodily abnormalities and temporary inhibition of reproduction, produced by treatment with cyanide, did not affect the transmission of the size character in *Paramecium*.

Inheritance and its expression. It is evident that,

under duplicated environmental circumstances, inherited factors for size express themselves in a highly uniform way. It should be kept in mind, however, that more than one generation of *Paramecium* may be required for the conservative "nuclear" characters for size to materialize in "somatic" expression. For instance, if *Paramecia* are transferred from 10° to 25°, the average adult body volume is reduced by more than half (figure 36). But obviously more than one binary fission is required to accomplish this reduction of size. The expression of the hereditary character is conditioned by the immediate past history of the organism; such conditioning has been demonstrated repeatedly in experiments on inheritance, and Jollos ('21) found that such bodily conditions may prevail through hundreds of generations of *Paramecium*. But the fact that such factors or "persisting modifications" are ultimately lost, so that the organisms return to their original bodily characteristics, shows that inheritance has not been indelibly affected.

In *Paramecium* no attempts have been made to measure sizes in successive generations. But in *Colpoda* (Adolph, '29) it has been found that there is a high correlation between the size of the parent and the sizes of its offspring, when they become adults (figure 25). The correlation is $+0.56$; the individual resembles its immediate parent more than it resembles the average size of the clone. Selection would obviously be effective as long as it were continued; but actually in only three or four generations after selection had ceased the selected line reverted to the average.

Inheritance in uniparental generations is certainly much simpler to trace than in biparental, yet one large complication is attached to micro-organisms in the fact that a very appreciable quantity of a daughter in-

dividual's body substance is actually furnished by the parent. For whereas the germ cells of most multicellular organisms furnish a negligible amount of actual

Figure 25. Correlation of body volumes between parents and their offspring in Colpoda. Body volumes are in thousand cubic micra. The coefficient of correlation is $+0.561$. The frequency curve for parents, which belongs on the ordinate scale, is transferred to the abscissae for easier comparison with the curve for the progeny. Means are indicated by vertical lines. (From Adolph, '29).

material to the developed offspring, unicellular organisms receive material which is usually half of the quantity contained in the adult. It is, probably, this ma-

terial contribution which perpetuates for a time traits that are not transmitted in the relatively inviolable hereditary constitution.

2. OTHER SOFT-BODIED SPECIES

Bacillus coli. Some of the earliest observations upon inheritance of size in unicellular species were made by Barber ('07) upon *Bacillus coli communis*. He first secured a clone by the isolation with a micropipette of one individual, allowed its progeny to multiply, and then selected individuals which appeared unusually large in order to see if their size was a genetic characteristic or not. Out of 140 large individuals which were isolated, only 16 survived in cultures. One of these 16 kept its long-bodied character; it bred true for 32 months at least. In another clone 50 large individuals were isolated, but only 14 multiplied in culture; yet one of these also maintained its large size. Incidentally a third long-bodied race was established by a single selection. In *Bacillus typhosus* none of the long individuals selected within a clone bred true. In a yeast, *Saccharomyces anomalus*; repeated selections within a clone were made; these also were negative.

Barber published no measurements of size in these organisms, and possibly a micrometer was not used. It cannot be ascertained just how significant the difference was between the three selected clones and the normal-sized sister cultures. That the size difference was marked to comparative inspection should, however, have made it a significant one. The possibility therefore remains, though unconfirmed, that differences of size biotype, which were independent of cultural conditions, arose.

That extensive work upon the inheritance of size in bacteria has not been carried out, merely indicates, it

Figure 26. Constancy of body length in a clone of *Trypanosoma lewisi* which remained in the same host for many days. The same clone is represented living in the blood of three different rats. (Redrawn from Taliaferro, '23).

may be supposed, the universality with which uniformity of size is taken for granted.

Trypanosoma. Among flagellates the inheritance of size has been investigated with particular results in the parasitic species *Trypanosoma lewisi* living in rats. Some of the evidences for the exact inheritance of size in this species were reviewed by Hegner ('21). Taliaferro ('23) proceeded to infect rats with single individuals of the organism, giving rise to a pure line which was transferred to many rats. It was found that as the infection increased in age the mean body length of the trypanosomes gradually increased (figure 26). After some days the parasites attained sizes which were not only maximal but also excessively similar (figure 1). The coefficient of variability after the twenty-fifth day of infection never exceeded 4.7, and was as low as 2.2. The low variability was taken to indicate that reproduction had ceased, while all of the organisms present were able to grow until they were adult in size. This interpretation was proved by counting the organisms which were undergoing fission, and by counting the concentrations of individuals in the host's blood (Taliaferro '24). Undoubtedly the low variability resulted also from the great constancy of the environment furnished by the host.

Several rats were infected with the same pure line of trypanosomes, and it was found that after the initial period of growth the identical mean length and mean width were attained by the parasites in each of the rats (figure 26). After the twenty-fifth day of infection the mean lengths of four infections were between 29.3 and 32.1 micra, and the mean widths, which always had higher coefficients of variability, were between 1.42 and 1.74 micra (fourteen determinations).

Actinophrys. In the naked heliozoan *Actinophrys* there exists one demonstration of size inheritance. Be-

lar ('24) measured the diameters of the spherical cysts which are formed by the organisms when food is denied them. In the formation of cysts reorganization occurs, and although most cysts are zygotes, a few are parthenogenetic cysts; and these latter show in the frequency curves for diameter (figure 27) as minor

Figure 27. Distribution of diameters in a clone of *Actinophrys sol* which had been allowed to form cysts. The skewness and minor modes are due to the admixture of parthenogenetic cysts among the usual conjugation cysts. The mean diameters are represented by vertical lines. (Redrawn from Belar, '24).

modes. The values at the minor modes correspond approximately to half the volumes of those at the major modes. Belar made four series of measurements, each based on 200 to 600 cysts, upon normal organisms cultivated under uniform conditions over a period of

more than two years. The means for diameters varied between 30.3 and 31.0 micra, but the modes were identical. The coefficients of variation for diameter were always large (11 to 12), as a result both of the presence of parthenogenetic cysts and of the starvation treatment.

Entameba. Concerning many species of unicellular organisms there exists presumptive evidence that diverse size biotypes exist, and therefore that size is inherited. Thus, in the parasitic *Entameba histolytica* (Dobell and Jepps, '18) and *Entameba coli* (Matthews, '19) cysts were shown to differ both in mean size and in frequency distribution very markedly from one human host to another. The cysts from several individuals also were similar, indicating that they might belong to the same one of the various biotypes. But, unfortunately, the environments of these amebæ may have differed in such a way as to produce these very size diversities, even though the distribution of sizes from one host was usually normal. Ujihara ('14) gathered data which tended to show that the cyst sizes were not correlated with the sizes of the free-living amebæ in any one host infected with *Entameba histolytica*.

Colpoda. The infusorian Colpoda furnished data upon the inheritance of constancy in size (Adolph, '29) entirely similar to the data for *Paramecium*. Due to the fact that a spherical cyst was formed in preparation for each fission, it was a simple matter to make sure that all the organisms measured were physiologically of one age, the age of maturity. Moreover, the organisms were measured while living, so that the progeny of the very individuals which had been measured could be compared with them. Under constant conditions of temperature (26.5°) and medium (an inorganic salt solution and a suspension of green flagel-

lates of a single clone as food) the variability was that shown in figure 20 above. Each distribution shown is based on measurements made on two to four successive days, and there was little difference in the distributions on each of the several days. The progeny of a single individual did not vary so widely as the progeny of all cousins or of all second cousins or of all daughters. But there were also slight differences between the sets of animals from different culture dishes; showing that with the utmost control of environmental conditions, environmental effects were not wholly excluded, though it happened that the environmental effects averaged out upon successive days. It is important to note that the range of variation was just as great among the animals in a single dish in the second generation from a single ancestor as in a large clonal population. There seems no doubt, therefore, that most of the variation is unavoidable by any possible isolation of the immediate ancestors.

The effect of conjugation upon the inheritance of size was not studied in Colpoda. Encystment in protective sheaths involves a considerable reduction of body volume which is largely accounted for by the expenditures in digestion and evacuation. After emerging from encystment the same sizes were shown by the animals as before it, when cultivated in the same way.

3. SHELLED RHIZOPODS

In contrast to the organisms so far considered, shelled organisms have a permanent size between one reproduction and the next. Although the size of the shell is not in reality the size of the organism, it is undoubtedly a proper measure of the organism at the time when the shell formed. At reproduction there is a rapid and great swelling of the protoplasm, so

that essentially the increase of size in the measureable mass occurs all at once in each generation.

Arcella. The genus which has been most studied is *Arcella*. Pearl and Dunbar ('03) studied the variability occurring in a single wild culture of *A. vulgaris*. The diameters proved to be unimodal in frequency, but the variation was great (table 5), perhaps due to lack of uniformity in the ultimate ancestry. Nevertheless there was no essential change in the mean size or in the variability throughout a period of three weeks in this culture.

Hegner ('20) compared the inheritances of size in several races of the one species *Arcella dentata*. The individuals of this species are characterized by having two nuclei each. He found unimodal frequencies for the diameters of shells, both in wild populations and in pure lines or clones; the variability among the latter being considerably smaller than among the former. Within a clone there was a high correlation (of +0.49) between diameter of parent and diameter of offspring, which is a fact of great importance.

Occasionally a very small or a very large specimen was produced; the progeny of the unusual individual were then also unusually small or large, yet always tended toward the mean. Four or five generations were required to return to the mean. Meanwhile the original small or large individual reproduced more first-generation progeny, all of which were like the first of its progeny in size. Similar regulations toward mean size, extending through four or five generations were observed by Hegner after a half or less of the shell, protoplasm, and nuclei had been cut away (figure 28), and again after an individual had regained the binucleate condition by means of the process of reproducing an empty shell without fission of the living material (figure 29).

Hegner ('19a) selected individuals of *Arcella dentata* which exhibited large and small sizes, and found that then the diversities were maintained through many weeks; and at least six weeks after selection had ceased. He supposed that this diversity within a clone was permanent.

Figure 28. Progression of shell sizes in two lines of *Arcella dentata*. Two single uninucleate individuals were cut into two between the zero and first generations, and all the progeny remained uninucleate throughout the observations. (Data of Hegner, '20b).

Selection for shell size was carried out by Jollos ('24) in three species of *Arcella*. He gives data only for *A. polypora*; in this species cultures were followed for eleven months after selections had ceased. Periodically he measured the sizes of the progeny, but he, like Hegner, does not state whether or not he counted with the later progeny those immediate progeny of parents whose shells had persisted from previous periods. Jollos's best experiments are represented in figure 30, and it is apparent that during many months

the mean size gradually came back to the original size, and then stayed there.

Reynolds ('23) found the variability in diameter of shells in a clone of *Arcella polypora* considerably greater among individuals which had an abnormality

Figure 29. Restoration of mean shell size in the progeny of 13 individuals of a clone of *Arcella dentata* which were rendered uninucleate by cutting the body and shell into two. In each of the 13 lines the progeny became binucleate again between the zero and first generations by the process of forming and casting off an empty shell. (Data of Hegner, '20b).

of shape than among normally shaped individuals of the same clone. He supposed that the abnormally shaped individuals were descended from a parent which was in reality two unseparated or fused individuals. The variability was then due to variation of

projected surface rather than to variability of body volume, and for this reason the data need not here be analysed further.

Figure 30. Inheritance of diameter of shell, in a clone of *Arcella polypora*. At A selection of the largest and of the smallest individuals was started, seven selections being made up to B, when selection was stopped. At C one unusually small individual was isolated, and the smallest of its progeny was selected after each series of measurements up to D, when selection was stopped. (Data of Jollos, '24).

Diffugia. A very extensive study of size was made in the species *Diffugia corona* by Jennings ('16). Variation of shell diameter was usually large in wild populations and in wild cultures, but very small (3.16 to 5.74) in pedigreed clones. The latter was not always the case, for in the largest family studied the coefficient of variation was 13.65. In ordinary pedigreed cultures, size differences were greater among distant relatives than among close ones, and diversities of size

obviously increased progressively with the generations. This was shown in particular by the close correlation for size between parents and offspring, which among ten families (Jennings's table 32) averaged $+0.37$, and in one very large family was $+0.67$. Since families gradually became diversified, it was possible to select very large and very small individuals. Since their progeny each tended to resemble the parents, the diversity endured for some time. Selection, therefore, increased the diversity. But it was apparent that the progeny always varied back toward the mean. Unfortunately the permanence of the selected diversities of size was not tested throughout many generations after selection had ceased.

It is possibly of significance that selection was able to increase sizes to very great extents, while selection was effective in decreasing the sizes only to small extents. The largest organisms showed a very high mortality; a fact which constitutes a distinct limitation to diversity of biotype.

In several instances considerable variations of size appeared suddenly, and were inherited for at least five generations of *Diffugia*. But in the light of Jollos's ('24) observations on *Arcella* this is not necessarily an indication of genotypic change.

Centropyxis. Another species in which the inheritance of size has been studied is *Centropyxis aculeata*, by Root ('18). Again clones proved to show permanent differences in shell size, but data upon variability within populations or clones were not published. In two families there were distinct correlations between shell sizes in parents and in progeny (of $+0.253$ and $+0.088$); but these disappeared in the second and further generations. In this species therefore the size of the race was more important than the size of the immediate parent; in *Diffugia* and *Arcella* the size

of the immediate parent was often as strong a determiner of body size. No experiments upon selection for size were carried out by Root.

Summary. In the shelled rhizopods there is, obviously, a somewhat complicated control of the size of offspring. On the one hand each individual tends to resemble its immediate parent; and on the other hand all individuals on the average tend to assume a size which is inherited through many vicissitudes and over years of time. The first sort of factor has been termed by Jollos ('13) a persisting modification (*Dauermodifikation*); the second is obviously inheritance in every possible understanding of the term. The first factor can certainly be distinguished from the second; whether it too is an inheritance is a matter of definition.

Persisting modifications in size (without selection) lasted only one generation in *Centropyxis*, but much longer in *Diffugia* and *Arcella*. When large and small individuals were repeatedly selected, then the progeny gradually reverted to the mean for the clone, yet persisted in *Diffugia* for at least eight generations, and in *Arcella* for a hundred or more generations. But finally (in *Arcella*) the mean size was reattained after six months (figure 30). It may easily be imagined that in the production of a shell, during the fission of one of these species, a large influence is furnished by the old shell or by the old volume of protoplasm. This picture is certainly true to some extent; it is a way of accounting both for the parental resemblance and for the requirement of four or five generations for reversion after cutting in *Arcella*. But it may be too simple to account for the six months' persistence of selected effects in *Arcella*. Jollos ('24) has shown that other sorts of persisting modifications are transmitted through many shell-less generations in this genus, and also in shell-less infusoria ('21).

Although some of these modifications are not dependent upon the shelled condition, it is likely that they are to be distinguished as having to do with "cytoplasmic activities" primarily, in the sense that they modify the expression or realization of genotypic characters without modifying the ultimately transmitted character. If the transmitted genetic constitution were changed, it would hardly find its way back after six months (figure 30) to its original quantitative size-value.

CHAPTER V

THE RATE OF MULTIPLICATION

THE STUDY of any living unicellular species first concerns itself with reproduction. Multiplication of individuals is, to all visible perception, the predominating business of micro-organisms. Multiplication rate is commonly used as the criterion of suitability of a culture medium and of a food material, and as the criterion of whether a given environmental factor influences the race. Multiplication rate, rather than size or any other character, came to be the criterion of life in these organisms because of its tangibility and convenience.

The rate of multiplication is the measure of how much time the individuals are allotted in which to grow. It is obviously a limiting factor in the acquirement of body size. Its ease of measurement presents the opportunity to investigate quantitatively how size varies in correlation with the rate of multiplication.

1. ENVIRONMENTAL FACTORS

Cultural conditions. For many species of unicellular organisms cultural conditions have been found under which the rate of multiplication is fairly constant. The rate has been universally studied by reckoning the average number of multiplications from counts of the number of individuals present upon successive days or at other convenient intervals. This method gives correctly the mean rate, but obscures most of the variations from the mean. Yet modifications of the mean rate were nearly always found in successive days or weeks or months. Were these changes inherent, or

due to conditions, or were some of the changes due to each?

It is recognized by most of those who have cultivated unicellular species that the more constant the rate of multiplication is, the more constant have been the conditions of cultivation as judged simultaneously by other criteria. For each race of organisms, therefore, it is probably useful to study only the modifications observed when the rate is most constant in control cultures.

In the past it has often been assumed that similarity of multiplication rate existed throughout a species. This assumption is known to be untrue in some cases; differences of race must, therefore, be recognized in order that reproducible results may be compared and not confused.

Constant conditions. Techniques have now been devised whereby a great many species of unicellular organisms may be cultivated so that uniform rates of multiplication result.¹ Uniformity of multiplication means, of course, that the number of organisms present at each equal increment of time shall be a uniform multiple of the number at the previous period. Such a relationship is a geometric progression; when the logarithm of the number present is plotted against the time, a linear relationship results. Certain limitations must be understood; for it would be possible to have reproduction occurring in rhythms, as so often occurs in green organisms, when the numbers may not increase for some hours or days which are occupied in growth of individuals. Roach ('26) studied such an

¹In bacteria: Barber ('08), M'Kendrick and Pai ('11), Penfold ('14), Chesney ('16). In yeasts: Slator ('13, '21), Clark ('22), Peskett ('24, '25, '27), Balls and Brown ('25), Richards ('28a). In ciliates: Cutler and Crump ('23, '24), Greenleaf ('26), Myers ('27), Beers ('28b). In green organisms: Hartmann ('21), Roach ('26), Jahn ('29). In sporozoa: Taliaferro ('25), Hartman ('27).

instance in *Scenedesmus*, and found that logarithmic increase of total bulk prevailed even though the increase of number of individuals occurred in steps.

It was the general rule with older techniques that a considerable "lag" occurred in the rate of reproduction just after transplanting to new medium.² It has been shown that this temporary inhibition of reproduction is due to differences between the old and the new medium; though it is not always agreed as to what factors of the medium and of the organisms are responsible for every instance of lag. It seems worth pointing out that a lag in rate of reproduction may be due in part to an increase in the size of each individual, and therefore not to any failure to accumulate protoplasm. This is certainly the case for *Bacillus coli*, relying on the data of Henrici ('23), which are given in table 3 and later on in figure 46.

Influential factors. A great many factors have been demonstrated to influence significantly the rate of reproduction of particular unicellular species. Internal factors such as conjugation³, various forms of parthenogenetic reorganization⁴, encystment, "life cycle", life history, reduplication, and regeneration, all play important parts. The more directly influential external factors of oxygen supply⁵, temperature⁶, age of culture fluid⁷, quality and concentration of food⁸, con-

² In bacteria: Müller ('95), Hehewerth ('01), Rahn ('06), Lane-Claypon ('09), Coplans ('10), Penfold ('14). In yeasts: Wildiers ('01), Carlson ('13), Slator ('13). In ciliates: Robertson ('21), Yocom ('27), Petersen ('29).

³ Maupas ('88), Hertwig ('89), Calkins ('02), Jennings and Lashley ('13a).

⁴ Woodruff and Erdmann ('14), Hertwig ('14), Fermor ('13), Calkins ('21).

⁵ Gutmanowna ('15), Medrkiewiczowna ('21).

⁶ Dallinger ('87), Maupas ('88), Ward ('95), Müller ('95), Woodruff and Baitsell ('11), Mitchell ('29).

⁷ Lane-Claypon ('09), Woodruff ('11a), Penfold ('14).

⁸ Beers ('28a, '28b), Penfold and Norris ('12), Vieweger ('23), Cutler and Crump ('24, '27), Ludwig ('28b), Adolph ('29), Butterfield ('29).

centration of various solutes⁹, likewise are almost universally potent.

Methods have been devised of controlling and holding almost constant every one of these factors; and it is when these conditions are controlled most accurately that the greatest constancy of reproductive rates has been observed.

The best control has been attained in the case of organisms such as green flagellates, which can synthesize their own organic compounds from a medium of inorganic salts. For biophytic species it is best to feed a pure culture of other organisms washed free of the medium in which they in turn were grown (Belar, '28).

Absolute invariability of cultural conditions can theoretically never be attained; and it will always remain a bare possibility that the seemingly inherent residuum of variability in rate of reproduction and in other functions is environmental in origin.

2. INTERNAL FACTORS

To study exactly how constant the rate of reproduction is, it is necessary not merely to count the numbers of individuals at intervals, but to time accurately the very minute at which each individual is produced by fission. This has been done only for the infusorian *Colpoda* (Adolph, '29); the variability found is shown in figure 31.¹⁰ The statistical consideration of fission rate leads to a useful conception of the processes of multiplication, and from it can be found, as will be

⁹ Nowikoff ('08), Ugata ('26), Hammett ('29), Peters ('04), Spek ('20), Hartmann ('24a), Packard ('26), Middleton ('28). Further references to many potent solutes and some physical influences are given by Ball ('25a).

¹⁰ Similar observations of the time required for one complete generation have been made on bacteria and yeasts, as in the work of Barber ('08) and Peskett ('25), but no frequency curves nor individual determinations were reported.

shown later, how reproduction is related to the size of the adults when ready to reproduce.

Inheritance. That rate of reproduction is inherited within each species might almost be assumed. That it is inherited within, and differs in value for, each of many races of one species, has been repeatedly demonstrated upon infusoria under controlled cultural conditions¹¹.

Under apparently identical conditions it is possible to select from a single clone fast-fissioning and slow-fissioning lines (Middleton, '15; Parker, '27). It may be that these differences are due to unequal distribution of bodily material (Petersen, '27) or of genes (DeGaris, '28) to the offspring, or that the diversities arise during "reorganization" processes of some sort.

Depressions. Over long periods of time, marked decreases in the rates of vegetative reproduction have often been observed. Whether these are due to external or to internal contingencies has been one of the the major problems of protozoology. Permanently downward trends in the rates of reproduction have been regarded as deteriorations of the internal capabilities of propagation. In every case investigated, however, some influences of environmental factors upon the rates of deterioration have been found¹², and in many cases the running down has been shown to be unnecessary and controllable¹³.

Reorganizations. Not only long-continued downward trends but also cyclic trends or rhythms in rates of reproduction have been observed. These were cor-

¹¹ Jennings and Lashley ('13a), Jollos ('21), Middleton ('15), Parker ('27).

¹² Woodruff ('08), Baitsell ('14), Jennings ('20), Beers ('26, '28a, '28b), Austin ('27).

¹³ Woodruff ('11b, '21, '25, '29), Enriques ('16a), Hartmann ('17), Mast ('17), Metalnikow ('22), Chatton and Chatton ('23), Belar ('24), Spencer ('24), Dawson ('28), Woodruff ('28).

related, in *Paramecium*, where fully adequate environmental influences were at first not identified, with internal structural reorganization processes¹⁴. But, in most species, such reorganizations as involve the nuclei occur only in encysted or other non-vegetative states of existence (Fermor, '13). In nearly all species both the trends in rates of reproduction and the nuclear reorganizations have been found controllable, and usually avoidable.

3. INHIBITION OF REPRODUCTION

The possibility of separating reproduction from growth in size, in unicellular organisms, has often been suggested, but the experimental realization of it has been surprisingly rare.

Separation from growth. The greater or lesser size of organisms which are cultivated under contrasting conditions has frequently been measured. The modification of adult size by temperature means that the ratio which reproduction bears to growth in volume differs at each temperature. Jollos ('13) showed that some inorganic substances also affected the size of *Paramecium* in such a way that reproduction and growth in size were influenced differently; therefore, he concluded, the regulators of the two are not identical.

Perhaps the clearest case of separation of reproduction from growth is that studied by Hartmann ('21) in *Eudorina*. In this green colonial flagellate, under certain conditions, the asexual reproduction of thirty-two new colonies from one parent colony occurred every fifth day, when light is supplied for twelve hours out of each twenty-four. When, however, the same light is supplied continuously, reproduction is has-

¹⁴ Woodruff and Erdmann ('14), Hertwig ('14), Erdmann and Woodruff ('16), Jollos ('16), Woodruff ('17), Young ('17, '18).

tened. For the first reproduction, sixty hours of light are again utilized for growth in one generation; but thereafter reproduction proceeds much more hastily, allowing less time for growth. Actually the individuals and the colonies became more and more dwarfed in each generation until they died out. In this species of organism, growth inhibited reproduction over short periods of time; and in fact it is well known that in the Volvocales reproduction usually occurs in the first few hours of darkness. When under the experimental conditions photosynthesis and accretion proceeded without intermission, then the balance between growth and multiplication became upset, and multiplication went too fast.

Premature fissioning. The instances in which reproduction goes faster than growth throughout more than a few generations are not known to be numerous. Something of the sort occurs in those instances of "depression" where reproduction keeps on. Starvation alone is said to allow the continued reproduction of smaller individuals in some species. Similar results may occur in response to low tensions of oxygen. Where there are diverse types of reproduction, as in the formation of spores, or of cysts, or of gametes, there may be multiplication without growth. And, of course, the performance of two or more fissions at one period of reproduction, which is common to many species, particularly of Volvocales, is a variety of reproduction without growth which is usual and "normal."

Various experimental methods have been employed in attempts to induce early fissioning. Specific organic food compounds have been reported to hasten reproduction (Ugata, '26), though simultaneous modifications of adult size were not looked for. Changes of osmotic pressure, acidity, and salt content have been found to modify multiplication in particular instances,

but in hardly any instances were changes of adult size looked for. The "mitogenetic radiations" from onion-root tips were said by Baron ('26) to increase the numbers of buds formed by yeast cells, but by Urbanowicz ('27) not to change the rate of multiplication in *Paramecium*.

Every factor which significantly modifies fission rate requires study in relation to adult size. If size is affected without the fission rate, then the influence is upon the rate of growth; if fission is affected, then growth may or may not be also affected.

Retarded fissioning. The hastening of fission under circumstances that are already adequate for the continued growth and multiplication of individuals is, therefore, of rare occurrence and experimentally difficult to induce. But the inhibition of reproduction under circumstances where growth continues, occurs frequently, and in response to definite stimuli.

In numerous cases double organisms, or organisms with excessive body substance, have been observed by chance. Such modified forms have been found to persist for many generations in *Paramecium* (Simpson, '01; Jennings, '08a; Stocking, '15), in *Didinium* (Mast, '09), and in *Oxytricha* (Dawson, '20). In most instances it is impossible to distinguish between mere abnormality of size and true failure of formed progeny to separate. Only in the extreme cases can the individual be defined; in the borderline cases no criterion has yet been discovered which will differentiate between single and multiple individuals, if in reality there is any objective differentiation.

Incomplete fissioning. In relatively few instances have methods been found by which incomplete fissioning can be induced.

After cutting. Balbiani ('91, '92, '93) found that injury by cutting might result in the formation of mon-

sters during subsequent regeneration. The forms of these monsters, in *Stentor* and in *Paramecium*, indicated that they were truly multiple animals; they had grown, but their protoplasts failed to separate. Some of the monsters contained single unusually large nuclei; others contained a separate nucleus and other distinct structures, for each of the individuals represented in the incomplete fissions. Balbiani ('93) also found that normal single individuals were given off by regular fission from one or another of the cohering "individuals" of *Paramecium*.

The two species used by Balbiani have yielded the most extensive results in subsequent cutting experiments. In *Stentor* incomplete and irregular fissions were observed to occur by Johnson ('93), Prowazek ('04a) and Stolte ('22). Irregular fissions were similarly observed in *Paramecium* by Lewin ('11), Calkins ('11b), and Peebles ('12). But in all these cases the abnormally formed or sized *Stentors* or *Paramecia* either die or within a few generations regulate back to normal, and not only in cytoplasmic structure but also in nuclear form and size.

In another ciliate, *Stylonychia*, monstrous forms resulted after cutting (Prowazek, '04b); but these individuals were not observed to undergo further fissioning.

Under chemical influences. Prowazek ('08) investigated the influences of some chemical substances upon fission in *Trypanosoma equinum*. He found that extremely dilute hydrochloric acid depressed cytoplasmic division while nuclear division went on, and big individuals were thus obtainable. In no cases, apparently, was Prowazek ('10) successful in hastening fission in *Colpidium* by means of chemical substances.

Crampton ('12) noted that *Paramecia* and *Stylonychia* which were confined in narrow glass tubes failed

to divide. Evidence could not be obtained that this was due to any shortage of oxygen or food or any chemical change in the culture medium. But the experiments do not entirely preclude these factors, and Kalmus ('29) was able to demonstrate that with less soluble glass and with higher concentrations of bacteria the *Paramecia* divided at the normal rate. Crampton's effect was probably of a chemical sort, therefore.

It was noticed by Chatton ('21) that the presence of sodium bromide in the culture medium of the ciliate *Glaucoma* caused subsequent fissions to remain incomplete. An effective procedure was to place individuals in a 1.6 percent solution of the salt for ten minutes, and then to transfer them back to the usual culture medium. Certain of the individuals became double, especially with respect to the micronuclei and to the mouth. Such a double condition then persisted through subsequent generations for at least five months.

The exact times of the generation cycle at which such treatments with sodium bromide were effective were worked out upon *Colpidium* by Ephrussi and Lwoff ('23). It turned out that two periods of susceptibility existed in each generation, one at the time of fission and the other about half-way since the previous fission.

Another method of inhibiting the completion of cytoplasmic fission was worked out by Chatton and Chatton ('25). When a certain strain of *Bacillus coli* as food was given to either of two species of *Colpidium*, two or four ciliates remained attached in the form of a chain. The effect was highly specific, for other genera of ciliates gave no such response. Moreover, other species of bacteria as food, and even three other clones of *B. coli*, did not produce the effect.

Treatment with very dilute hydrocyanic acid induced formations of multiple individuals in *Parame-*

cium caudatum (DeGaris, '27). These monsters reproduced individuals of normal size, but the formations persisted for several generations. Similar formations were induced by ultraviolet radiations, according to Hinrichs ('28) and MacDougall ('29). The possibilities of all these methods of inhibiting reproduction appear to be great enough to warrant their extensive use in investigating the nature of cytoplasmic size regulations.

Numerous observations of the inhibition of fission, in media containing chemical substances, have been made upon bacteria. In the presence of urea or of human urine, *Bacillus coli* formed filaments up to 120 micra in length (Wilson, '06). Salts such as calcium chloride and magnesium chloride stimulated the formation of large individuals in various species (Hata, '08). Similar influences were exerted by modified hydrogen ion concentrations (Reed and Orr, '23). Various dyes were effective in producing the formation of long filaments; the experiments of Vay ('10) seem to indicate that the effects were highly specific. Revis ('12) described the appearance of long, branching filaments of *Bacillus coli* when grown on agar media containing malachite green. These filaments were said to contain no cell walls, as though formed by the growth of bacilli which did not multiply.

These are a few cases out of a number which have been described for various bacteria. Apparently in none did the observers follow out what might be implied in "Koch's laws", obtaining back normal individuals by subsequent culture. But in numerous instances where monstrous "involution forms" of various species of bacteria have been obtained, the more familiar forms appeared again after return to normal fission rates upon new and adequate medium (Löhnis, '21; Henrici, '28).

Under mechanical factors. Among rhizopods there are reports of inhibition of reproduction, in the presence of certain mechanical conditions¹⁵. Levy ('24) believed that separation at fission in *Ameba proteus* was successfully prevented by confining the dividing individual in a very small lane of water upon a slide or by applying an appropriate mechanical stimulus. Belar ('21) found the viscosity of agar medium a determining factor in the separation of the progeny in various species of *Chlamydomorphys*.

Unequal or eccentric fissions can be produced by a number of different influences. Such were noted by Ryder ('93b) in *Euglena* which were kept for some days under a coverslip. He attributed the inequality in mitosis to the localized shortage of oxygen in the body, a conception which was supported by the additional fact that sister individuals which were lying side by side became mature for fission at widely diverse times.

Centrifuging of *Stentor* was found by Popoff ('09) to lead to subsequent unequal fissions. The inequalities persisted for at least one week, during which a fission occurred. The evidence is that such a modification was ultimately restored to normal size (Burnside, '29).

Popoff ('09) also tried the effect of cold, and stated that some unequal progeny resulted. It is not clear that such giants would persist when restored to the original conditions of culture. It seems only inevitable that more than one generation should be required for complete restoration of size.

Multiplication of nuclei only. The fates of individuals in which cytoplasmic division failed to accompany nuclear fission are various. Usually, it may be said,

¹⁵ Blochmann ('88), Stolc ('06, '10), Kühn ('16, '20), Levy ('24), Breuer ('17), Belar ('21).

multiple progeny were maintained for several generations, but usually this was true in only one of the progeny of each fission. In the instances investigated, the unusual individuals either regulated back to normal by multiple fissions, or died. There are no exact measurements of size in such progeny. Kühn ('16) noted that in a general way the cytoplasm was distributed proportionally to the number of nuclei in multiple *Amebæ*.

Sometimes the fission of the cytoplasm is absent while nuclear division occurs. Blochmann ('88) described the rejection of mitotically produced half-nuclei in *Euglypha*, and remarked on its resemblance to the formation of polar bodies in eggs. In *Euglypha* a new shell was also formed, the rejected half-nucleus being deposited within it. Hegner ('20) described the formation and rejection of shells alone in *Arcella*. This was observed to occur only in individuals which had been deprived of one nucleus, the binucleate condition being usual. The rejection of a shell always accompanied the formation and retention of two nuclei from the single one. That cytoplasm and nucleus together should go through such a partial process is striking.

Among some heliozoa and a few other multinucleate species, all numbers of nuclei between one and several hundred nuclei per individual can be found. The factors that determine when the cytoplasm shall divide are apparently here wholly unrelated to the factors for nuclear division, except of course for the limitation that no cytoplasmic portions separate without nuclei. It is usual in these species for both cytoplasm and nuclei to divide into two equal or nearly equal aggregates.

Chamber progression. Some data upon the relative volumes of individuals exist in the instances of what may be interpreted as a very interesting variety of inhibition of fission occurring normally in the shelled

foraminifera. During growth, larger chambers are successively formed by the individual. Measurements of the linear dimensions of these chambers show that the ratio between the last old one and the first new one tends to be constant (table 6). In a number of

TABLE 6.

RATIOS OF LINEAR DIMENSIONS IN SUCCESSIVE CHAMBERS OF FORAMINIFERAN SHELLS. DATA OF RHUMBLER ('09-'11)

Species	Dimension	Number of ratios measured	Extremes	Mean
<i>Pulvinulina menardii</i>	length	14	1.07-1.52	1.19
<i>Rheophax nodulosa</i>	"	8	0.88-1.76	1.18
<i>Rheophax nana</i>	"	10	1.00-1.70	1.23
<i>Rheophax dentaliniformis</i>	"	4	1.38-1.45	1.40
" "	breadth	4	1.20-1.31	1.27
<i>Biloculina bradyi</i> , form A	"	4		1.28
" " form B	"	8		1.28
<i>Trioculina rotunda</i>	"	8	1.25-1.38	1.33
Mean				1.27

instances the linear ratio is, at least on the average, very nearly 1.26, as was shown by Rhumbler ('09-'11). Przibram ('13) pointed out that this number is the cube root of 2; in other words that the volume of the protoplasm exactly doubles at each new chamber-formation. It is the peculiar fact that the volume doubles, rather than increases by some other multiple, which makes this resemble a sort of reproduction which is really expressed only as growth; an *Arcella*, for instance, likewise suddenly doubles the volume of its protoplasm by imbibing water at the time of reproduction, and differs from the foraminiferan in that it separates into two parts soon afterward.

The nuclear conditions among the foraminifera have not been thoroly described. According to Schaudinn ('03) a multinuclear condition may prevail in the species *Polystomella crista*. He also described nuclear divisions as occurring at times other than the

reproduction of individuals. From this it could be hypothecated either that a foraminiferan is analogous to a multinucleate heliozoan, or that it is analogous to a colonial flagellate whose individuals have not become separated.

Nuclear division. Finally, it is worth-while noting that nuclear fissions in protozoa may be regulated entirely separately from cytoplasmic fissions. This is the case in all multinucleate species which have no definite number of nuclei per individual. And it is significant that in many such species all the nuclei divide at one time. This was noted by Pfitzner ('86) in *Opalina*, and by Gruber ('87) in *Holosticha*, and by many others. In *Actinosphaerium*, individuals may exhibit an arrangement of the various phases of mitosis in zones, showing that, for instance, the prophase started in a few nuclei of one region and then spread progressively throughout the protoplasm until, apparently, all the nuclei were induced to divide in sequence. This is an extraordinary degree of coordination.

Summary. Instances have now been cited in which cytoplasmic fission, nuclear fission, growth in bulk, and aging in time stood in all the possible relationships of dependence and independence. In any particular instance these four functions may or may not be concatenated. And those combinations out of the four that normally coexist may become dissociated at least in part. The regulation of size is not always bound up with any one of the other three. Where it is so bound, a tangible factor is present, capable of setting the limit to size. Where none of these factors has to do with size, it is possible that other internal mechanisms are present to control size, without their having actually been influenced by size itself. If no internal factors limit growth in the time before reproduction, then size as such remains as its own determiner.

CHAPTER VI

SIZE IN RELATION TO MULTIPLICATION RATE

IS REPRODUCTION merely an expression of growth, as Johannes Müller ('40) postulated? In unicellular organisms does reproduction occur every time a certain size is attained, or are conditions other than increase of size required? This latter should be a simple question for which to find an affirmative answer, but a hard one to prove negatively, for a single case of reproduction without growth in the absence of any change in external conditions would indicate the possible independence of these two factors.

1. GROWTH AS A PREREQUISITE TO REPRODUCTION

The uniformity of adult size, which has been already evaluated, indicates that growth ordinarily proceeds to a fixed extent between two reproductions. When growth is modified in various ways, is reproduction also affected?

Starvation. Reproduction is usually inhibited in individuals that are starved during any considerable portion of their period of individual growth. This was illustrated particularly well in the starvation experiments with *Paramecium* recorded by Estabrook ('10). Jickeli ('02) found some exceptions to this generality, and put forth the contrary hypothesis that fission is stimulated by hunger.

In many instances, as that cited in figure 14 above, refeeding after starvation allows a resumption of growth. In such cases reproduction has been much delayed as compared with fed controls. In these cases,

therefore, growth in size may be regarded as a prerequisite for reproduction.

Hartmann ('21), in a species of green flagellate, *Eudorina*, found cultural conditions under which growth decreased in rate but multiplication increased in rate, as compared with the normal conditions for indefinite survival in cultures. The conditions consisted in the continuous exposure of the organisms to light, so that food could presumably be synthesized faster than while the usual day-and-night rhythm prevailed. This would in some respects represent the opposite effect from that produced in *Paramecium* by starvation. But probably in neither case was the food supply the essential factor.

Hertwig ('89) noted certain cultural conditions which brought about what he termed "depression" in *Paramecium* and other protozoan species. In certain phases of depression, growth of individuals was apparently retarded, but rate of multiplication increased. Similar changes were observed by Kasanzeff ('01), Wallengren ('02), Chainsky ('06), and Lipska ('10) in *Paramecium*.

Excision of cytoplasm. Increase of body size may be prevented not only by starvation and by unfavorable cultural conditions, but also by repeatedly cutting away protoplasm which has been accumulated. Hartmann ('22) periodically cut away portions of the body of *Stentor*, and found that reproduction was inhibited for as long as the attainment of the normal adult volume was prevented. But in *Stentor* both nucleus and cytoplasm were removed together; Hartmann ('24b, '26, '28) therefore did similar experiments with *Ameba*, in which only cytoplasm was removed. In *Ameba* he was able to culture single, continually growing individuals, without reproduction, for several months; while sister individuals which were not cut went through

Figure 31. Correlation between body size and the time required to grow to this size in 155 individuals of Colpoda, clone C, at 19.5°C. No significant mass correlation is to be found. Triangles and frequency curve *A*=quadruplets which produced twins. Unmarked and curve *B*=quadruplets which produced quadruplets. Squares and curve *C*=twins which produced quadruplets. Arrows indicate that the second limit of generation time was undetermined, but lies a brief time in the direction pointed from the tail of the arrow. (From Adolph, '29).

many generations. Phelps ('26) likewise prevented *Ameba* from reproducing for as long as one month through this expedient of cutting.

From these experiments upon *Stentor* and *Ameba*, the conclusion may be drawn that the attainment of a certain size is a prerequisite for reproduction. But, conversely, reproduction is not essential to continued vitality and growth of individuals. This would seem to differ from the dictum of Rubner ('12) for yeast, that "only through such processes as result in fission can they live indefinitely". But since growth of the individual under normal conditions does bring on fission, Rubner's emphasis upon the processes leading up to fission, rather than upon fission itself, was undoubtedly the correct one.

Age at fission. In *Colpoda* the body sizes of a population of adults were compared with the durations of life which were occupied in the attainment of these sizes (Adolph, '29). The mass correlation, which is shown in figure 31, gave a slightly negative coefficient; that is, the individuals with shorter lives were on the average somewhat larger than were the longer lived ones. But the coefficient was within the error caused by the normal variabilities for duration of life and for adult body size.

TABLE 7.

CORRELATION OF ADULT BODY VOLUME WITH AGE AT FISSION AMONG THE FOUR MEMBERS OF QUADRUPLT LITTERS IN COLPODA. THE COEFFICIENT OF CORRELATION IS +0.271. (FROM ADOLPH, '29)

	Order of duration of generation					
		1	2	3	4	Total
Order of body volume	1	11.5	3.	3.5	5.	23
	2	6.5	9.5	2.	6.	24
	3	3.5	5.	8.	8.5	25
	4	2.5	5.5	9.5	6.5	24
	Total	24	23	23	26	96

When, however, four sister progeny from a single parent, growing in a single dish of culture medium, were compared with respect to age and to size attained, then there was a small but significant correlation (table 7). The individuals which lived longest were those that grew to the largest size. This held true in two clones which were very different in absolute sizes; and the correlation coefficient of $+0.271$ was apparently of a significant value.

The interpretation of this plain result is slightly involved. If the small individuals had been those which divided later, then evidently they had delayed in order partially to come up to a standard size. If the small individuals divided first, then size would be regarded as having practically no influence upon the initiation of fission. As it actually turned out that size and age were positively correlated, it is just to conclude that the normal variation of duration of life was not influenced by the size of the individuals. When the processes which have to do with age came to a certain point, fission occurred regardless of how much body substance was present.

As a result, the older individuals accumulated slightly more body substance for the reason that they had more time in which to acquire body substance.

It must be stated, however, that the age of maturity is not independent of size when environmental factors come in to retard the growth of individuals. When increase of body size was inhibited through lack of food, it turned out that partially starved individuals delayed their reproduction by several hours (figure 41, later on). Within the extra time they were able to make up the deficiency of size in small part; but ultimately they divided at sizes significantly smaller than those which were adequately fed.

2. REGENERATION AND REPRODUCTION

Regeneration, as a type of growth in size, has been studied in relation to reproduction in a number of protozoan species. The invariable result was that reproduction failed to occur so soon in mutilated individuals as in uncut individuals. But in how far this is due to injury as such, to the mechanical stimulation, and the like, rather than to the loss of body substance itself, is uncertain.

Rate of regeneration of bulk. Measurements of the rates at which the volumes of individuals are restored after part of the body has been cut away are very few. Sokoloff ('24) attempted some in *Spirostomum* and in *Dileptus*; he found that eventually adult size was completely regained, and that it took longer to regain this size, the smaller the piece which was regenerating. The rate at which body volume was regained cannot be compared with the usual rate at which the volume was increased by growth, because no data on the normal growth of individuals were obtained for either of these species. It is known that growth in volume is not always proportional to the mass which the individual already has at various times (Chapter 2). Whether the rate of regeneration would correspond to the rate of growth at each size, or at each age since cutting; or whether the curve of regeneration differs essentially from the curve of growth, has not yet been decided. Data upon this point would serve to ascertain the relative importances of these two factors in increasing the body size.

At the present time it cannot be said whether reproduction by an individual cut to half its size will occur earlier or later than that of an individual reduced by normal fission to half its size.

Will more total protoplasm result in cultures where either reproduction or growth, or both, is hastened

by frequently cutting each individual into two viable ones, than in cultures which reproduce in the normal fashion? Slight evidence bearing upon this question may be adduced from an experiment of Phelps ('26). By cutting, about one quarter of the cytoplasm was removed from an *Ameba* every second day, and this prevented the *Ameba* from reproducing for 32 days. The total amount removed was about equal, it was reckoned, to 2.4 adult *Amebæ*. But the control animals meanwhile reproduced 3.5 times, giving 12 nearly adult *Amebæ*. Whether in addition to the factor of initial mass there was also some retardation due to the operation itself, one is not justified in calculating from these approximate data. Evidently *Ameba* is not a suitable organism with which to find out whether more total protoplasm will form after cutting. An organism, such as *Stentor*, which will grow and reproduce in both pieces resulting at each cutting, is needed.

Effect of fission on growth. The soft-bodied unicellular organisms are usually characterized by growth up to a certain size, after which fission into two individuals occurs and growth is renewed. Growth is necessary in most instances before fission occurs. But fission is clearly separated from growth, as previously noted; fission is not a slow and continuous process which goes on while growth is proceeding, but comes on after certain requirements have been fulfilled. Apparently growth stops in some species several minutes or hours before fission visibly sets in.

Were there less separation between growth and fission than actually exists, it would be hard to conceive how the adult size could vary so little as it has been shown to do; there is no rigidity either morphological or mechanical which visibly decides when adult dimensions have been reached. The presence of a definite cycle in the growth of individuals of some species

seems to furnish a tangible determiner of adult size. This growth cycle is still more evident in the many species where more than two individuals, usually four, are reproduced at one time.

From this point of view, fission constitutes a "rejuvenescence"; it enables growth, which came almost to a standstill before fission, to be resumed, and to be resumed most rapidly at once. Thereafter the growth intensity gradually diminishes until the next fission. It might be assumed that assimilation goes on at a fairly uniform rate, but that it is slowly caught up with by dissimulation; for it is seen in some of the data upon rate of growth that a level of maximum body size is reached shortly before fission.

Fission is undoubtedly a major factor in growth in some species. In other species, such as the filamentous bacteria, where growth is in one dimension only, fission has less effect upon growth (see figure 16), and in a few species the effect is not measurable.

On the other hand, reproduction is never regulated wholly by growth. Growth has been shown to be one prerequisite, yet not always indispensable; for age or other factors may become potent enough, at least in some species, to overcome its absence. There is evidence, now to be presented, that special factors have different effects upon reproduction and upon increase in mass.

3. REGENERATING CAPACITY AND SIZE

Thus far the rate of regeneration has been considered in relation to the onset of reproduction. But size factors are also concerned in the ability of cut individuals to survive and regenerate.

Criterion. The criterion of regenerating capacity among unicellular organisms has often been merely to observe whether they were able to reproduce. But in

metazoa such a requirement would never be connected with regeneration; because it is there easy to observe other processes which are more to the point than the ability to have progeny. Among protozoa, in fact, types of reorganization are well known to occur quite without conferring the ability to survive indefinitely by means of multiplication. When the statement is made that the nucleus is indispensable for regeneration among protozoa, it means that only nucleated fragments will reconstitute individuals which will multiply. This is universally the case among species possessing discrete nuclei.

But some species allow the nuclei to be fragmented too, and this is probably what occurs when species without discrete nuclei are injured. In many such instances, two or more individuals, each of which will give rise to endless descendants, may be artificially produced at any time from a single vegetative organism.

The presence of some cytoplasm is generally believed to be necessary for regeneration. This problem is an academic one, since it could always be argued that some small amount of cytoplasm accompanied any possible nuclear fragment.

Least size. A question of frequent interest has been: how small may a fragment be and still grow and reproduce? Average results for the smallest viable pieces are listed in table 8. All those species which show recovery in pieces constituting 1 to 7 percent of the original size are those characterized by possessing long, beaded or bacillary nuclei. In species with globular nuclei it is almost impossible to trim away more than one-fifth of the body (as in *Frontonia*) without injuring the nucleus; and globular nuclei seem to be peculiarly poor at quickly forming new surfaces and thus preserving their contents, in contrast to elongated

nuclei. A nucleus alone may constitute five percent or more of the body volume. It would be possible to regard the presence of many, or multiple, or elongated, nuclei in large unicellular species as conferring of itself a high capacity for regeneration.

For technical reasons the large species of organisms have alone been used to study the sizes of "minimal reorganization masses". The absolute volumes of these pieces are not small, therefore, as compared with

TABLE 8.
LEAST PORTIONS OF PROTOZOA WHICH ARE CAPABLE OF COMPLETE REGENERATION

Species	Number of pieces tested	Approximate bulk of one normal individual	Least portion which regenerated	Investigator
		cu. mm.	per cent	
Cut once:				
Stentor polymorphus	..	0.006	4.2	Lillie ('96)
Stentor coeruleus	..	0.011	3.3	"
Stentor coeruleus	..	0.04	1.3	Morgan ('01)
Spathidium spathula	16	0.000,01	6.6	Moore ('24)
Spirostomum ambiguum	30	0.06	1.4	Sokoloff ('24)
Dileptus anser	29	0.01	1.3	"
Bursaria truncatella	30	0.02	2.4	"
Frontonia leucas	..	0.006	20.	"
Cut repeatedly:				
Stylonychia mytilus	..	..	0.1	Dembowska ('25)
Ameba proteus	..	0.005	1.2	Phelps ('26)

species which are normally small. Moreover, in some species there exist clones which are as diverse in size as the volumes of these pieces and as the volumes of the original organisms from which they were cut. In fact, as will be shown later, starvation alone may reduce the volumes of individuals in numerous species as much as the cutting reduced these.

Repeated cuttings appear (table 8) to result in reductions which are viable in considerably smaller sizes. But in none of the experiments performed is it clear that cutting alone was responsible for the result, for starvation prevailed, and perhaps other chemical factors were potent.

The age factor. It has been concluded by some investigators that age of individuals is a factor in re-

generating capacity. Popoff ('08) in *Frontonia*, and Calkins ('11a) and Young ('22) in *Uronychia* found that larger percentages of individuals survived if cut at times other than during the process of fission. But such differences can be definitely correlated with differences in the physical state of bodily materials. In fact the size of minimal reorganization masses depends far more upon the time required to form a new surface layer on cytoplasm or on nucleus than upon the amount of material required for other functional processes. This is perhaps best shown by the large difference in the result whether cutting is done slowly or rapidly, whether over a large surface or a small, whether all at one time or in successive portions.

Genetic factors. The readiness of formation of new body surfaces varies among different clones of a single species. This was particularly noted in *Paramecium caudatum* by Calkins ('11b). It is probable that it also varies with cultural conditions.

Chemical factors. But the largest factor in the velocity of formation of new protoplasmic surfaces appears to be the chemical conditions which prevail. This was long ago appreciated by Kühne ('64) as a result of his experiments in placing *Amebæ* in various solutions of salts. Recently Mast ('26, '28), Chambers ('26), and Reznikoff ('28) have begun to discover the role of particular ions in particular concentrations upon the viscous, cohering, and imbibing properties of the cytoplasm of *Ameba*. In *Stentor*, Heilbrunn ('27) noted the particular role of calcium ions in determining the rapidity with which new surfaces are formed when cytoplasm is squeezed from the body. Similar tremendous variations in speed of "surface precipitation" in response to crushing in calcium salt solutions can be found in *Ameba*, *Paramecium*, and undoubtedly many other species of unicellular organisms.

CHAPTER VII

THE FUSION OF INDIVIDUALS

IMPORTANT INFORMATION concerning the regulation of size, comparable to that obtained from the cutting of individuals, might be obtained from the fusion of individuals. No systematic studies of fusion have been carried out; the possibility of fusing or transplanting protoplasms has often been visualized, but the experimental difficulties have proven deterring. Whereas numerous means have been found of preventing the separation of the progeny at the time of fission, the reuniting of progeny that have once separated is immensely more difficult to control.

1. AGAMIC FUSION

But occasionally fusions of individuals occur without any experimental manipulation. What are the relationships existing between individuals, which govern their individuality and their power to reunite?

Degrees of fusion. The union of cells without nuclear fusion was termed plastogamy by Hartog ('91). It is convenient to distinguish "complete plastogamy", in which fusion is so thorough as to form a doubly endowed individual of normal form, from "pseudopodial plastogamy", in which the protoplasm is continuous, but separate bodily masses are preserved. There are, also, unions of individuals with nuclear fusion which are clearly not conjugations or gametic unions or any other type of reorganizations. The origins of these fusions are very various and their persistences show many gradations.

The degree to which protista maintain their indi-

viduality is surprising. Schultze ('63), Cienkowski ('63) and DeBary ('64) remarked how Myxomycetes came into intimate superficial contact without establishing protoplasmic continuity. The same has caused surprise to the observers of most kinds of organisms that have flowing protoplasm.

The more or less permanent fusion of individuals has been observed in all classes of protozoa, a majority

TABLE 9.

SOME SPECIES OF PROTOZOA IN WHICH FUSION OF INDIVIDUALS OR THEIR PARTS WAS OBSERVED, WHERE IT SEEMS REASONABLY CERTAIN THAT THE FUSION WAS AGAMIC AND THAT THE INDIVIDUALS WERE ORIGINALLY SEPARATE

Species	Observer
<i>Heliozoa: Actinophrys sol</i>	Siebold and Cohn ('51), Gruber ('83a) Penard ('89), Schaudinn ('96) Belar ('22), Looper ('28)
Actinophrys oculata	Sondheim ('16)
Actinosphaerium eichhorni	Cienkowski ('65), Greff ('67), Brandt ('77), Gruber ('83b), Johnson ('94), Howland ('28)
Sphaerastrum fockei	Doflein ('11)
Acanthocystis myriospina	Stern ('24)
Raphidocystis infestans	Wetzel ('25)
<i>Myxomycetes: species undetermined</i>	Cienkowski ('63), DeBary ('64), Prowazek ('01), Bruck ('08)
<i>Monothalamia: Euglypha alveolata</i>	Blochmann ('88), Reukauf ('12)
Trinema sp.	Penard ('90)
Cyphoderea margaritacea	Rhumblar ('95)
Centropyxis aculeata	Schaudinn ('03)
Diffugia lobostoma	Rhumblar ('95, '98a), Goette ('17)
Diffugia urceolata	Zuelzer ('04)
Diffugia, five other species	Kepner and Reynolds ('23)
Arcella vulgaris	Elpatiewsky ('07), Khainsky ('11)
Arcella polypora	Reynolds ('24)
Chlamydomorphys stercorea	Schaudinn ('03)
Chlamydomorphys grata	Breuer ('17)
Chlamydomorphys, four other species	Belar (and Schüssler) ('21)
<i>Foraminifera: Orbitolites complanatus</i>	Verworn ('91), Jensen ('95), Rhumblar ('02)
Orbitolites duplex	Rhumblar ('02)
Amphistegina lessonii	Jensen ('95)
Patellina sp.	Schaudinn ('95)
<i>Flagellata: Polytoma (?)</i>	Dallinger and Drysdale ('74)
Myxochrysis paradoxa	Pascher ('17)
Tetramitus rostratus	Bunting ('26, '29)
<i>Ciliata: Paramecium putrinum</i>	Doflein ('07)
Stylonychia mytilus	Doflein ('07)
Chilodon uncinatus	MacDougall ('25, '29)

of the species for which it has been described being listed in table 9. In most of the instances included in this table the fusion was actually watched. In some the fusion was so complete that a single nucleus was formed, with double or more the number of chromosomes; and the body was of the normal shape but of enlarged size. Actual measurements of the dimensions were made in only one or two of the studies referred to; these are discussed elsewhere. The enlarged size was said to be sometimes maintained for several generations, but apparently tendencies existed toward the formation of individuals endowed with normal nuclear and cytoplasmic quantities. Belar ('21) noted that fused individuals tended to die, especially when more than two nuclei were present; so that there are distinct disadvantages in the multiple condition, and natural preventatives to continued propagation of such forms.

Grafting. The artificial grafting of two cut individuals to one another was first performed by Cienkowski ('65) in the heliozoan *Actinosphærium*. In one case he fused together five individuals, holding them in contact by paper strips; soon afterwards the giant individual underwent fission. This was possibly in response to the large bulk of its substance. Judging from the frequency with which *Actinosphæria* fuse spontaneously, it is questionable just how much the manipulation had to do with the success of Cienkowski's grafts. Prowazek ('01) got three *Myxamebæ* to fuse after cutting, but the fate of the resulting giant was not described. Other attempts to induce fusion by cutting have been conspicuously negative in result, as in the early investigation of Verworn ('91) on the marine *Thalassicola*.

In *Ameba* the possibility of inducing fusion has been investigated several times. Approximation and cutting have so far yielded no continuity of cytoplasm,

even between sister individuals.¹ Locomotion and form changes in *Ameba* are accompanied by the continued formation of new surface films; at least in the plasmagel (Mast, '26). Some experiments in which *Amebæ* were crushed suddenly under a coverglass led to the bursting of old films and within limits to the formation of new ones, though it could not be ascertained directly that the plasmalemma as well as the plasmagel was broken by this treatment. This makes it possible that successful fusion could be brought about by happening to crush two individuals simultaneously in such a way that the crushed portions came in contact with one another in the incredibly brief interval before new surface films form. The conditions for the formation of these films have been little investigated in protozoa; but it is known that they vary widely among clones, and with changes in the inorganic composition of the medium.

Among the hard-shelled marine foraminifera the fusion of pseudopodia has been often observed. Verworn ('91) attempted to produce such fusions artificially without success, though he discovered fusions which had occurred spontaneously. Jensen ('95) observed fusion of separated pseudopodia, and found that sister individuals (Rhumbler, '02) fused unavoidably if they came into contact. But there was never any significant acquirement of cytoplasm on the part of one or another individual. Random individuals of the same species did not unite their pseudopodia; on the contrary their pseudopodia either broke or withdrew whenever they actually touched one another.

The genetic relationship among plastogamic individuals has been demonstrated to be exceedingly important in the case of shelled rhizopods. Schultze

¹ Kühne ('64), Berthold ('86), Rhumbler ('98b), Jennings ('04), author.

('63) found that, in *Gromia*, pseudopodia of the same individual would fuse with one another, but not with those of different individuals. Kepner and Reynolds ('23) found for various species of *Diffugia* that pseudopodial fragments cut off from an individual were not only reincorporated with the cytoplasm from which they came, but that the individual reacted toward the fragments in such a way as to creep in their direction. When fusion between individuals did not occur, there was, on the contrary, a shattering of the protoplasmic surface. Fragments from one individual were sometimes incorporated by certain other individuals; probably they belonged to the same clone. In *Arcella*, Reynolds ('24) showed clearly that the hereditary relationship among individuals determined whether the fragments would fuse or shatter. Starting from a single parent, the descendants became gradually diverse enough so that fusion occurred rarely or not at all. Diverse environments were effective in hastening the attainment of diversity in the cytoplasm.

Among ciliates, where most is known about size-regulation and pedigree's, the instances of cell fusion are particularly rare. This in itself may be an evidence of more efficient control of size. Illustrative of experiments which have been tried are those of Prowazek ('01), who obtained very temporary fusions in response to cutting in *Glaucoma*; and those of Morgan ('01) who transplanted without success cut pieces of *Stentor*. Greater hope of success in grafting lies in first modifying the physical properties of the cytoplasm by radiation (MacDougall, '29) or by chemical conditions (Heilbrunn, '27).

Significance of fusions. In order to evaluate fully the significance of an observed or induced fusion of individuals, one must know the genetic relationship of the individuals, and then follow the individuals and

their progeny through many generations. In this way the fusion may be identified as agamic, or gametic, or plasmodial, or as related to some other event concerned in a morphologically known life cycle.

The possibility suggests itself of building large individuals by transplanting protoplasm, by means of a micropipette, from the body of one individual to the body of another. To one unacquainted with the properties of cellular materials this seems to be a perfectly feasible sort of experiment. In actual fact cytoplasm of egg-cells and other cells with which experiments have been made does not remain liquid when being handled, and any transplanted portion remains discrete from the host cytoplasm. It is never incorporated but is finally ejected.

It is obviously important to know that two vegetative protozoan individuals can fuse or be induced to fuse under some circumstances. The fact gives a practical method by which the size of an individual may be increased without the intervention of growth processes. Thus far no consistent attempt has been made to find how such individuals, either multinucleate or polyploid, regulate their body sizes. It is probable that they exhibit sorts of regulation, with respect to further fission and to liability to death, similar to those exhibited by the fused individuals which have been observed under various accidental conditions, as above enumerated.

The fusion under controlled conditions of unicellular individuals is, on the whole, almost as difficult to obtain as the fusion of neighboring cells in multicellular organisms. To investigate the attainment of union among individuals should become all the more important because of this extreme degree of individuality which is ordinarily manifested by them.

2. THE CONDITIONS FOR CONJUGATION

It is difficult to distinguish some kinds of plasmogamy from true conjugation, for it is not certain that every fusion of nuclei is a true union of gametes. It has often been supposed that conjugation might be a mechanical process, occurring whenever the superficial cytoplasm became sticky. This view would require the corollary that in species where an external condition produced the stickiness, some mechanism caused the nuclei of fused bodies also to fuse.

The conditions for conjugation are partially known in some species². It is fairly certain that deep-seated physiological changes accompany whatever physical condition of the cytoplasm is requisite, at least in cases of typical conjugation.

Occurrence. The phenomena of conjugation, in those species of ciliates upon which studies have been made, lead to the following picture of its occurrence. The ability to conjugate as gametes is, probably, wholly absent in some species or clones. Where present it can be entirely prevented by appropriate cultural conditions. Where inducible, the conditions for induction differ not only for each species, but for clones within the species. With proper means of induction, conjugation can be induced very frequently, and therefore with little reference to any particular interval of time since it last occurred.

It is to be supposed that conjugation can occur in any species where there is a morphological differentiation of gametes, even though such conjugation has not been seen. But the union of the gametes, of course, need not occur under all conditions under which they are formed.

² Stein ('54), Everts ('73), Bütschli ('76), Maupas ('89), Verworn ('00), Enriques ('07, '08, '10 '16b), Woodruff ('08), Jennings ('10), Zweibaum ('12), Hopkins ('21), Dogiel ('25), Ball ('25b), Austin ('27).

A method. The problem of size-regulation may receive important elucidation if and when it is found possible to control conjugation more accurately than has heretofore been attained. Plastogamies might be induced at will if the nuclear fusions, or merely the nuclear reduction divisions, could be inhibited after union of cytoplasm has been completed. Stolte ('24) described in *Blepharisma* certain naturally occurring "conjugations" which gave rise to reorganization of the nuclei without any fusion of or exchange of nuclear material. Thus the nucleoplasms of the two individuals remained distinct while the cytoplasm united; it is necessary to study further the conditions under which the union will persist when once it has thus spontaneously begun.

What constitute the differences between agamic fusion and gametic fusion? The answer, when found, may carry with it important corollaries concerning the regulation of size of the entire body, of mass of nuclear material, and of capacity for multiplication or further synthesis of more protoplasm in the race.

3. CANNIBALISM

When an individual engulfs another individual of its own species the result resembles, in some respects, a fusion of the individuals. Although in no cases the acquired bodily material is directly incorporated, the bulk of the body is apparently increased more rapidly thereby than by any other means. Whereas, with a few exceptions, those food materials which are acquired in living or in solid form are small in bulk compared with the assimilating individual, those acquired by cannibalism form a relatively large bulk of nutritive substances in exactly the proper proportions for growth. A mass approximately equal to the bulk already owned by the cannibal is acquired at one gulp.

Cannibalism is not widespread among protozoa, perhaps because of the mechanical difficulties of handling a portion of food equal to the individual's own bulk. Cannibalism was observed among ciliates in *Oxytricha* by Haime ('53) and J. A. Dawson ('19), in *Onychodromus* by Maupas ('88) and Joukowsky ('98), in *Pleurotricha* by Joukowsky ('98), and in *Stentor* by Gelei ('25). Among naked rhizopods it was reported by Leidy ('79), Penard ('02), Jennings ('04), Lapage ('22), and Ivanic ('27).

Oxytricha. A few interesting data upon body size and upon reproduction in cannibals were recorded by Dawson ('19). In *Oxytricha* the cannibalism was repeated very often after the first victim had been ingested. Cannibalism occurred chiefly when other food was not abundant; and as a result of previous underfeeding both the cannibals and their victims were much smaller than well-fed individuals of other cultures. Cannibals which were digesting three victims at one time had about twice the volume of a well-fed individual, but seven times the volume of the average starved individual. This may be taken to indicate that the cannibals were, to begin with, larger than their victims. Pedigrees showed that the fission rates of cannibals were raised only barely perceptibly by the acquirement of body substance from the victims. The victims were always digested like other food substances, in vacuoles. Even after digestion was complete there was hardly any tendency to undergo fission. Neither the swelling of the body, nor the addition of living substance to the body, therefore, was a stimulus to reproduction. In one case Dawson observed fifteen victims inside one cannibal, and measurements showed a total bulk of twenty times the volume of a starved individual.

Relation to growth. Cannibalism is a method by which large individuals arise, by which substance is stuffed into the body at a much higher rate than ordinary feeding could accomplish. Yet, for the most part, the body waits its usual time to age, before it undergoes fission. But since cannibals have hitherto not been observed among well-fed individuals, it is evident that the benefit of ordinary feeding is lost whenever the conditions for cannibalism prevail. It is not clear whether body substance built up from this incomparably appropriate kind of food is incorporated faster, or with less expenditure of "age", than it is during the increase of size under usual conditions of growth.

CHAPTER VIII

INTERNAL CHARACTERISTICS AND SIZE

1. ASSORTATIVE MATING

CONJUGATION, signalized by fusion and nuclear exchange, occurs in a great many unicellular species; but only in ciliates the act of mating has been studied in relation to body size.

Conjugants are smaller. In some species, conjugating individuals are characteristically smaller than non-conjugants. Maupas ('88) measured the lengths of both kinds; in *Paramecium aurelia* conjugants were 75 to 145 micra long, while non-conjugants of the same cultures were 125 to 200 micra long. Similar smaller sizes were found in the conjugants of other species; while in a number of species, as *Paramecium bursaria* and *Colpidium* (Maupas, '89), the sizes of conjugants did not differ from those of the rest of the population. Pearl ('07) found that in a general population of *Paramecium caudatum* the inferiority of size amounted on the average to 15 per cent in length, and 18 per cent in breadth; therefore to about 43 per cent in volume. But within a clone the difference was somewhat smaller (Jennings, '11), amounting to 8.5 per cent in length; which is nevertheless a highly significant difference.

This smaller size might represent an actual reduction of body volume through the expulsion of water, perhaps due to changes in the physical and chemical characters of the body substances in preparation for conjugation. Zweibaum ('22) found an increase of about 300 per cent in oxygen consumption per *Paramecium* individual at the initiation of conjugation, pointing not only to a chemical modification but also

to the possibility that part of the disappearing bodily material may be burned up. Again, conjugation in most of the cultures which have been measured was induced by inadequacy of food, and the smaller sizes may be due to cessation of assimilation. Either this factor or some other stops growth, and conjugants may characteristically be immature individuals. Jennings ('11) showed, in fact, that conjugants grew to adult size after conjugation was completed.¹ Pearl ('07) demonstrated that a change of cultural conditions modified the sizes of non-conjugants greatly, but not the sizes of conjugants. This is likely to have been merely because the conjugants were not growing nor undergoing fission; and the food supply was of no effect, once they had been induced to conjugate.

Variability. All conjugating individuals in a culture were found by Pearl ('07) to be slightly more alike in size than all non-conjugating individuals. This is shown in figure 32, curve B. In four cultures the coefficients of variation averaged 7.8 for conjugants, and 8.3 for non-conjugants. This small difference is easily attributed to the fact that the non-conjugants were of more diverse ages. It was not due to change of form, since the breadths were in proportion to the lengths. Jennings ('11) confirmed these differences of variability, and brought evidence to support the view that the low variability of conjugants was entirely due to the uniformity of ages or lack of growth. Remarkable reductions of variability in the body lengths of conjugants as compared with non-conjugants were demonstrated by Poljansky ('26) in the parasite *Dogiella*.

¹ More marked growth of ex-conjugate individuals immediately following conjugation was measured by Dogiel ('25) and Poljansky ('26) in two parasitic genera of ciliates, *Cycloposthium* and *Dogiella*. Corresponding reductions in adult size were found to characterize one or more generations just preceding conjugation.

Assortation of size. Pearl ('07) discovered that *Paramecia* that were conjugating with one another were more alike in size than were random pairs either of other conjugants or of non-conjugants (figure 32).

Figure 32. Assortation of lengths among conjugating as compared with non-conjugating *Paramecium caudatum*. A, conjugating pairs; B, conjugating individuals paired at random; C, non-conjugating individuals paired at random. In each case 101 pairs were measured. (Data of Pearl, '07, pp. 257-259).

This similarity of mates is termed "assortative mating". The coefficients of correlation for the lengths of the two members of a pair were between $+0.43$ and $+0.79$ in five cultures studied by Pearl. Similar coefficients, averaging $+0.38$ were found by Jennings ('11) in mixed populations.

But even within a clone there was considerable assortment, giving a coefficient of $+0.25$ (Jennings, '11).

Assortation of body lengths between mates was found not to occur in *Chilodon* by Enriques ('08).

In the parasitic ciliate *Anoplophrya* Collin ('09) observed it; but here the possibility remained that the extremely different-sized individuals, which did not mate, belonged to different clones.

Watters ('12) demonstrated in the ciliate *Blepharisma* that conjugants are smaller and that they mate assortatively. Calculations from the frequency curves which were published show that the variability in length for non-conjugants was 16.9, for all conjugants was 10.3, and for actual pairs was 7.2. This genus is entirely similar in assortment, therefore, to *Paramecium caudatum*.

Assortative mating with respect to body length was found and measured in several species of parasitic infusoria by Dogiel ('25) and Poljansky ('26). Although the hereditary origins of the individuals were not controlled, remarkable uniformity of sizes occurred throughout the general populations studied.

Mating, then, presents a physiological process which requires a high degree of similarity between the mates for its successful accomplishment. Through the size prerequisite, mating tends to bring together not only similar ages, but also like hereditary constitutions. Size is a visible means of discouraging the mixing of races.

Its causes. The cause of this assortment of sizes in mating is not fully known. Pearl ('07) believed it was due to the requirement that the oral grooves of the two pairing individuals must fit in order to unite fully. Jennings ('11) made more complete observations and confirmed this belief, observing that animals which did not fit upon coming together, often separated and sought other mates.

Both Pearl and Jennings sought for evidence of a change in length during the process of union in conjugation, but demonstrated that such did not exist for *Paramecium*. But in *Chilodon*, Enriques ('08) found that one individual of each conjugating pair did shorten during the process of pairing. On the average the shortening in length amounted to 18 per cent. The shorter length was kept by the ex-conjugant, but not by its progeny, if one may judge from the variabilities of size measured among the non-conjugants. It is not clear whether the breadth and the thickness of a conjugating individual decreased with the length; it cannot be definitely concluded that the volume of the body diminished.

It is probable that similarity of size is a mechanical necessity for mating. Whether this is the only factor, in such species as show assortment, is uncertain. It must be remembered that there are some species, even among ciliates, in which gametes are differentiated that are always of very diverse sizes. Such gametes have no difficulty in uniting.

2. THE EFFECTS OF CONJUGATION

Before conjugation. Some observations indicate that in the period just before conjugation there occur modifications of size which are distinct from the inhibition of growth at mating. But, again, it is not proven that these are essential accompaniments of con-

jugation rather than due to those circumstances very unfavorable to growth by which conjugation is usually induced. The most representative observations were made by Maupas ('89), who noted that under starvation conditions several successive fissions were likely to occur, without intervening growth, as a preamble to conjugation. Such he believed to be the case in the ciliates *Didinium*, *Enchelys*, *Leucophrys*, and *Vorticella*. Of course, growth was impossible without food; and Maupas did not record the actual number of generations which did not grow, nor insure that the starvation was absolute.

After conjugation. Just after conjugation fission has been observed to proceed at slow rates for several generations. Joukowsky ('98) noted this to be the case in *Stylonychia*, Jennings ('08b) in *Paramecium*, and Calkins ('19) in *Uroleptus*. During such a period the individuals were observed to be unusually large. But after a few generations, in Jennings's cultures, the dimensions were the same as they subsequently were in their descendants which flourished several months later. In these few generations which were unusually large, the readjustment of the nuclear conditions by the formation of new macronuclei occurred, and slow fission as well as large size accompanied this process. Other observers failed to find any deviation of fission rate after conjugation, particularly in *Paramecium* (Simpson, '01); evidently conditions need to be highly controlled in order to evaluate it. In later generations conjugation is recognized to lead to increased rates of reproduction in a number of species, particularly in *Uroleptus* (Calkins, '19), *Spathidium* (Woodruff, '25), and *Blepharisma* (Woodruff, '28).

Calkins and Gregory ('13) followed further the influence of conjugation in *Paramecium* upon the sizes of the descendants. They cultivated separately lines

from each one of the four progeny produced in the second generation after conjugation; this generation marking the completion of segregation in the nuclear materials. Sixteen sub-lines were cultivated, four from each of the four initial progeny; and large numbers of individuals from each of the sixteen lines were fixed for measurement upon each of two separate days. The measurements of length are difficult to evaluate, but it seemed that upon the first of these days the descendants of one of the four progeny differed consistently and significantly from those of the remaining three. But in the second set of measurements the difference was not present in at least two of the four sub-lines, of this progeny. The variability due to other conditions appears to have been great enough to mask whatever true genetic differences might have prevailed among these four sets of descendants.

Biparental inheritance. How is size inherited when individuals conjugate which have distinct size genotypes? This important question remains unanswered, because mating has never been obtained between genotypes which were known to differ (Jennings, '11). Not only does the tendency to assortation discourage the mating of such individuals, but probably other physiological differences thus express themselves. All the studies of the effects of conjugation therefore have to do so far as is known with biparental inheritances within a clone.

Can one demonstrate that inheritance is or is not from both parents, under these conditions? Only in rare instances have differences within a clone been found which are transmitted to offspring. The diversities within the limits of "normal variations" are not transmitted for more than three or four generations, at least in ciliates, unless some influence, such as temperature change or treatment with a chemical sub-

stance, has been exerted upon a part of the population. Jennings and Lashley ('13a) believed that the ability to survive in cultures and the rate of reproduction by fission could be demonstrated to be inherited from both parents. They found that the coefficients of correlation between the two sets of progeny of a pair were always greater than between other sets of ex-conjugant lines "mated" at random. But the past performances of the conjugant lines, before they paired, were not known.

Biparental size. With respect to the inheritance of size in ex-conjugants there are data also upon *Paramecium*. Jennings and Lashley ('13b) compared the variabilities in the dimensions of mates with the variabilities among their progeny. Among the progeny of 43 pairs of individuals which had conjugated in wild cultures, the body sizes were much more homogeneous (after a lapse of 25 days since conjugation) than in the two members of each pair. In the particular race studied, the coefficient of correlation for length between mates was $+0.39$; this much was due to assortation in mating. Between the two sets of progeny from each conjugated pair, the coefficient of correlation was $+0.57$. It seemed from this that the progeny of two mates were more nearly alike than the mates themselves. This was interpreted to be due to the presence of hereditary influences from both parents. But it seems inadequate to compare in the case of the mating parents the sizes of single individuals, and in the case of the progeny the mean sizes based upon 16 to 66 individuals. It would be better to know the mean sizes for many individuals in each of the parent lines before conjugation occurred. For, all frequency curves of size in *Paramecium* show that any one individual may depart by 30 per cent or more from the mean length which it represents genotypically. It may be recalled

also that conjugants are not obtained under the same environmental conditions as the most healthy non-conjugants.

Observations more nearly of the sort indicated were made by Jollos ('13, '21) on *Paramecium*. In one instance he showed that after four conjugations had intervened the vegetatively reproducing clonal population had the same size distribution as that originally present. In these observations, it may be said, time was allowed for the genotypic constitution to express itself as phenotype. In Jennings's observations, of course, it seems as though 25 days would allow enough time for any variants to come back to normal in their descendants.

If Jennings's data are to be taken to indicate genetic segregation among the ex-conjugants belonging to a clone, it would be necessary to rule out the effects of conjugation upon the manifestation of size in all the lines studied.

Size mutation. In two cases, Jollos ('13, '21) observed sudden changes of size within mass cultures which were clones (figure 33). Jollos considered it impossible that these could be due to contaminations, and so he believed them to be mutations of the genotype. One size-mutant race differed in susceptibility to temperature and to arsenious acid as well as in size. The altered sizes were likewise accurately transmitted through at least three subsequent conjugations. This is the type of occurrence which every biologist would like to witness.

In both of the mutations which Jollos observed the size jumped to a smaller value. It is perhaps of interest that the modal body length of the mutant line *b* (figure 33) is 80 per cent of the length of the original line *a*. If all the linear dimensions are proportional, a reduction of 20.6 per cent in them is equivalent to a

halving of the body volume. Was the "mutant" race a "hemi-haploid"?

3. ENDOMIXIS AND RHYTHMS

Endomixis was identified at its discovery as a nuclear reorganization which occurred periodically, in step with transient decreases of fission rate. Since

Figure 33. Distribution of body lengths in a clone of *Paramecium caudatum*, comparing the original line *a* with mutant line *b*. Culture *a+b* grew from individuals of both lines in one dish. Culture *bc* represents line *b* after conjugation had intervened. (Redrawn from Jollos, '21).

most changes of fission rate have proven to involve changes of adult size, it was natural to look here too for changes of size. This was done by Erdmann ('20) by fixing large numbers of individuals at known days of an endomictic "cycle" and measuring them.

Size between endomixes. Under the prevailing conditions of culture endomixis occurred approximately every thirty days. The results, in three lines belonging to two clones, are shown in figure 34. For com-

paring the sizes, the mean size at 14 days after endomixis is taken as 100 per cent, because the body length happened to be greatest in all lines on this day. Erdmann believed that these data demonstrated the existence of a regular progression of sizes, increasing from endomixis up to 14 days, and then declining.

Figure 34. Fluctuations in body length, and in body volume (calculated as $l \times b^2$), in a clone of *Paramecium aurelia* at various phases of the cycle of endomixes. Each point is the mean from the measurement of 120 individuals. Temperatures 24° to 26°C. (Data of Erdmann, '20, p. 99).

But the small amounts of change which were actually found do not indicate that the differences were significant. They become of a surety not significant when body volumes are calculated by taking into account the breadths as well as the lengths of the individuals (figure 34). If the differences were even larger they might still be due to inequalities of cultural conditions from day to day.

Size at endomixis. What it would be most desirable to know is whether there are size differences upon the day or two immediately at endomixis and just after it.

For, according to Jollos ('16), this is the period in which the rate of reproduction is affected, and hence the period in which irregularities of size are most probable. So far modifications of size in the endomictic cycle cannot be considered as demonstrated.

Figure 35. Inheritance of body length, and of body volume (calculated as $l \times b^2$), in a clone of *Paramecium aurelia*. Between each of the four series of measurements endomixis occurred once. (Data of Erdmann, '20, p. 133).

Effect on inheritance. Has endomixis anything to do with the subsequent inheritance of body size? It seems certain that endomixis occurred many times during the cultivation of Jennings's lines of Para-

medium; so that from figure 24 it might be taken that intervening endomixis is without effect upon size.

Erdmann ('20) made a particular study of this point. Two lines of clone 0 were kept, and after each of four endomixes the sizes were measured in various populations. As shown in figure 35, the changes of mean size at different periods were no greater than the diversities among sister cultures at some one period, with respect to either length or volume of the body.

Endomixis or a similar nuclear reorganization occurs in numerous species when protective cysts are formed. The effect of intervening encystment was noted in Colpoda (Adolph, '29) and the body size was found to be the same before and after it (see figure 20).

The conclusions to be drawn from the data upon the after-effects of conjugation, of endomixis, and of encystment must be purely negative. For, each population whose size was measured was in reality descended from a single ex-conjugant or single ex-endomictic; a great number of such populations must be measured before the assurance becomes great that some few of the reorganized individuals did not suffer modification with respect to the inheritance of size.

Depressions. "Degeneration" of protozoan races, which is a term meaning that the fission rate is progressively decreasing, has been noticed by many observers to accompany decrease of body size in successive generations. Maupas ('88) was the first to measure the sizes at intervals during the progressive failure of his cultures. In the case of *Stylonychia pustulata*, the length of the body shortly before the death of the last individuals of the race was only one-fourth of the original length.

"Rhythms" which have not been identified with endomixis have been studied in relation to size in a few instances. Woodruff ('13) found in continued cultures

of *Oxytricha* that larger body sizes prevailed whenever fission was slower. Popoff ('07) stated that the opposite relationship held true in *Stylonychia*. In some of Jennings's cultures of *Paramecium* the size increased; in some it decreased, with slower fission. It must be obvious that the result depended not only upon the species observed, but also upon the conditions which led to the modification of fission rate. Few observers of living protozoa believe that changes of fission rate, whether they occur in rhythms or not, are wholly independent of external conditions. As has been shown extensively in an earlier chapter, all sorts of circumstances may retard the fission rates, as compared with the rates prevailing under standard circumstances. Some of these conditions encourage assimilation or increase of size, and some do not.

Summary. All the events that were above discussed might be regarded as due to internal factors in the regulation of size. But unicellular organisms, of all organisms, are at the mercy of environments. The events are controllable to a very large extent, if not entirely. If the visible reorganizations are responses to external conditions, then the size changes may be either in response to the reorganizations or directly in response to the same external conditions. It is a healthy point of view to realize that internal modifications are in response to many kinds of conditions; some can be seen and some cannot be seen. Who then shall say whether a modified size or fission rate is the result of an unseen and unknown internal change or of a perhaps known external change? But both views reduce to an identity when a measured modification is simply correlated with a measured change, whether it be external or internal; and as little as possible is said about cause and effect.

CHAPTER IX

SOME EXTERNAL FACTORS AND SIZE

THE INFLUENCES upon size of a number of factors have now been reviewed; these factors may be conveniently grouped as internal factors. Those considered were: reproduction, growth, regeneration, transplantation, inheritance, conjugation, and other reorganizations. In a subsequent chapter some mention will be made of the effects of various cell structures such as the nuclei.

With a knowledge of what results and variabilities are to be expected from factors which, so far as now known, are internal, it is possible to analyze the effects of some definite external influences. In very few instances have the effects of a controlled influence upon single individual unicellular organisms been studied. Practically all the results are therefore of a statistical nature, obtained by comparing many individuals, some of which are under experimental conditions and some under control conditions.

1. TEMPERATURE

Variations in temperature produce striking changes with respect to body size. The changes were measured adequately by Popoff ('08) and Rautmann ('09) upon three species of infusoria. Their procedure was to measure the sizes of many individuals taken from a culture at room temperature, then to set the same culture at another temperature for 24 hours or more, finally measuring many other individuals from the culture. Their mean results, in which each point was based on ten to a hundred individuals, are represented

in figure 36. For *Paramecium* and *Stylonychia* the results were quite uniform; the body volume diminished to one-half or less with an increase in temperature of fifteen degrees.

Allescher ('12) also showed that *Paramecia* were larger at a lower temperature than at a higher one,

Figure 36. Effect of temperature upon the main body volumes of three species of ciliates. At the temperature of 15°C. the volumes were taken to be 100 per cent. The broken line represents approximately the relative rates of reproduction in the three species. (Data of Popoff, '08; Rautmann, '09).

though her results were complicated by the added factor of starvation. She found that *Stentor* (figure 37) was influenced in the same way by temperature as were *Paramecium*, *Frontonia*, and *Stylonychia*. So also was *Colpoda* (figure 39 below) and, so far as is known, all species of unicellular organisms.

Rate of change of size. How long does it take to accomplish such a change of size, and how permanent is the change? Popoff and Rautmann, on the basis of

Figure 37. Combined effects of age of culture, hunger, and temperature, upon the mean body volumes of *Stentor coeruleus*. The sizes when in a flourishing culture at 15° to 22° were taken as 100 per cent, and the measurements shown were made after 3 to 7 days standing at one of the three temperatures indicated. (Data of Allescher, '12).

a very few observations, concluded that the change of size was completed in about the space of time required for one whole generation of the organisms to grow up.

Such might well be the case when the shift of temperature was one of only five degrees, as happened in their experiments; but if the organisms were transferred from 25° to 10°C. the amount of growth required to attain the new size would be so great that at least a much prolonged duration of one generation would be required to carry it out.

The observations of Middleton ('18) indicate that not only size but also fission rate may be persistently modified by temporary subjection of *Stylonychia* to temperature differences. What is needed is a simultaneous study of size and of fission rate in the same series of individuals, in order to find which of the two characteristics is most susceptible to the influence.

Jollos ('13) found that the size responses of *Paramecium* to temperature were of several varieties, depending, he believed, upon the particular clone studied. When put into a higher temperature some maintained their characteristic adult size, or became slightly larger; others became rapidly and permanently smaller (just as in Rautmann's experiments); still others became smaller at first and after many generations regulated back to about the original size. Jollos seemed to find that only in the last case, which is illustrated in figure 38, did the organisms remain healthy at the higher temperature; others died within eight weeks. This is evidence that body size is of vital importance, and closely correlated with ability to survive.

When put into lower temperatures *Paramecium* either became permanently larger (just as in Rautmann's experiments), or else became larger at first and later returned to the original size (illustrated in figure 38). The regulation back toward the original size occupied two to four months in the lower temperatures as compared with one to eight weeks in the higher temperatures. So far as Jollos observed, the fission

rate changed whenever the size changed, and inversely to it; except that the rate remained greater in the high temperature for some time after the size had returned to normal.

It was noted in one instance that the smaller body volume of Colpoda at a high temperature was maintained for at least one generation after transfer to a

Figure 38. Effect of temperature upon the mean body length in a clone of *Paramecium caudatum*. Frequent subcultures were made, and kept at the temperatures indicated. (Data of Jollos, '13 and '21).

lower temperature (Adolph, '29). In subsequent generations the adult size of the body enlarged. On passing from the lower temperature to the higher, the smaller size was attained very speedily, for the organisms which were in process of growth at the time of transfer ceased assimilating and underwent fission at an early age.

Assimilation rates. Evidently a variety of adjustments may be made by diverse species and clones of ciliates in response to temperature changes. A useful picture of the adjustments may be based on the fact

that adult body size is the quotient of assimilation rate and fission rate. A smaller size obviously means that fission rate increased relatively more than assimilation rate. The consequences to the population, expressed as rates of growth at various temperatures, may thus be calculated from the mean sizes and fission rates which have been measured, as in table 10. In

TABLE 10.

COMPARISON OF THE RATES OF MULTIPLICATION AND OF INCREASE IN BULK AT THREE DIFFERENT TEMPERATURES. COLPODA, CLONE A. (FROM ADOLPH, '29)

Temperature <i>degrees C.</i>	19.3	21.6	26.5	Derivation
A. Mean duration of generation <i>hours</i>	24.0	17.8	13.0	measured
B. Mean duration of division cysts <i>hours</i>	4.6	3.8	3.1	measured
C. Mean adult body volume <i>thousand μ^3</i>	2073.	1700.	1189.	measured
D. Gain of volume per genera- tion <i>thousand μ^3</i>	1555.	1275.	892.	$3C/4$
E. Generations per week	7.00	9.44	12.92	168/A
F. Number at end of one week <i>thousand</i>	16.4	489.	60,300.	$4E$
G. Total bulk at end of week <i>billion μ^3</i>	34.4	832.	71,700.	$C \times F$
H. Relative rate of multiplication	1.	1.35	1.85	$\frac{E/7}{D} \times \frac{19.4}{1555.}$
I. Relative rate of active growth per individual	1.	1.137	1.123	

this particular clone of Colpoda it follows from the measurements of fission rate and of adult size that assimilation rate per individual is very little increased by temperature compared with fission rate. A change of ten degrees in temperature multiplies the assimilation rate 1.2 times and the fission rate 2.3 times.

The conception might be formulated that assimilation is limited by some physical process such as the rate of beat of cilia, while fission rate is governed by a chemical process. Since mechanisms of assimila-

tion differ among diverse types of unicellular organisms, some species may yet be found in which size increases with temperature.

2. FOOD

Kinds of living food. Random observations have frequently been made which indicate that qualitatively different foods modify the characteristic body sizes of bacteria and protozoa. Only those instances where pure clones were present need be seriously considered. Yet without this precaution the results of Maupas ('88) are probably reliable. In *Onychodromi* which were fed on "large infusoria" the mean body length was 300 micra; fed on "small *Cryptochila*" the length was 150 micra; the breadth being in proportion. In *Stylonychia* when fed *Cryptochila* the mean length was 160 micra, while when fed the bacteria of wheat infusion it was 125 micra; and the fission rate was only 37 percent of that on the first food. Of course various chemical conditions in addition to the food differed in two such contrasting cultures.

The heliozoan *Actinophrys* is markedly larger when fed upon *Gonium* than when fed upon *Chlorogonium* (Belar, '22). This difference is not entirely due to larger or more numerous food vacuoles.

Cutler and Crump ('27) observed that the soil rhizopod *Hartmanella* undergoes fission at different sizes when fed upon diverse species of bacteria. When feeding on certain bacteria called *YB*, the mean volume just before fission was said to be 274 cubic micra; while on bacteria *SE* it was 190 cubic micra. No idea can be obtained how many measurements were made to arrive at these values.

Observations under highly controlled conditions were made upon *Colpoda* (Adolph, '29). The ciliates were in a uniform solution of very dilute inorganic

salts, and in it one or another of three flagellates, from clonal cultures, were fed to them. The differences in body size were considerable, as shown in figure 39. Particularly between individuals feeding upon the two green flagellates *Gonium* and *Chlorogonium* was the

Figure 39. Influence of temperature and of quality of food, upon mean adult body size in Colpoda. *G* were feeding on *Gonium*, *CI-IV* on *Chlorogonium*, and *PI-II* on *Polytoma* (From Adolph, '29).

contrast striking; at either 16.7° or 26.4° the Colpoda had twice the body volumes upon the former food as upon the latter food. In this instance there was little chance of chemical differences other than those of the food, for both these flagellate species were grown in the absence of organic substances; they obtained their

nutritional energy entirely from light and their chemical materials entirely from the same inorganic medium.

Duration of photosynthesis. The energy which green flagellates receive from light is equivalent to assimilated food. Absence of light is therefore exactly equivalent to starvation. Hartmann ('21) found an interesting effect of prolonged supply of light upon the colonial species *Eudorina elegans*. Standard cultures were maintained at maximum rates of growth in inorganic salt solutions which were radiated by strong artificial light for twelve hours daily. When light was furnished continuously, then reproduction occurred faster. Whereas the colony of thirty-two individuals ordinarily produced thirty-two new colonies at one time every fifth day, or every sixty hours of radiation, the colonies now reproduced at first after sixty hours, then faster and faster. Insufficient time was taken for growth, and so the individuals and the colonies became smaller with each generation until finally they died. This is an effect quite similar to that of a high temperature; but the effects of temperature are usually adjusted or compensated within the organisms.

Kinds of organic compounds. In the green alga *Scenedesmus* a great variety of foods can modify the size of the individuals, according to Roach ('26). Some of the results are shown in table 11, though it is not certain that this investigator would wish those cultures which were made at different times compared very closely with one another. Not only the mean volumes of individuals were calculated, but also the total bulk which resulted after various numbers of days. Our comparison at the ninth day is shortly before the end of "logarithmic" multiplication in all the cultures.

The hexose sugars and sucrose hastened growth

very considerably, compared with the cultures in which the algae synthesized their own materials from light. Increased oxygen supply (row 2) had the marked effect of reducing the mean size of the cells but at the same time inducing them to multiply just enough faster to make the total bulk in the culture the same. In the dark the body size increased markedly over the size in the light. One is left in some doubt as to why the total bulk was the same in the light as in the dark; perhaps there were actually materials in the medium

TABLE 11.

BODY SIZE AND TOTAL BULK OF *SCENEDESMUS COSTULATUS* GROWING IN A BASIC INORGANIC MEDIUM AT 24.5° IN STRONG LIGHT, TO WHICH MEDIUM WERE ADDED VARIOUS SUBSTANCES IN ONE PER CENT CONCENTRATION. THE VALUES LISTED ARE THOSE FOUND ON THE NINTH DAY OF AGE OF THE CULTURE. (DATA OF ROACH, '26.)

Medium	Mean diameter in micra	Mean volume in cubic micra	Number of cells per cu. mm.	Total bulk in cubic micra per cu. mm.	Log. of same	Relative rate of log bulk increase in per cent of its own glucose control
Glucose	7.5	243	4,800	1,168,000	6.068	100
Glucose + aeration	6.3	145	6,280	910,000	5.959	99
Galactose	...	...	...	127,400	5.105	94
Sucrose	...	...	...	89,300	4.95	84
Fructose	...	...	...	9,850	3.994	73
No addition	8.5	325	48	15,620	4.194	60
In dark	10.0	583	27	15,770	4.20	40
Glycerin	...	...	...	7,970	3.902	43
Mannite	6.2	163	.85	943	2.975	13
Xylose	...	...	...	...	...	0

utilizable without photosynthesis. Roach concluded that glycerine, mannite, and xylose actually inhibited what growth and multiplication would otherwise have occurred.

It is surprising to find that clear-cut effects of single nutrients have not been measured upon the sizes of bacterial individuals.

Judging from the results that have been obtained with different kinds of living or synthetic foods, it appears inevitable that sizes should be diverse at different times in cultures of organisms such as ciliates which are feeding upon unknown mixtures of bacteria.

Quantity of food. The amount or concentration of

food is an important factor in controlling the sizes of unicellular individuals.

The influence of starvation has been investigated, but rarely under well-controlled conditions. With most mass-culture methods it is impossible to distinguish lack of food from other influences such as progressive chemical changes in the medium. Only where nutriment is furnished either in the form of pure cultures of visible organisms or as known concentrations of dissolved substances, or where all nutriment is suddenly removed and sterile distilled water is substituted, can effects be evaluated.

It has been abundantly proven,¹ at least qualitatively, in spite of inadequately controlled cultural conditions, that starvation caused a tremendous reduction of size of *Paramecia*, and particularly in breadth. Thus in two experiments in Jennings's clone D, starvation lasting seven days gave individuals with only 37 percent of the original body volume on the average, and starvation lasting eleven days gave individuals with only 29 percent. Refeeding in the first case for only 24 hours gave recovery to 92 percent of the original volume. What Jennings designated as starvation was, of course, complicated by other cultural conditions.

Similar reductions of size occurring under what were termed conditions of starvation have been noted in numerous species by many investigators, notably in *Stentor* by Sosnowski ('99) and Allescher ('12), and in *Arcella* by Hegner ('19b).

With the older methods of culture, starvation must have prevailed as often when it was unsuspected as when it was known to be present.

Total starvation. *Colpidium* was found by Wallengren ('02) to respond to complete lack of food by a

¹ Kazanzeff ('01), Wallengren ('02), Jennings ('08), Chainsky ('10), Lipska ('10), Allescher ('12).

tremendous reduction of body size. In ten days, body length diminished from 100 micra to 25, and breadth from 33 micra to 12; so that about 97 percent of the original body volume was lost.

Mass cultures of single clones of *Colpidium colpoda* were studied with respect to size by Vieweger ('25).

Figure 40. Effect of total starvation upon the body volume of *Colpidium colpoda* at 20°C. The progressions in three different cultures are represented. (Data of Vieweger, '25).

When a large population had formed, the organisms were transferred to boiled water, and this water was renewed daily. Within the first two days (figure 40) tremendous reductions of body volume occurred, and within a week the organisms averaged only one-fif-

teenth the body volume of their progenitors. There was no indication that any reproduction occurred during the starvation period, so that probably the original individuals gradually lost bulk. Mean body volume appears to have diminished progressively in a logarithmic sort of way. This might be taken to mean that the

Figure 41. Effects of concentration of *Chlorogonium* as food upon *Colpoda*. In the lower curves are indicated the fates of the various individuals; in the upper two curves are the body sizes and the durations of life from hatching to division-encystment. The two body sizes shown at the relative concentration 1 are of permanent cysts which were formed at the end of 18 hours; all other sizes are of division cysts which gave quadruplet progeny. (From Adolph, '29).

expenditure of bodily substance for energy was proportional to the amount of body left to expend it. Students are constantly surprised by the endurance non-encysting ciliates show under conditions of hunger.

Graded concentrations. Cutler and Crump ('27) noted that *Hartmanella* divided at smaller sizes as the counted concentration of bacterial food decreased. They found that both *Hartmanella* and *Colpidium* attained unusually large sizes just after copiously re-feeding.

Graded concentrations of food flagellates were tested upon *Colpoda* (Adolph, '29). The results, shown in figure 41, indicate that all graduations of size could be obtained in response to various conditions ranging from starvation to an adequate supply of the food organisms. Whereas at concentration 1, the lowest, nearly all the *Colpodae* refused to grow and formed protective cysts, at concentration 4 most of them tried to grow and died in the attempt. But those that did grow could not attain the size characteristic of well-fed individuals, though they took longer times in which to gather what food they could.

As was the case with temperature, food appears to influence body size primarily through fission rate. In some species of unicellular organisms reproduction keeps on for some generations after the food supply is inadequate. In other species reproduction ceases as soon as assimilation becomes inadequate for growth. In the latter, size will decrease only in proportion as bodily materials are used up in metabolic processes.

3. AGE OF CULTURE MEDIUM

The study of reproduction in unicellular organisms has in reality been for the most part a comparison of methods and conditions of culture. The chief impediment to progress has been that most of the cultural conditions were such as could not be described nor exactly reproduced. Most of the studies of size in protozoa have been made under such unknown circum-

stances. It was only after the more or less successful device of Jennings ('08b) that pure lines of holotrophic organisms under experimental and control conditions could legitimately even be compared while being so cultivated. His device was to cross-inoculate daily all parallel cultures with a mixture of the bacteria present in each culture. Thus the culture media were much more similar to one another than by earlier procedures; nevertheless the variations from day to day were tremendous. In spite of improvements to date, such as those of Hartmann ('21) it remains true of all species of unicellular organisms that, as postulated by Jennings ('08b) "a small mass of fluid constitutes a relatively large and varied environment for *Paramecium*. A watch-glass of hay infusion is a microcosm to this animal."

The studies now to be reviewed are mere beginnings at the analysis of chemical conditions. In future days when media can be fully controlled they will look crude indeed; yet they serve at least to demonstrate the existence of inconstancy of cultural conditions.

Ciliates. Pearl ('06) studied the variability of size in wild cultures of *Chilomonas paramecium*. This species has a fairly constant elongated ovoid form. It reproduces by binary fission; but division is longitudinal, not transverse as in *Paramecium*, so that growth in length is not a marked feature. Normal frequency curves of variability were found upon fixed preparations made at a single time, and the co-efficients of variation for length and for breadth were 10.3 and 10.9 respectively; about the same therefore as in *Paramecium* from wild cultures. Pearl compared *Chilomonas* taken at two times from the wild culture, once when food was abundant and multiplication was rapid, and once when circumstances were "unfavorable." In the latter case the body was smaller both in length and in

breadth, representing a mean decrease of twenty per cent in body volume.

Similar but more marked results were found by Jennings ('08b) upon *Paramecium* in cultures of single clones. The organisms were largest when taken from new cultures, gradually diminishing in size as the cultures aged. When transferred from old cultures to new ones, restoration of the full original size occurred in one or two days (table 12).

TABLE 12.

EFFECT OF FOOD AND OF AGE OF CULTURE UPON THE DIMENSIONS OF *PARAMECIUM*. CLONE *D*, SECOND SERIES. DATA OF JENNINGS ('08b.)

	Mean body length in micra	Coeff. of var.	Mean body breadth in micra	Coeff. of var.	Relative body volume
24 hrs. in fresh hay infusion	184.1	8.8	46.0	11.4	100.0
7 days starved	146.1	7.0	31.2	12.5	36.6
24 hrs. in fresh hay infusion	163.9	12.8	46.7	28.9	91.7
6 days in unchanged medium	174.4	8.5	44.8	17.4	89.6
48 hrs. in fresh hay infusion	191.4	8.9	54.9	14.3	147.9

The progressive changes of size in ageing mass cultures of *Colpoda* (figure 42) and of *Stylonychia* (figure 43) were measured by Enriques ('09). The diminutions of size were irregular, perhaps depending upon the reflowering of food organisms in the first two weeks of culture.

In cultures of *Colpidium* it was noted by Wachen-dorff ('12) that mean body length declined during four weeks from about 200 micra to about 50 micra.

A more complete analysis of the ageing of *Colpidium* cultures was made by Vieweger ('25), who followed the changes of size in clonal mass cultures. The relative densities of population were simultaneously measured (figure 44). An important result was that the size decreased very markedly some days before reproduction ceased. The progression of this decrease

in size was similar to that during complete starvation (figure 40 above), but was much slower.

Heliozoa. As long as unknown food was used as nutriment for protozoa, no distinction could be made between the nutritional factor and other properties of

Figure 42. Effect of age of culture upon mean body length in *Colpoda steinii*. The progressions in two different cultures are represented. (Data of Enriques, '09).

the medium. It is now known that most unicellular organisms create in culture fluid substances which are detrimental to their further multiplication. Organisms supplied with visible food, such as green organisms raised on inorganic salts plus light, can have their food supply accurately controlled independently of other chemical factors. When there is no lack of food,

Figure 43. Effect of age of culture at 22° upon the mean dimensions of the body of *Stylonychia pustulata*. Volumes are calculated at $l \times b^2$. (Data of Enriques, '09).

formation of cysts was induced in cultures poor in metabolic products the cysts were smaller than those induced in the ordinary way by ageing and starvation of the cultures. Richness in metabolic products here

Figure 44. Effect of age of culture at 20° upon *Colpidium colpoda*. A, Relative body volume of one individual; B, Relative concentration of the *Colpidia* in the culture fluid; C, Total substance of all the *Colpidia* present, i.e. A multiplied by B. (Data of Vieweger, '25, expt. IIIa).

gave rise to increased sizes, representing a mean body "old" culture fluid has by this means been found to be detrimental to reproduction.

Belar ('24) demonstrated the effect of products of metabolism in the heliozoan *Actinophrys*. When the volume 49 percent larger. Diversities in hydrogen ion

concentration were shown not to be responsible for the size difference.

Algae. In *Scenedesmus* the progression of body size was studied by Roach ('26). Under the conditions studied, adult individuals were small in young cultures,

Figure 45. Progression of body length in three species of bacteria after implantation from a 24-hour old culture into new broth. Each point is an average of ten measurements of fixed individuals. (Redrawn from Clark and Ruehl, '19).

that is, during the period of logarithmic multiplication. When reproduction slowed up, the mean size increased. Whereas in the young cultures most adults reproduced four progeny at one time, in the old cultures 8 to 16 progeny resulted from about half of the reproductive divisions.

Yeasts. In yeast the sizes of individuals were found by Richards ('28b) to diminish progressively during the early history of a culture fluid, just as was the case in *Scenedesmus*. Later the size slowly increased to a level which was maintained for several days.

Figure 46. Progression of body length in *Bacterium coli* after implantation on fresh agar. Volumes are calculated at $l \times b^2$, in which b was derived from area divided by length. (Data of Henrici, '23).

Bacteria. Only recently have changes of size with age of culture been studied in bacteria. Whereas a culture of ciliates or algae may require a week or two to lead to significant changes of size, the heavy inocula customarily used in bacterial cultures, together with faster metabolism and multiplication, lead to critical changes within a few hours. Observations were made upon a number of different species by Clark and Ruehl

('19); three representative sorts of results are reproduced in figure 45. In most species the size increased suddenly after transplantation to new medium, and gradually decreased to the original level after twelve

Figure 47. Progression of mean body length in *Bacillus megatherium* as related to the age of the parent culture. At zero hours a culture was implanted from a 12-hour old parent culture. From this one, subcultures were made at 2, 4, 6, and 8 hours; individuals from each being fixed and measured every hour. (Redrawn from Henrici, '24a).

hours. But in *B. diphtheriae* and related organisms the reverse was the case.

Extensive analysis of these changes in bacteria has been undertaken by Henrici ('23-'28). He first correlated the size changes with the rate of multiplication

(figure 46). Obviously the size was largest when multiplication was most rapid. In finer analysis, Henrici ('28, p. 70) showed that the maximum size was reached

Figure 48. Progression of body length in *Bacillus megatherium* in relation to the concentration of nutrients. Agar media with varying concentrations of peptone and beef extract were compared, the strongest concentration being 16 times the weakest. Concentrations 1 and 2 were not shown, but the progressions were very similar to that for concentration 4. (Redrawn from Henrici, '24b).

exactly when the maximum rate of multiplication was reached, both diminishing thereafter. The enlargement of the body did not take the place of multiplica-

tion in the "lag" period of multiplication; but body growth of individuals was fastest when reproduction was fastest. There was a lag in the accumulation of total bulk as well as of numbers of individuals.

Figure 47 shows that the maintenance of a large size was very transient, as in *B. megatherium*. Transfer to new medium at the end of each four hours would keep the body length above 8 micra, but by the end of six hours the body size would have to start enlarging all over again. Henrici ('24a) showed that the amount of inoculum had a large influence upon size attained. It is possible that a very few individuals, preferably of a motile species, in a large volume of medium, would be able to maintain the maximum size and maximum reproductive rate for many hours as has been shown to hold true for yeasts and ciliates.

Henrici ('24b) investigated the effect of concentration of organic nutrients upon body size, with the result shown in figure 48. More food material had relatively little effect upon body size, though it had a marked effect in prolonging the period of maximal reproductive rate.

Henrici ('28) formulated evidence contrary to the possibility that "metabolic products" accumulate to the point of beginning to inhibit growth and reproduction, and suggested that internal changes ("physiological youth") were induced by the mere presence of fresh medium. The suggestion was made that the factor of cell size which varied may be the surface tension at its boundaries, this being dependent in some way upon influences of the medium upon metabolism or chemical constitution.

Henrici ('28) presented a considerable amount of data illustrating the effects of various properties of culture fluid upon several species of bacteria. Particularly in relation to variability of size at different

cultural ages can his results not be adequately treated here. Suffice it to point out that the lowest variabilities occurred slightly after the maximal sizes had been attained; this might be taken to mean that the individual and the race were under most nearly "normal" conditions when it was of largest size, was assimilating fastest, and was reproducing fastest.

The relation between body size and the maximum population attained in a uniform culture medium was studied by Butterfield ('29), with the important outcome that the total bulk of protoplasm was approximately uniform for diverse species. This seems to mean that any one of five species of bacteria or the ciliate *Colpidium*, which were the kinds measured, would multiply until the food material had diminished to some definite small amount. The material utilized would yield the same mass of protoplasm whether transformed by one species or another.

Experiments concerning the influence of the volume of the medium and of the ratio of the number of organisms to the volume and surface of the medium have not been performed in such a way as to distinguish fully between nutritional, physical, and specific chemical factors. The influence of these factors upon size has hardly been analyzed even to the extent of finding whether such factors exist. It might be assumed that one organism in a cubic millimeter of fluid is equivalent to a thousand individuals in a cubic centimeter of fluid, with respect to size and fission rate. But is it true that such is the case? So far it can be stated only that the contrary has never been satisfactorily observed.

4. CHEMICAL ENVIRONMENT

Are those modifications in organisms which result from ageing of the culture medium entirely due to changes in the food supply, or can significant influ-

ences result also from changes in the chemical nature of the medium? A culture medium is not an easy object of chemical analysis. It is simpler and more satisfactory to make up new medium with definite differences from the old, and find the result of the transfer. To do this it is necessary to use large volumes of medium, or else very frequent replacements of medium.

Concentration of salts. Most studies of the effect of osmotic pressure or total concentration have been carried out by placing comparable samples of organisms into various dilutions of a salt solution.

Paramecium. The effects, upon the rate of growth of individual Paramecia, of various concentrations of sodium chloride were tested by Estabrook ('10), with results which have already been given in figure 18. In concentrations above M/30 the bodies were smaller at all ages than the controls in hay infusion. At concentration M/10 growth apparently ceased. The question naturally arises whether the smaller sizes in M/10 solutions were due to an inhibition of assimilation or due to a reduced water content.

The answer to this is apparently given by tests which Estabrook made upon adult Paramecium. When, as shown in figure 49, individuals were put into M/10 NaCl, they shrank markedly during the first 15 minutes. Then they began to recover in volume, though probably not to the original value. This is the sort of progression of body volume which occurs in response to transfer to a similar solution on the part of frogs and most other aquatic organisms which have been tested. Curiously enough, this was earlier stated by Enriques ('02) to be the course of events on the part of certain protozoa; yet it is not recorded that he actually made any measurements of size.

To lower concentrations of sodium chloride, the response was apparently similar but of less degree. Fig-

ure 50 shows that, after acclimatization to the salt solution was complete, the mean bulks were just as large in concentrations up to $M/20$ as in the control medium. This is evidence, for one species of ciliates, that changes of total concentration are not significant

Figure 49. Progression of body length and body volume (calculated as $l \times b^2$) in a culture of *Paramecium* which was transferred, at zero time, into a 0.10 M solution of sodium chloride made up in hay infusion. At each of the times indicated 50 individuals were fixed and measured. (Data of Estabrook, '10).

factors in the influence exerted by ageing of medium. But in concentrations above $M/20$ sodium chloride, the osmotic pressures of the medium have marked effects upon body size; evidently reversible dehydrations or swellings are produced.

Yeast. Similar changes of volume have been demonstrated in yeast. Paine ('11) studied the progression of cell volume with time, when the yeast individuals were transferred to very strong solutions of several substances. The results in two solutions which gave contrasting size modifications are represented in figure 51. In less than one hour the body volumes dropped

Figure 50. The influence of various concentrations of sodium chloride (made up in hay infusion) upon body-volume (calculated as $l \times b^2$) in a clone of *Paramecium caudatum*. Each point represents the average of 50 individuals which were fixed at the end of 48 hours in the solution, within which time fission occurred on the average once in each of the original animals. Concentration is expressed as molar. (Data of Estabrook, '10).

very greatly; how greatly we cannot tell. Thereafter the individuals in the urea solution recovered their original volumes and maintained them; but those in the sodium chloride solution recovered only slightly, and later shrank further. It is possible that the differences in the various solutions were due in part to modifications in the rate of multiplication of individuals with time, for the body volumes were reckoned from hematocrit determinations that were carried out upon cul-

tures which were initially equivalent in being inoculated with similar samples. Paine believed that the urea entered the bodies, while NaCl did not, which is the customary assumption made in plasmolysis experiments.

Figure 51. Progressive effect of molar solutions of urea and of sodium chloride upon the relative volumes in *Saccharomyces cerevisiae*. The volumes were measured by a hematocrit. (Data of Paine, '11).

The amount of shrinkage of yeast in various concentrations of sodium chloride was determined by Drabble *et al.* ('07). In this work the diameters of individual cells were measured. As shown in figure 52, the shrinkage was greater with each increment of concentration. Apparently all the samples of yeast were measured within the same time limits, but in the light

of the later work of Paine, which was just discussed, one would like to know the mean time elapsed before the recorded results were obtained; the investigators merely state "at least 15 minutes" elapsed before measurements were begun.

Almost identical with the results of Drabble are the

Figure 52. Effect of various concentrations of sodium chloride upon the mean volume of *Saccharomyces cerevisiae*. The yeasts were placed in each solution directly from a sugar medium where the mean volume was 402 cubic micra. From 16 to 31 different individuals were measured for each point. (Data of Drabble, Drabble, and Scott, '07).

changes of volume in yeast which may be derived from data that Rubner ('24) obtained by an entirely different method. He placed samples of a uniform stock of yeast into various concentrations of sodium chloride, and measured the ratio of water to nitrogen at the end of 24 hours. When calculated as relative body volumes, the data fall along the curve of figure 52. This fact makes it probable that the yeast cells measured by

Drabble had already attained their equilibrium volumes in the various solutions.

In measurements of relative volumes at the end of 45 minutes, Seliber and Katznelson ('29) observed shrinkages in various concentrations of NaCl which were approximately half those found by Drabble. But

Figure 53. Effect of various concentrations of sodium chloride upon the mean body volume of *Pleurococcus vulgaris*. The algæ were placed in each solution directly from the scrapings of tree trunks; and at least 15 minutes elapsed before the diameters were measured. From 124 to 211 different individuals were measured for each point. (Data of Drabble, Drabble, and Scott, '07).

in shape the curve was similar to figure 52. The time factor proved to be important in such determinations of yeast size, from the fact that very different size relationships appeared when the organisms were allowed to multiply in media of various concentrations for one or more days.

Green Algae. Drabble ('07) also measured the changes of size in individuals of the green alga *Pleuro-*

coccus when placed in various solutions of sodium chloride. The results are indicated in figure 53, each point of the graph representing measurements of diameter made upon over a hundred cells. The curve is of the same shape as for yeast.

In the green colonial flagellate *Gonium*, Hartmann ('24b) obtained giant individuals by cultivating in "Knop solution" of 0.02 or 0.05 osmolar concentration instead of the usual 0.001 osmolar. Upon transfer to the more concentrated medium, reproduction ceased for several days while growth proceeded. When a larger size had been attained, then reproduction began in a few colonies; but most continued to grow until each individual had three times the usual diameter. This increase of about 27 times in volume required many days, and the individuals which became largest never reproduced, but died. If large individuals were returned to the dilute medium, they divided very rapidly, and thus gradually restored the size of the individuals. In this process there was a sudden increase in the number of fissions per colony, for whereas each individual normally divided four times in quick succession, they now divided five times, thus producing colonies of 32 individuals instead of the usual 16 individuals.

Mechanism. We may compare the amount of shrinkage in solutions of equal osmotic pressures in the several species of unicellular organisms which have been measured. Fortner ('25), whose results were given earlier in figure 23, found that *Paramecium* lost 73 percent in volume when transferred from water to a solution of sucrose having an osmotic pressure of 10 atmospheres. The time required for this degree of shrinkage is not known. In sodium chloride solutions of equal osmotic pressure (0.24 M), yeast lost only 26 percent and *Pleurococcus* 54 percent. The conclusions

to be drawn from this comparison are far from clear; it is probable that the conditions of experiment do not sanction the comparison at all.' But it might be that the concentrations of the bodily contents are inversely proportional to the losses by shrinkage.

Any interpretation of the mechanism governing shrinkage in solutions would involve assumptions. The usual conclusion is that no solute can penetrate the boundary between body and medium, and that the water is then distributed between the two in proportion to the relative osmotic pressures of body and of medium. The data also allow the probability that other forces are present, namely, forces such as maintain considerable concentrations of salts and organic compounds inside the bodies when the medium has zero concentration.

The effects of strong solutions of sodium chloride, of urea, and of sucrose upon body size are important in themselves. But they are certainly not factors which explain the differences between aged culture medium and new culture medium.

Acidity. Hydrogen ion concentration is known to affect unicellular organisms in various physiological respects. It is abundantly known that this concentration changes significantly with time in most culture media. Its effect upon body size has been studied in one species by Chalkley ('29).

Body volumes in *Ameba proteus* were measured by Chalkley, either by calculation from the areas of individuals viewed simultaneously from two angles, or by measurement of the lengths of individuals drawn into capillary tubes of known bore. In solutions of lactose, glycerol, and urea which were certainly hypertonic, body volumes always diminished, the changes increasing with concentration and with time. In Ringer's solutions buffered to various hydrogen ion

concentrations from pH 6.4 to 8.0 there were always losses of volume, for the individuals came into the solutions from nearly pure water. The losses were most rapid at pH 7.0. But in pH 6.4 to 6.0 the body volumes always increased. The modifications of volume after complete acclimatization to the various acidities, as in succeeding generations, were not recorded; they might be very different from the initial changes which were observed.

Attention is eventually also required to the correspondence and lack of correspondence between the hydrogen ion concentration of the body substances and that of the medium, and its effects.

Metabolic products. Apparently nothing is known with respect to the influence of specific chemical compounds, either inorganic or organic, upon body sizes. Yet it is generally supposed that the accumulation of some metabolic products, possibly organic, is the effective factor of ageing in a culture medium. The different types of substances which are end-products of metabolism in organisms are not so numerous that they cannot be tested with respect to their effects, both separately and in combination, both upon fission rate and upon body size.

Trypanosome hosts. The apparent complexity of the sort of analysis of various conditions which is ultimately required, may be pictured from the work with parasitic trypanosomes. Wendelstadt and Fellmer ('10) infected various species of cold-blooded animals with *Trypanosoma brucei* from rats. In the blood of grass-snakes the infecting individuals were much smaller after four days of infection, especially at the eighth day, than they were in the blood of the rats. When put back into rats from the snakes, the parasites were much larger than in the original rat infection. Moreover, the large size persisted not only for many

generations of trypanosomes but throughout several passages into new rats. The observations were continued through twenty passages, and it was found that gradually the size returned to normal. Taliaferro ('24) found the influences upon the parasites of the same host at different ages of infection to change markedly with time; the effects of different host species required to be reinvestigated with this in mind. The changes of size observed might be considered as the resultant of continuation of growth during inhibition of multiplication and vice versa.

Wendelstadt and Fellmer also passed *Trypanosoma brucei* from rats into tortoises, lizards, salamanders, beetles, and slugs. In several of these species the trypanosomes could not be found, but when the blood was inoculated into new rats trypanosomes, sometimes of immensely modified sizes, reappeared. Similar results were in several instances found with *Trypanosoma lewisi*.

The species *Trypanosoma rotatorium* showed analogous changes of size when Nöller ('13) inoculated it into tadpoles and frogs. Moreover, the sizes were tremendously greater in the tadpoles than in the frogs, so that if a tadpole bearing the parasites underwent metamorphosis, its trypanosomes would become much smaller. Various factors were investigated by Nöller to account for the size diversity, without finding a crucial one.

It may be pointed out that the diversities of sizes among populations of trypanosomes which are derived from different hosts, as in the observations of Bruce ('11, '13), are completely paralleled by the diversities of size in clones of protozoa at different ages of the culture, or at different temperatures, or on different foods. Yet Pearson ('14) postulated, from the premises of statistical significance, that in a particular in-

stance the chances were less than one in a hundred thousand that two selected frequency curves of size represented the same species of parasite in different hosts. The biological responses to environments must be taken into account more seriously than the differences which appear to exist in the "internal" differentiation of unicellular individuals.

Other parasite hosts. A number of parasite species were inoculated by Hegner ('29) from the intestinal tracts of various birds into the intestinal tracts of parasite-free chicks. He measured the size frequencies when in the original hosts, after the various inoculations, and after cultivation on artificial media. Large differences of mean sizes were found in each situation. The size of the organism is, obviously, just as much a characteristic of the medium in which it finds itself as of the species to which it belongs.

5. RADIATIONS

The studies of the effects of radiations upon size are at present wholly inadequate and primitive. Pauli and Hartmann ('24) observed that *Paramecia* swelled after being treated with cathode rays. The treatment was evidently severe, since new surfaces and new vacuoles formed in the cytoplasm. Calkins ('29) measured a sudden and large diminution in the size of *Uroleptus* ten minutes after exposure to a mercury-vapor lamp. After this shrinkage the organisms did not change in volume, though it was 48 hours before death occurred. For reproducible results monochromatic radiations of measured intensities must be used, and significant numbers of organisms must be measured at various periods of time after treatment has been administered.

CHAPTER X

SIZE IN RELATION TO BODY SURFACE

A FAVORITE CONCEPTION with those who have thought about the limitation of body size, is that somehow the ratio of surface to volume is important. Spencer ('66) for instance, suggested several ways in which surface could be significant in physiological activities. But with one exception no specific instance has been demonstrated in which, for example, the supply or excretion of some particular material proved to be the limiting factor for body size.

1. DIFFUSION OF OXYGEN

The supply of oxygen might be such a factor. Does a reduction of the tension at which oxygen is supplied to the surface of the body influence the sizes attained by unicellular individuals? The possibility was tested in *Colpoda* (Adolph, '29). Certain numbers of young individuals were placed upon microscope slides in drops of culture fluid containing high concentrations of food organisms. Half of the slides and drops were placed in a gas-chamber where they were kept for 24 hours in an atmosphere of one part of air and three parts of hydrogen. The other half of the slides and drops were kept in a moist-chamber in room air. The frequencies of sizes among the individuals which became adults were then measured, and are represented in figure 54. In the low tension of oxygen the individuals were all smaller than any in air tension. They were small enough to produce only two progeny each, while the larger control individuals had four progeny each.

The fact that a low tension of oxygen both shortened the lives of the individuals, and caused the adults to be smaller, adds another factor to the long list of those already found to be effective in the control of the size of unicellular individuals. But the pressing question is, was there a lack of oxygen within the body?

Supply of oxygen. Within recent years methods of calculating how far substances will diffuse into tissues

Figure 54. Effect of low oxygen tension upon adult body volume in *Colpoda* at 19°C. Half of the individuals in generation II were kept in a tension of 40 mm. oxygen, from 16 hours after the formation of division cysts in generation I, until new division cysts formed at 21 to 23 hours. Division cysts in the higher tension were formed only after 25 hours. One unit of the ordinates represents a frequency of 5 individuals. (From Adolph, '29).

which are using them up or producing them have been devised by Krogh ('19b), Warburg ('23) and others. It is necessary first to measure the rate at which oxygen, for instance, will diffuse through water, a gel, or some tissue; to measure the rate of oxygen consumption of the particular tissue; and to measure the dimensions of the tissue. In the case of *Colpoda* the oxygen consumed was found to be 4 cc. per cc. of body per hour. The body of a free-swimming *Colpoda* is nearly that of an ellipsoid, and Harvey ('28) showed

TABLE 13.
RATE OF OXYGEN CONSUMPTION OF VARIOUS UNICELLULAR SPECIES

Species	Number of measurements	Temperature, °C	Volume of one individual in cubic micra	Surface of one individual in square micra	Cc. oxygen per billion individuals per hour	Cc. of oxygen per body per hour	Cc. oxygen per square meter of body per hour	Investigator
Collozoum inerme (colony with gelatinous mass)	1	16	10 ¹⁴	..	..	0.078	..	Vernon ('95)
Colpidium colpoda	2	17	152,000	16,000	210	1.38	13	Wachendorff ('12)
"	6	25	..	..	250		..	Bledowski and Zweibaum ('15)
"	..	..	..	..	200	10	..	Peters ('29)
Paramecium caudatum	20	..	433,000	33,000	737	1.70	21	Zweibaum ('22)
"	4	19	..	..	3850	...	..	Necheles ('24)
"	2	25	..	..	..	0.47	..	Grobicka and Wasilewska ('25)
"	4	..	..	..	568	..	..	Ball ('25a)
"	10	22	..	..	5600	..	..	Kalmus ('28)
Colpoda steinii	10	19.7	140,000	14,000	550	4.0	39	Adolph ('29)
Blepharisma undulans	..	20	..	..	..	0.5	..	Emerson ('29)
Ameba proteus	..	20	..	..	..	0.16	..	Emerson ('29)
Saccharomyces cerevisiae	..	26	180	150	0.191	11.	14	Pütter ('24)
B. coli commune	9	22	0.47	3.9	0.090	191.	23	Müller ('11)
B. fluorescens liq.	17	22	0.19	1.9	0.534	1960.	196	Müller ('11)
B. fluorescens non-liq.	20	15	0.06	1.26	0.126	2100.	100	Pütter ('24)
Photobacterium phosphorescens	..	21.5	1.7	7.6	0.030	17.5	3.9	Harvey ('28)

that the oxygen tension at the center of such a body, which is consuming oxygen uniformly throughout, will fall to zero when the shortest radius of the ellipsoid

$$a = \sqrt{\frac{5D \times C}{A}}$$

D being the coefficient of diffusion which was determined for frog tissues by Krogh ('19a), C the tension of oxygen at the surface of the body, and A the rate of oxygen consumption. The radius required turns out to be

$$a = \sqrt{\frac{5 \times 1.4 \times 10^{-5} \times 60 \times 0.209}{4}} = 0.0148 \text{ cm} = 148 \text{ micra},$$

when the culture fluid is in equilibrium with room air.

When the tension is reduced to one-fourth that of room air, the radius will be one-half as great, or 74 micra. The shortest radius of an ellipsoidal Colpoda, whose volume is the mean of those individuals in figure 54 which were exposed to 40 mm. oxygen tension, is 40 micra; for the individuals exposed to room air it is 50 micra. There seems to have been no absence of oxygen anywhere in the bodies, of either control or low-oxygen individuals.

Oxygen consumption. It is worth while to find out whether there are any other unicellular species where oxygen will barely penetrate to the center. This can be judged for those species whose rates of oxygen consumption have been measured. In table 13 the available determinations have been listed.¹ Among the ciliates, which are the only species whose body size is sufficient to bring them into consideration, none has a greater rate of oxygen consumption than Colpoda has,

¹ Numerous other determinations of oxygen consumption have been referred to the dry weight of the organisms used, as by Callow ('24), Meyerhof ('25), and Copping ('29). Still other determinations have not included any measure of the quantity of organisms used.

and the shortest radius of none of these forms is greater. Complete lack of oxygen apparently does not occur anywhere in the bodies of individuals in culture media which are in equilibrium with air.

The lowered tension of oxygen, in the experiment of figure 54, therefore exerted its effect not by denying oxygen to the tissue. It may have acted by lowering the concentration of oxygen in the tissue in connection with some process which was thus limited. One possible way in which a low tension might be conceived to have a significant influence is through its liberation of the processes which form acid in tissues, such as was investigated, particularly by Meyerhof ('25) and termed by him the Pasteur effect.² The effect is present in yeast and probably a great many other species and tissues. Shoup ('29) found that a very considerable tension of oxygen was required at the surfaces of certain bacteria in order that their oxygen consumption might remain maximal. From this it appears that in the Colpoda the tension of oxygen was certainly low enough to make its shortage felt, even though oxygen was not actually absent in any part of the body.

But, oxygen is not necessary for survival, as was shown by Spallanzani (1803), and by Pasteur ('61) to be true for many kinds of unicellular organisms; this has since been widely investigated. Although *Paramecium* is killed in a few minutes by lack of oxygen when suspended in a drop of medium (Zoethout, '99; Pütter, '05), it and other ciliate species survive for many hours or days when put into large volumes of fluid without oxygen (Pütter, '05).

Whether oxygen penetrates to the interior of an individual by diffusion alone can hardly be strictly assumed. It is conveyed by water in those species which have unidirectional migrations of water, certainly in

² Pasteur ('76).

all those with contractile vacuoles; and it is circulated by protoplasmic cycloses in many more species.

Whether oxygen is utilized equally fast by all regions of a unicellular body is not known. It is possible that some of the visible structures do not respire at all. The conception has been widely prevalent that nuclei consume oxygen faster than cytoplasm; this is based on experiments with nucleoproteins and with microchemical tests. Child and Deviney ('26) reasoned from the localized effects of cyanides, and Kalmus ('28) concluded from microchemical tests with methylene blue, that oxidations might be more rapid in *Paramecium* at the two ends of the body. The contractile vacuoles of various organisms have been supposed to be essential in assisting in the supply of oxygen (Roszbach, '72; Degen, '05) and presumably in the localization of the utilization.

If the functioning of cilia uses up oxygen, then the superficial layers of the body may consume it in higher proportion than the rest of the body. Actually the work done by cilia calculates out to be less than one percent of the oxygen consumption of *Paramecium*, according to Ludwig ('28a), even allowing only 20 percent efficiency. For locomotion and ingestion by flagella, the same relationships hold as for ciliary functions. Angerer ('19a) calculated that the work done by the flagella of a bacterium in locomotion amounted to less than one ten-thousandth of the energy obtained by oxidations.

2. DIFFUSION OF OTHER SUBSTANCES

The limitation of the supply or removal of substances other than oxygen has not been tested in relation to body size. Carbon dioxide, due to its high solubility in water, can hardly accumulate under natural conditions. Urea, in concentrations up to 0.14 M, had

no marked effect upon body size in Colpoda (Adolph, '29). Other specific products of metabolism have barely been identified in protista.

Organic consumption. The rate at which organic substances are transformed in unicellular organisms is enormous. Pütter ('24) calculated, from the rate of oxygen consumption of *Bacillus fluorescens non-liquefaciens* (see table 13), that an individual must consume its own weight of oxygen every 20 minutes, and its own content of organic substances every 4.3 minutes at 15°C. It may be assumed that some of the organic constituents are preserved from burning, to serve as structural machinery; whence it follows that the actual turn-over of the fuel contained in the body is really still faster. In no particular instance is the site of combustion known; it is possible that most of it is near the surface and that compounds of greater diffusivity are then formed to furnish energy to interior points. But in any case the rate of combustion must be near the limit of the rate of penetration of the organic compounds, even with the tremendous surface present. In this species of bacteria the specific surface amounts to 210,000, using the term as defined by Ostwald (see Thompson, '17, p. 32); that is, 210,000 square centimeters for each cubic centimeter of body substance. This is something like 170 times the specific diffusing surface of the blood-vessels of human muscles (Krogh, '19b). Angerer ('19b) pointed out that specific surfaces of this order of magnitude were especially prevalent in spirochaetes.

3. LOCOMOTION

One of the possible relationships of surface is implicated in the carrying out of locomotion by cilia or flagella. Given cilia of a certain size and structure, the force exerted by all of them is proportional to their

number, and the number on an individual is proportional to its surface. But the mass of the body, which is, if of the same density, proportional to three-halves power of the surface, has to be propelled against gravity as well as horizontally. To keep from sinking it is necessary to increase its velocity with its size; the force expended to do so must be proportional to the seven-fourth power of the surface (Thompson, '17).

Power required. The force developed in locomotion in *Paramecium* was estimated by Jensen ('93) from the centrifugal force necessary to prevent its characteristic progression away from gravity. From this force he calculated the power expended in normal movement, which depended also upon the difference in density between body and water. Jensen estimated the relative density as 1.25, a value higher than is now tenable for almost any living tissue. Allison ('24) by a more reliable method arrived at a mean value of 1.042 for a particular lot of ciliate bodies. From his erroneous estimate Jensen obtained a figure which was much too high for the power exerted during locomotion by cilia.

It was shown by Bresslau ('13) that the power exerted by the individual to lift its own body can be calculated without knowing its specific gravity. He found that the limiting length for a *Paramecium* would be about 2 mm.; which is far beyond the actual dimension of any living *Paramecium*, but barely beyond that of a *Spirostomum*. Bresslau pointed out that among rhabdocoele worms, all those species which are over 3 mm. long characteristically creep, and only those under this length use their cilia in swimming. This ratio of ciliary power to momentum may be a concrete limiting factor for species in general, but it has not been tested experimentally as a factor of size within a species.

The power required for locomotion was considered as a function of body size by Angerer ('19a) and Ludwig ('28a), using rod bacteria and *Paramecium* as types. They concluded that the expenditure for locomotion would be proportional to the square of the body surface, which is a fairly close approximation to Thompson's value. The possibility always remains that types of cilia may differ so that in larger individuals more power is exerted by each cilium. When sea-water is the natural medium, the density of the body may equal that of the medium, thus avoiding this consequence of size.

Assimilation. In an analogous way it can be supposed that a ciliary mechanism of ingestion which is adequate for a small race would be inadequate for some larger race. The power of the cilia will be proportional to the body surface, instead of to the body volume which is to be supplied with food. No one has conceived a utility for measuring growth as a function of body surface; yet from the standpoint of most mechanisms of assimilation, increment of body substance would be expected to be proportional to surface. The tacit assumption seems to be that somehow an organismal type will increase its surface in order to keep up with the demands of its mass.

4. FORCES PECULIAR TO SURFACES

The principle of similitude is the recognition of the fact that of physical forces, a certain number act at the surface or are proportional to the surface, while certain others act upon all the units of the mass and therefore are proportional to bulk. For instance, the density of a body is wholly the expression of the intensity with which gravity acts upon its mass; but the velocity with which the body sinks through water or another

medium is proportional not only to the density difference, but also to some function of the surface.

Surface tension and size. It was pointed out by Thompson ('17, p. 34) that micro-organisms differ most essentially from large organisms because the forces which are important to them are surface forces, or forces on a molecular scale, instead of the forces such as gravity which act upon bulk. This fact expresses itself in the prevalence of spherical forms, which are particularly common among unicellular species. The force which prevails in the attainment of such a body form is, of course, surface tension.

Surface tension may be said to be effective over distances of about 0.05 micra (Thompson, '17, p. 43), so that an organism with this radius is *all surface*. Differentiation of structure within such an organism would require tremendous forces of other sorts, to combat the intermolecular tensions in the regulation of the distribution of substances. The equilibrium of forces which accompanies a given set of bodily dimensions may be regarded as the resultant of surface tensions opposed to internal or massive forces. Some of the massive forces are measurable as cohesion, elasticity, and viscosity.

Viscosity. In general, the larger the departure from the spherical form, the greater are the massive forces prevailing in a liquid or partially liquid mass. The absolute viscosity of one species of unicellular organism has been measured; *Paramecium* was found by Fetter ('26) to have a value 8000 times water. It is a stiff gel instead of a true liquid. But Jacobs ('22) showed that the viscosity of *Paramecium* and of *Colpidium* was readily modified; the production of a higher intracellular acidity by the administration of carbon dioxide caused at first a decrease and later an increase of viscosity. It is not recorded whether sig-

nificant changes of bodily proportions accompanied the change of viscosity. The viscosity of *Stentor* was found by Heilbrunn ('23) to be markedly modified in either direction by the presence of certain cations in the medium.

In contrast to the high viscosity of these ciliates stands the value for the internal mass or plasmasol of *Ameba*. Heilbrunn ('29) ascertained that this was only twice the viscosity of water.

In *Ameba* there is a continuous local change of viscosity of the body substance; in this sort of organism it is doubtful whether size could be a simple correlative of the ratio of surface forces to internal forces. Chalkley ('29) made some measurements of relative volumes of fluid and solid portions under various conditions; but it seems probable that some ratio which would affect size would be much less than the errors of his measurements.

Surface tension and fission. One of the favorite "theories" of cell division has been that the lowering of surface tension relative to massive forces results in fission. Perhaps such a hypothesis is only a description which adds nothing to direct observation. But the hypothesis leads to a definite type of experiment; namely, actually to lower the surface tension between medium and organism to see if smaller body sizes then prevail. Up to the present time, however, the only experiments of this sort have not measured body size, but rather shape, rate of multiplication, flotation, infection, and viability (Larson, '28). The rate of multiplication of some species of bacteria was found to be decreased by the addition of soaps, which can equally readily be explained as an effect of some other sort than surface tension. Indeed, to avoid the surface effect of the reagent, the body may easily contain a concentration of the substance proportional to that of

the medium, except in the first few seconds of treatment. Moreover, the effect of soaps and the like upon liquid:air surface is hardly equalled by their effect upon liquid:liquid surfaces.

Paramecia were reported by Bauer ('24) to swell when sodium taurocholate was added to the culture fluid. No measurements were recorded in support of the conclusion, and rounding-up of the bodies was observed, which would make it difficult to compare accurately the total body volumes under the two conditions.

Perhaps the only striking evidence for the importance of surface forces is the generally observed fact that fission occurs in the equator of least area. An elonguated body is usually fissioned at right angles to the long axis. There are some apparent exceptions to this rule, which rule originated with Hofmeister ('63). The exceptions may be taken to indicate that surface forces are not in every case the most intense ones which are operative.

In a number of unicellular species it has been observed that the growth of individuals is almost wholly in length, and is not equal in all directions. Paramecium, and certain, perhaps many, bacilli are examples (Jennings, '08b; Schmalhausen '26; Henrici, '28, p.93). The ratio of surface to mass is thus kept almost constant between one cell division and the next, and so the total energy of the surface keeps pace with the mass. Unless, therefore, the tension in the surface varies with absolute size, there is no corollary of similitude which would cause a crisis to bring on fission. At present there is nothing to show why growth is disproportionate in the various dimensions. Reasons need urgently to be discovered as to why most kinds of unicellular organisms are not spherical, and why even

though not spherical their proportions are not maintained constant as the individual enlarges.

Electrical charge. Another force of little importance in limiting the form and size of large organisms, but of great importance to micro-organisms, is electrical potential. Whereas to a frog differences of electrical potential may have to do with the equilibria of substances between body and medium, in a bacterium they have to do not only with these local processes but also with bodily individuality and with resistance to many sorts of death-producers, which depend upon stability in suspension. In other words, a potential may compensate for a high force of cohesion or a small surface tension. A potential is one more means by which the balance of surface and internal forces may be adjusted.

It is known that the preservation of bodily integrity by unicellular organisms does not depend so much upon chemical differences between body and medium as upon physical boundaries. One of the ways in which lethal substances produce their effects is by modifying the electrical properties of the surface. This was shown extensively by the observations of Hardy and Harvey ('11), who measured the potential by cataphoresis of the organisms which had been treated in various ways. It is hardly enough to realize that electrical properties are significant in organisms of small size; modifications of size must be sought in correlation with changes of potential.

CHAPTER XI

SIZE OF NUCLEUS

DATA UPON the modifications of size of various portions of the unicellular body are, in the nature of the organisms, very few. For that reason this chapter will include nothing upon the less visibly differentiated kinds such as bacteria and yeasts. Some of the studies which have been already reviewed contained information upon the variability and growth of the several dimensions of the body. But, morphologically speaking, almost the only distinct region or portion of the body which has received study is the nucleus.

1. SOME INTERNAL FACTORS

It was a natural expectation, supported by general observation, that various intracellular structures are proportionate in bulk to the whole body. The analogy of these structures to the organs of the metazoan body made this assumption more than gratuitous. But it could be tested only by actual measurements. Two questions had to be investigated; first, did such proportionality hold within a species, and second, did it hold upon comparing different species?

Relative size. The first measurements of nuclei and of the bodies to which they belonged were made upon a wild population of *Paramecium* by Hertwig ('89). He ascertained upon each individual the two linear dimensions, length and breadth; and drew the conclusions that length and breadth varied in proportion to one another. He gathered that each of the two dimensions was correlated between the body and its nucleus. When the relative volumes are calculated

from his measurements (figure 55) the correlation between body and nucleus is far from striking; most of the measurements, however, were made upon individuals which had just finished fission, and it is certain, at least, that these were smaller both in nucleus and in body than the older specimens.

Figure 55. Correlation between nuclear volume and body volume in *Paramecium caudatum*. The volumes of the macro-nucleus and of the body are calculated at $l \times b^2$. (Data of Hertwig, '89).

Theory of fission. At the time, these measurements seemed merely to demonstrate what might be expected, namely, that large and small individuals were built approximately in proportion. But in later years some

observations (of Gerassimow, '02) upon polyploid Spirogyra and (of Boveri, '02) upon cells of sea-urchin eggs, brought to light more exact proportionalities between nuclear quantity and body volume. Hert-

Figure 56. Growth in volume of the nucleus and in the ratio of body volume to nuclear volume during one generation in *Frontonia leucas*, at 25°C. (upper) and at 14°C. (lower). Volumes were calculated as $l \times b \times t$. Each point represents one individual fixed and measured; compared to its own sister individual fixed at the time of fission. The points were averaged at intervals to give the lines drawn. The body volumes of the same individuals are shown in figure 13. (Data of Popoff, '08).

wig ('02) saw in this a relationship which was possibly a general one. He ('03) gave to it a theoretical importance, for he suggested that the size of the nucleus controlled the size of the body. A certain bulk of nucleus presided over the accumulation of so much cytoplasm; when this much had been attained the nu-

Figure 57. Growth in volume of the nucleus and in the ratio of body volume to nuclear volume during one generation in *Paramecium caudatum* at 25°C. Volumes were calculated as $l \times b \times t$. Each point represents one individual fixed and measured. The body volumes of the same individuals are shown in figure 8. (Data of Popoff, '09).

cleus was forced suddenly to double itself, and fission of the body occurred. This was, therefore, a possible mechanism of fission which included in it a means of regulating body size; though ultimately it left open the question as to what controlled the rate of assimilation on the part of the cytoplasm, a physiological ques-

tion which might be temporarily shoved aside by supposing that assimilation automatically proceeded as fast as ingestion could be accomplished.

Growth. The real test of the hypothesis was to see how the nucleus grew as compared with the body during the life of the individual. This study was undertaken by Popoff ('08) upon the large ciliate *Frontonia*. Individuals which were growing at 25°C. and at 14°C. were killed at various intervals of time after they had emerged from fission. The relative sizes of the nuclei of these single individuals are plotted in figure 56, while the sizes of their bodies are represented earlier in figure 13. The bodies were relatively larger than the nuclei at all ages up until shortly before fission, when the nuclei underwent great enlargement.

This was a surprisingly good fit to the theory. Popoff ('09) proceeded in the same way with *Paramecium* (figure 57), and with only fifteen individuals to measure from, arrived at the identical result.

The agreement of Popoff's results with a theory announced five years earlier must have been so convincing as to deter other investigators from studying the rate of growth of nuclei. But, now twenty years after, it would be interesting to see how the measurements result when made by an investigator who had no theory upon the subject. Examination of Popoff's linear measurements show that an error of one scale division in the values here and there would have made large differences in the outcome. In other words, at each time interval enough determinations are required so that they can be treated statistically.

Multinucleate species. Another way of testing whether nuclear bulk was proportionate to size of body was to study species having variable numbers of nuclei (more than one). Such a species was the heliozoan *Actinosphaerium* which Hertwig ('98) had previously

studied with respect to reproduction, and nuclear relationships of which Geoffrey Smith ('03) proceeded partially to analyze. In this species up to 650 nuclei were present in a single vegetative individual. The number of nuclei was found by Smith to be closely correlated with the dimensions of the individual. As far

Figure 58. Correlation between the number of nuclei and the cube of the diameter (in arbitrary units) in *Actinosphaerium eichhorni*. Individuals were grouped according to their diameters, and the modal numbers of nuclei in each group are here plotted. (Data of G. Smith, '03).

as the data indicate (figure 58) the number was probably proportional to the volume of the whole individual, and not to its diameter or its surface.

The formation of cysts which was induced by lack of food caused the death of most of the nuclei, leaving a few, each of which then formed one cyst. The number of cysts formed also was found to be a function of the size of the individuals in which they formed and

therefore of the total number of nuclei originally present. In warm temperatures fewer cysts formed and in cold temperatures more cysts formed, from similar-sized individuals; nevertheless the sizes of the cysts themselves were unaffected by the temperature.

In this species it was possible to distinguish to some extent the volume of the nucleus from the volume of the chromatin condensed within it. In the warm temperatures the nuclei became larger, but the chromatin masses smaller. This is an instructive warning concerning the deduction of correlations for nuclear materials. Smith made no measurements of size frequencies in nuclei or chromatin.

In several species of *Arcella*, similar observations were made by Hegner ('20). In the species *A. poly-pora* the number of nuclei varied up to seven, and the size of the shell increased with the number. The number was very closely correlated with the volume of the individual, as figure 59 shows, on the assumption that the volume was proportional to the cube of the diameter of the disk-shaped shell. This was exactly the situation found in *Actinosphaerium*. In the *Arcella dentata*, where no more than two nuclei occurred, measurements of a large population (428 individuals) showed that the modal diameter for binucleates was 125 percent of that for uninucleates. This ratio of diameters corresponds to a ratio of cubes or body volumes of 197 percent; the individuals with two nuclei had therefore almost exactly doubled the bulk of the individuals with one nucleus.

Growing *Opalinae* from the intestines of tadpoles were studied by Hegner and Wu ('21) with the result that the volume of cytoplasm appeared to decrease as the number of nuclei per individual became greater. Nevertheless the correlation of body volume and number of nuclei was very high. On the basis of substan-

tial numbers of measurements, the exact volume of cytoplasm which must accumulate before each nucleus began to divide was calculated.

Excision of cytoplasm. The effect upon the size of the nucleus of removing cytoplasm has been studied in

Figure 59. Correlation between cube of diameter of shell and the number of nuclei, in two lines, *A* and *B*, of one clone of *Arcella polyopora*. The most divergent point (in *A*) represents only two individuals. (Data of Hegner, '20b, pp. 49 and 50).

a number of species. In a few instances studied by Popoff ('08), *Frontoniae* which were cut early in their individual growth were found not to decrease the absolute sizes of their nuclei. The cytoplasm underwrote prolonged growth, so that the customary adult

body sizes were fully attained before fission occurred. If cut just before fission, then the fission proceeded at the location already determined for it, in each of the two instances studied. In these individuals also the nuclei did not shrink, and the cytoplasms underwent prolonged growth in the next generation. That the nuclei shrank during regenerations of cytoplasm in *Bursariae* was believed by Sokoloff ('24), but accurate measurements were impossible in this species. It is also stated by Dembowska ('25) that the nuclei of *Stylonychia* shrank during regeneration, but no measurements were given.

Studies upon *Ameba* have been carried out repeatedly. Removal of cytoplasm led, according to Gruber ('12), to shrinkage of nuclei; and, although measurements of the nuclei were not published, the relative numbers of individuals which showed shrinkage and which did not, were reported. The fact that the cut *Amebae* did not survive and reproduce in most cases, may mean that cultural conditions were unfavorable. These experiments were repeated by Phelps ('26), measurements being given for one individual in which the nucleus diminished in diameter after three days. But the shape and proportions of the nucleus also changed in that time. Moreover, the diminution was greater after five days than after the first three, showing that regeneration was not going on. Any responsive adjustment of nuclear volume should be looked for in the first few hours, one would suppose. In another experiment by Phelps ('26) cytoplasm was cut away on successive days, until the organism had only about one-eightieth of the usual adult volume. But in this instance the data regarding the size of the nucleus show that the nucleus was then absolutely somewhat larger than in the average full-sized *Ameba*. With the use of favorable and controllable cultural

conditions it was reported by Hartmann ('28) that, after the artificial removal of cytoplasm in *Ameba* and during subsequent regeneration, the size of the nucleus remained unchanged.

Excision of nuclei. Variations of the number of nuclei present in *Arcella* were produced by Hegner ('20) by cutting away some nuclei. As already shown in figures 28 and 29, the body volume was, within a few generations, regulated in approximate proportion to the number of nuclei present. But very frequently the number of nuclei was restored to its original value by a process of fission which consisted of nuclear division and empty shell formation without cytoplasm separation. This sort of adjustment is not known to be connected in any simple manner with body size, and offers a further problem in regulation.

Pieces of *Stentor*, in which both the nucleus and the cytoplasm had been diminished by cutting, always gave rise to progeny of the body size normal to the race (Burnside, '29). The size of the nucleus, as well as the number of nuclei and the volume of the cytoplasm, was apparently regulated at its old value by any unit of the body that was capable of carrying on at all. Hence it is probable that the regulator is not itself any structure of definite size.

Summary. It may be concluded that within a multi-nuclear clone the number of similar nuclei and the volume of cytoplasm are usually proportional. This implies nothing about the mechanisms of control of the size of the whole body, for it is also found that under constant conditions the size is preserved so that each individual has about so many nuclei. Some observations given in table 5 upon *Actinosphaerium* cultivated under highly constant conditions, show that the variability of body volume is hardly any greater than in the uninuclear *Actinophrys*; yet each *Actinosphaerium*

had a hundred or more nuclei and each Actinophrys had only one.

Ratios in different races. The question remains whether the ratios of nuclear volume to body volume are similar in various unicellular species which have discrete nuclei. This is far from being the case. The nuclei may form 8 per cent of the body or they may constitute much less than 1 per cent.

Within a species the ratio also differs tremendously among different clones. This was demonstrated by Popoff ('09) by comparing the various results upon *Paramecium* in the Munich laboratory, all the measurements having presumably been made under similar conditions. Whereas Rautmann's ('09) cultures showed 15 times as much body as nucleus, Popoff's showed 48 times as much. Intermediate lay the observations of Hertwig ('89) and Gläser (quoted by Popoff) at about 30 times.

2. SOME EXTERNAL FACTORS

Temperature. Temperature has been said to modify slightly the volumetric ratio of body to nucleus. Evidence on this point is contained in the data of Rautmann ('09), which are reproduced in figure 60. It appears that the ratio is greatest at 20° in *Paramecium*. But the differences are only slightly greater than the standard deviations within the groups measured. In *Stylonychia* (figure 61) the same observer found no significant change with temperature. In *Frontonia* the ratio was found by Popoff ('08) to be slightly greater at 25° than at 14°.

In all species (figure 61) the absolute volume of the nucleus was markedly diminished at higher temperatures. This was the case with the bodies of the same three species (above in figure 36). In view of all the errors involved in the data it is safe to conclude that

Figure 60. Effect of temperature upon the ratio of body volume to nuclear volume, and upon the volume of the nucleus, in *Paramecium caudatum*. Two clonal cultures (A and B) were used, each being kept successively for at least 24 hours at each of the three or four temperatures. From 8 to 19 individuals were measured for each point, and in the graph the extremes are all shown. Volumes were calculated as $l \times b^2$. (Data of Rautmann, '09).

the ratio of body to nucleus is almost constant over a large range of modifications of body size caused by temperature.

Hunger. Starvation has been found by many to be a potent factor in nuclear size. Most of the studies of this factor have been of organisms cultivated under

Figure 61. Effect of temperature upon the ratio of body volume to nuclear volume, and upon the volume of the nucleus, in three species of ciliates. Only average results are shown, including those for *Paramecium* which are shown in detail in figure 60. (Data of Popoff, '08, and of Rautmann, '09).

rather unknown conditions; lack of food was not absolute but the individuals observed were simply in old cultures which did not allow multiplication to proceed.

Interest in this factor was aroused by the observation of Hertwig ('98, '00, '04), upon the heliozoan *Actinosphaerium*, that a few of the many nuclei were greatly enlarged in old cultures. While normal in-

dividuals had nuclei of 14 micra diameter, these individuals had nuclei of 70 to 196 micra, which represented an increase of about 1,000 times in volume.

Figure 62. Effect of starvation upon the volumes of the body and of the macronucleus in *Paramecium caudatum*. Volumes were calculated as $l \times b^2$. This experiment is one of three; on each day ten individuals were killed and measured. (Data of Kazanreff, '01).

Such nuclei were stated not to be forming cysts; they did not recover their former size, nor did the individuals survive. It is possible to regard the change as irreversible and of no physiological significance in the

regulation of size. But these facts stimulated other studies.

In starving *Stentor*, various nuclear transformations were observed by Sosnowski ('99). The macronuclei divided irregularly during such fissions as occurred, so that in some individuals the nuclei were now very large and in some very small. But it was not feasible to estimate accurately the relative sizes.

More reliable measurements were yielded by hungry *Paramecia* when studied by Kazanzeff ('01) in Hertwig's laboratory. In certain cultures the nuclei actually enlarged, as time went on, while the bodies were becoming smaller, as is shown in figure 62. This result was substantially confirmed in this species by Wallengren ('02) and Lipska ('10). Similar increases in the volumetric ratio of the nucleus to the body were demonstrated in other species of ciliates, as *Dileptus* (Hertwig, '02) and *Colpidium* (Wallengren '02). In *Ameba* a like result was found by Prandtl ('07), though control individuals of the same culture were not studied.

Thus far one might be quite convinced that starvation leads to hypertrophy of nuclei, or at least to preservation of nuclear substance while cytoplasm disappears. The crucial test would have been to observe the return to normal relationships when food was again supplied. This was never done.

Later studies have not confirmed the above results. Perhaps the diversity of fact really depends upon the separation of food shortage from other influences. Borowsky ('10), working in Bütschli's laboratory, arrived at the result that hunger leads to the presence of fewer and smaller nuclei in *Actinosphaerium*, the very species upon which Hertwig's first observations were made.

The effect of progressive starvation was studied

most completely in *Colpidium colpoda* (figure 63) by Vieweger ('25). There was always a reduction in the volume of the nuclei; the nuclei were reduced even more than the cytoplasms, as indicated by the ratios

Figure 63. Effect of total starvation at 20°C. in *Colpidium colpoda*. The numerals I, II, III, represent three different cultures. (Data of Vieweger, '25).

plotted. This is just the opposite effect from that observed by Hertwig and Wallengren. Vieweger showed that the reduction in nuclear size during the ageing of mass cultures was much slower than that during true starvation; yet qualitatively and relatively it was the same.

Depression. Comparable with the effects of hunger and of age of the culture medium, are the effects of conditions of cultural "depression." The full definition of these conditions must be left to the investigators themselves. In *Stylonychia*, Popoff ('07) stated that nuclei increased in size while the bodies grew smaller and reproduced less frequently. But in *Actinosphaerium* Borowsky ('10) believed that the nuclei became relatively smaller. In *Tillina* and *Oxytricha*, (Gregory, '09; Woodruff, '13), there were no significant differences in the ratio of nucleus to body at periods of slow reproduction as compared with periods of rapid reproduction.

Significance of nuclear size. Something definite is known, therefore, about the size of the nucleus and the relative sizes of nucleus and cytoplasm under several sets of conditions. It appears from the data that the ratio between the sizes varies only within a limited range under any one set of conditions. The ratio changes with those conditions which have been tested. In experiments where nearly equal proportions of nucleus and of cytoplasm have been removed by cutting, as in *Stentor*, *Spirostomum*, and *Dileptus*, it was not noticed that reproduction occurred without the restoration in size of both nucleus and body; rather the contrary (Hartmann, '22, '28; Sokoloff, '24). It seems fair to conclude that, although nuclear growth does not occur independently of body growth in protista, it is not any progressively changing function of it. Hertwig regarded the nucleus as the conservative structure which controlled the cytoplasm. This is perfectly possible, and has received new significance from the inter-relations known to govern the expression of heritable characters. But no evidence has been found, particularly in the protozoa, for an actual causal relationship of the size of the cytoplasm to the size of the

nucleus. The sizes are both regulated, but that both are controlled by the same factors is as likely as that one is a factor in the regulation of the other.

Neither the nucleus nor the cytoplasm is homogeneous; each contains many structures and substances. In each structure the chief substance is water; a measurement of size is mainly a measurement of the relative amount of water present. It is unlikely that any other substance is always present in the same proportion to water throughout growth and all other variable circumstances. Growth or other change in any other one substance may or may not involve a proportional change of volume. The same difficulty is present in all studies of size; it is possible that size is a mere resultant of the constancy or fluctuation of many constituents which in turn are primarily the regulated quantities.

Size of some other structures. The nucleus, when present, is the most conspicuous of the differentiated structures of a unicellular organism. In most it is the only structure which can be measured with accuracy. In a few isolated instances other structures have been correlated in size with body size. Thus in *Diffugia*, Jennings ('16) found that spine size and spine number were unrelated to body size and to nuclear size. But in *Arcella*, Hegner ('19a) found spine number positively correlated with body size within a clone. In *Ameba* the size which contractile vacuoles attained before emptying was correlated positively with the size of the nuclei, according to Botsford ('26). Comparisons of this kind are required in great variety before factors effective in regulating structural size can be sifted out.

CHAPTER XII

CONCLUSION

1. SIZE REGULATION AND OTHER REGULATIONS

THE FACTS which have passed in review in these chapters are easily summarized qualitatively, by the statement that practically every factor which has been investigated has been found to modify body size in some species or another of unicellular organisms. Only quantitative results can be used to decide how the individual organism "knows" when its proper size has been attained.

Why variability? Some facts indicate that size is not wholly a question to be answered by the individual but rather by the racial population. For instance, in a certain population one per cent of the individuals have double the mean body volume and another one per cent have half the mean volume. Such a statistical outcome might be regarded as evidence of a factor beyond the control of the single member of the group.

From another aspect the variations may be regarded as due to differences in some physiological function other than the rate of building up of body substance. Perhaps size is controlled very accurately by a pattern of functions, while the functions in turn are the primary variants. This also leads at first to an apparent impasse, for then these constituent functions have varied in spite of the genetical and environmental uniformity.

Or, shall it be supposed that the inheritances and the conditions are not uniform? With respect to inheritances it is clear that any one of the variant individuals can be the progenitor of a population whose

variability and mean are exactly those of the original population. With respect to conditions there will always remain room for doubt, since no two individuals can live in exactly the same place at the same time. In truth it is no less remarkable that a number of populations belonging to one clone should show the same degree of variability, than that they should all exhibit the same mean of size or other character.

Interdependence. It must be obvious that size cannot vary independently of all other characters. Size has been found to be correlated with fission rate, with growth or assimilation rate, with ancestral reorganizations of various sorts. Under a standard set of conditions it was indicated, in the genus *Colpoda*, that fission rate was the primary variable, so that size variability might result from it. Under conditions of partial starvation, members of some species kept on reproducing almost as rapidly as in the presence of plentiful food, in spite of considerable decrease in assimilation; body size decreased accordingly. Under apparently uniform conditions, large sizes and high rates of fission followed soon after the act of conjugation in some races. Obviously body size is not invariably connected with any one internal factor, any more than it is with any single external factor.

Body size, then, is a characteristic which varies in its own right, at the same time that it, like all other characteristics, does not vary wholly independently of the other characteristics. The intimate control of size is, in a sense, incompletely understood until all the functions and dynamic equilibria of the particular organism have been described. But one can, *a priori*, get just as far by beginning the description with size, as by beginning with reproductive rate, or assimilation rate, or structural differentiation rate. Size can be measured in most kinds of unicellular organisms; it is

a real difficulty that few other characteristics (except fission rate) can be measured, for the purpose of correlating them with it.

This interrelation of diverse activities is brought out in various experiments which have been described in detail (as in figures 21, 28, 41, 46). Subjection to a new set of environmental conditions does not wipe out a particular activity and leave the rest untouched. Instead, it modifies quantitatively, not one activity, but several of them. The organism does not put one activity above all others. Neither the rate of growth nor the frequency of fission nor the attainment of a certain size has an inviolable right-of-way; instead, each of them compromises with the others. Assimilation which is inadequate to maintain a standard rate of growth is cooperated with by a retardation in the onset of fissioning. This sort of picture is familiar to every student of the functioning of living objects.

Racial regulation. Every individual is responding to a complex of factors resulting from past generations as well as of factors immediately impinging. For this reason it is not surprising that the whole interplay of characteristics is not completely upset or destroyed by a change in one environmental factor, such as lack of food or low tension of oxygen. Each individual has a certain momentum from its race. This shields it from being completely dominated by immediate conditions; it also adds to the factors controlling the character which is being studied. To find whether a given condition has influence is easily accomplished by keeping everything else constant. But to find how the factors interact to constitute a regulation, requires a highly complicated experimental analysis. It has not been carried out for any biological regulation; for the particular one of the regulation of size it is only possible at the present time to visualize simultaneously

some of the interactions which have been observed. It is inadequate to find that a significant correlation exists between total oxygen consumption and the duration of life; between amount of food ingested and the size of the body at maturity. The existence of one correlation does not indicate the mechanism of causation. The essential problem lies in the quantitative interaction of all the factors whose resultant is the phenotypical realization of a genotypical character.

Partial factors. One picture of the interacting factors that might be derived from the facts which have been set forth in these chapters is somewhat as follows. Every individual is a member of a chain of generations which are succeeding one another at regular intervals of time. Assimilation is the chief activity of each organism; this activity is punctuated periodically by fissioning. But the fissions occur not merely after the passage of so much time but rather after the passage of certain quantities of certain activities. Each of these activities is compounded of many physiological functions; so that it is necessary to correlate not merely so much assimilation, not merely so much respiratory metabolism, but also so much pressure of oxygen, so much tension of acid, so much mass activity of incoming food materials. Each of these constituent functions and their like is in turn modified by temperature, diffusing capacity, locomotion, reorganization, and other characteristics.

How shall these correlatives be evaluated? Evidently each must be treated, for a specific clone of organisms, in the manner that size has been evaluated. Instead of having a few data upon fission rate in one clone under one set of conditions, upon oxygen consumption in another species under a second set of conditions; some ten or twenty activities must be measured in relation to some one or two or more condi-

tions or functions which are allowed to vary. When it is found how many independent variables are present, if any, then the interdependences can be described in relation to them. The investigator has not the discouraging problem of measuring ten or twenty processes simultaneously, for organisms are not built of discrete activities. Instead, organisms represent highly co-ordinated combinations of activities; they secure many "degrees of freedom" through subjection to multifarious conditions. This statement involves no assumption as to whether the co-ordination is or is not of a kind existing only in organisms.

While it is possible in a few species that size is determined by a single factor such as duration of life, in most species that have been studied it has been demonstrated that numerous factors and correlatives are concerned in the regulation of body size. Hence the property of specific size is of the character of an "emergent." It is not observably the outcome of any combination of influences and endowments; yet it is concatenated with them. This by no means precludes the possibility that cause and effect can be traced when the implications of each of the influences and endowments are fully known.

Unanswered questions. The data available allow of no description of a mechanism by which each individual "knows" when its size has attained a value proper to the frequency of sizes in the race's population. But in certain specific cases it can be stated that the oxygen concentration at the center of the body, or the capacity to ingest food faster than it is required for mere maintenance, or the body mass which will barely be moved by the cilia, has in each instance attained a limiting value. Whether the same factor which prevents further growth will at the same time induce fission is not known; growth possibly may come to a stop at one

time, and fission begin later when a normal age has been attained.

The data also furnished no decision upon the various theories of size. Is the theory of Spencer realized in any specific instance? Does it matter that expenditure is proportional to volume and supply is proportional to surface? Is it minimum work for a race to increase the sizes of its individuals instead of regulating their diffusing capacities more precisely? None of these questions can be answered now; they await investigation.

2. INDIVIDUALITY

One of the classical problems of biology with which the regulation of body size is intimately connected is the question: what constitutes an individual? Among unicellular organisms, less than among any other kinds, does an individual always correspond to a discrete portion of living material. A series of generations shares characteristics; an entire population is intra-dependent. With respect to size, how does a double monster compare with the normal individual, or how does the double-sized individual which arose by living at a low temperature or by receiving ultraviolet radiation compare with one which is normal at a higher temperature or protected from radiation?

Limits of divisibility. A partial answer seemed to fit these questions at the time when it was believed that the divisibility of a protozoan individual was limited by some invisible organization in the body mass. To the present-day investigator, however, it seems more likely that divisibility is limited only by the conditions under which living material is kept from dissipating into its fluid environment. This is a physical limitation rather than one which is significantly physiological or morphological. Considerable experi-

mentation will be required to ascertain whether surface tension, surface precipitation, micellar size, or some other quantity, represents the indispensable or limiting property in a least-sized living unit.

Why fission? It would be possible to avoid the consideration of individuality by merely stating that not only a body but also an environment is necessary for life; therefore the individual consists of much more, unlimitedly more, than the body. But a more useful way of putting the question is, what requires unicellular organisms to be subdivided into many small individuals instead of fewer large ones?

This sort of question is difficult to investigate, because the regulation of subdivision processes was set up at some remotely ancestral time. In existing organisms many other activities cannot be carried on without it. But so was the whole regulation of body size, or of fission rate, or of rate of oxygen consumption set going in remote ages. Without a past there is no organism. It is possible that at some by-gone time environmental selection eradicated individuals of a given species which were smaller than some limit and larger than some other limit. But in the sequence of observable generations of individuals, even extreme variants are apparently well within the lethal limits. The normal variabilities for size are tuned to values which seem to keep out of reach of any selective factors which may possibly have once determined their survival value. No traces remain by which such factors can be detected.

What sort of mechanism is ruling the sizes of individuals? In each generation the body is first being mensurated, and then being inhibited from further increase in bulk. Each individual is one unit which is manifesting the numerous innate qualities of its race. In the unicellular body there is little "internal medi-

um"; yet regulations go on which are of the same sorts as are performed by huge organisms whose master cells and organs never experience gross inconstancies of the environment.

Co-ordination of constituent functions. Individuality seems to be less a self-sufficiency than a co-ordination. It is unessential to the problem that two individuals can be obtained by cutting one into halves, or that two individuals form from one, at a certain time under certain conditions. The biologist's goal is to describe what functions go to constitute the simultaneous complex of activities which is the individual. When these functions and their correlation have been described, individuality will have been defined. The regulation of each function, such as that of size, will be both part and whole of the co-ordination.

APPENDIX

SPIROGYRA CELLS

FILAMENTOUS ALGAE have probably less right to being considered as unicellular organisms than have metazoan egg cells. But at least two studies of fundamental importance have been carried out upon *Spirogyra*, of a kind which needs to be done upon ciliates and bacteria. The first is that of Gerassimow upon the dimensions of polyploid cells; the second is that of VanWisselingh upon the accuracy with which cells divide into equally-sized daughters.

Spirogyra cells are contrasted with most protista primarily by the presence in them of cell sap. Each cell has a large mass of discrete material, which is morphologically not a part of the cytoplasm; it occupies the core of the cell, leaving the cytoplasm as a shell spread on the inside of the cell wall. The sap or vacuole enlarges the cell's volume and surface, representing a saving, perhaps, of an equal bulk of metabolizing materials. It is quite impossible to measure the sizes of cells apart from the sap; indeed, the volume of the sap is carefully regulated by the cells.

Growth. *Spirogyra* lives in fresh-water as filaments of cylindrical cells which are joined end to end. All the cells of one thread are usually derived from a single parent cell which may or may not have been a sexual zygote. The diameter of a given thread is fairly uniform throughout its length, and is constant from one day to another, so that the rate of growth in bulk can be studied with fair accuracy by merely measuring the lengths of filaments or cells from time to time.

Spirogyra filaments were measured by Hofmeister

('74) at intervals of a few minutes, and his data led him to the surprising result that increase of length occurred

Figure 64. Growth in volume of normal and of double-nucleated cells of *Spirogyra bellis*. Each point represents the average of 20 to 80 cells, most of which were measured on two days besides the initial day. The normal cells were all members of the same filaments with double-nucleated cells. Volume was calculated as $l \times b^2$. (Data of Gerassimow, '01).

in jumps. These jumps were at very irregular intervals; as is brought out by the graphs of the data which

were made by Ostwald ('08). The intervals became longer as the observations continued, indicating perhaps that conditions were not entirely favorable to growth. These intervals were not related to the rhythms of cell division. Apparently the observations have not been corroborated.

When *Spirogyra* filaments are measured at intervals of a day or more, growth is found to be roughly proportional to length already present. In other words, growth is logarithmic; this is shown by the data of Gerassimow ('01, '02) and VanWisselingh ('08, '14, '20). All the descendants of an initially single cell are considered together, as though no cell-divisions had occurred, as in the data illustrated by figure 64. It was believed by VanWisselingh ('20) that a temporary retardation of growth could be detected whenever cell divisions occurred, similar to the retardation observed in filaments of *Bacillus ramosus* (above in figure 16). But the retardation is very small, and growth rates may be considered to be little interrupted by multiplication of cells.

Growth appears to be a prerequisite for reproduction in each cell of *Spirogyra*. Conditions of cultivation of *Spirogyra* have never been worked out to the point where they can be thought of as ideal; but under the best conditions of laboratory life, the sizes of adult *Spirogyra* cells vary only slightly more than the sizes of various ciliates and flagellates, and less than the sizes of some heliozoa and bacteria. The variabilities of *Spirogyra* dimensions are illustrated in figure 65.

Polyploid cells. Gerassimow ('01) found that by subjecting *Spirogyra* filaments to cold, irregular cell divisions were often produced, so that some daughter cells received all the nuclear material and their sisters no nuclear material. He found that the former became and remained much larger than the normal cells; the

Figure 65. Frequency of size among 26,596 cells of *Spirogyra bellis*. *A*, normal cells; *B*, cells with one double-sized nucleus; *C*, cells with two nuclei. The doubling of the nuclei was produced by previous exposure to low temperatures. The mean sizes are in each curve represented by vertical lines. (Data of Gerassimow, '02, p. 229).

latter in fact stopped growing. The cells with double quantities of nuclear material might exhibit two discrete nuclei, or one nucleus of a double size, or one nucleus made up of two connected lobes. In all cells the nuclei were suspended in the cell sap and were usually discoidal in shape, so that their dimensions could be measured and their volumes calculated.

The homogeneous character of the nuclear sizes is shown in frequency tables contained in Gerassimow's ('01) publication. Gerassimow based his comparisons upon mean volumes; double nuclei had on the average

TABLE 14.

MEAN DIMENSIONS OF NUCLEI IN NORMAL AND IN DOUBLE-NUCLEATED CELLS OF SPIROGYRA BELLIS. (DATA OF GERASSIMOW, '01 AND '02.)

	Normal	Double
Number measured	192.	107.
Diameter in micra	27.3	40.7
Thickness in micra	7.1	8.1
Volume in cubic micra	720.	1397.
Diameter of nucleolus in micra	10.3	12.5
Thickness of nucleolus in micra	6.9	6.9
Volume of nucleolus in cubic micra	140.	206.

1.94 times the volume of the normal nuclei. In the case of cells containing two discrete nuclei the volume of each nucleus was exactly the same as of a normal nucleus. The nuclei, therefore, exhibited their inherited quantitative characters with respect to their own sizes (table 14).

Gerassimow ('02) also measured the sizes of the cells themselves, which contained single and double quantities of nuclear material. For this purpose he measured the lengths and diameters of about 26,600 cells. The distribution of the various dimensions is shown in the series of frequency curves (figure 65) constructed from his data. It appears that the percentage variability in volume was very much larger in the bivalent cells; evidently the length and the surface were more

accurately regulated than the volume. Gerassimow ('02) based his comparisons upon the mean for each dimension, with the result shown in table 15. The same relation would be reached by comparing the modes, except in the case of cell volumes, where there was no distinct mode. The conclusion is that the lateral cell surface is proportional to the nuclear volume, and not the total cell surface nor the total cell volume.

The lateral cell surface in *Spirogyra* is characterized by having a number of ribbon-like spiral bands of chlo-

TABLE 15.
RELATIVE MEAN DIMENSIONS OF NORMAL, DOUBLE-NUCLEATED, AND TWO-NUCLEATED CELLS OF *SPIROGYRA*. (DATA OF GERASSIMOW '02, p. 233.)

	Normal	Double	Two
If volume of nucleus is	1.	1.	1.
Length of cell	0.15	0.09	0.08
Volume of cell	544.	809.	885.
Lateral surface of cell	31.8	31.3	31.2
Total surface of cell	42.1	43.5	45.3

rophyll. Gerassimow ('02) counted the number of complete spirals in these bands and reached the result that the number was nearly twice as great in the bi-valent cells. He also counted the number of pyrenoids or centers of starch formation in each ribbon of chlorophyll, arriving at the result that double-nucleated cells had 204 per cent and two-nucleated cells had 181 per cent of the number in single-nucleated cells. Thus on the average the number of pyrenoids was proportional to the nuclear mass, and to the lateral cell surface.

In the cases of the cytoplasmic or cellular surface, the chlorophyll bands, and the pyrenoids, it is evident that their quantity was conditioned directly by the amount of nuclear material present, because half of all the original cell contents except the nucleus went to form a non-nucleated cell at the time of the irregular

cell-division. The non-nucleated cells, Gerassimow observed, lived many days, carrying on photosynthesis, but not growing or reproducing.

Gerassimow ('01) also gathered data to show when and how the bivalent cells increased their sizes. In a number of filaments he followed the increases of the lengths and breadths of the bivalent cells and of several neighboring normal cells. The first measurements were taken two to five days after the treatment with cold, and before the bivalent cells had divided; second and often third measurements were made at intervals of three to ten days thereafter. The relative volumes found in one series of measurements, all of which were begun upon two adjacent days, are plotted in figure 64. It is seen that for normal and for enlarged cells both growth, as expressed by the logarithm of the volume increase, and cell reproduction, are very nearly proportional to time. It is evident that the bivalent cells grew faster only in the first few days; that thereafter they merely multiplied their original masses at the same rate as normal cells. In reaching this conclusion it is somewhat disconcerting to find that in both cases growth was slower in the first three days of the measurements than later; the temperature was not fully controlled, however, and in fact the initial delay of growth is not shown in measurements which were started at other times. Indeed, Gerassimow was very careful to compare growths only among the various components of a single filament. He concluded that the bivalent cells continued for many days to have increased rates of growth, a conclusion hardly justified by his data. Rather, after the initial process by which the cytoplasmic surface was adjusted to the nuclear mass, the enlarged cells approximately just maintained their double sizes while they reproduced in number and volume at the normal rate.

In another species of *Spirogyra*, Gerassimow ('04a) obtained some cells with nuclear contents of three, four, and five times the normal. These arose by the unequal division of the bivalent cells. In these too it turned out that the lateral surface of the cells was nearly proportional to the nuclear volume, and that cell division was delayed initially only.

In *Spirogyra*, therefore, it is clearly evident that cytoplasmic mass is correlated with nuclear volume. But no factors are so far known which in turn control nuclear or chromatin volume. Gerassimow('04b) made a particular analysis of this question. In some cases very small nuclei were distributed to daughter cells through a process of nuclear fragmentation resulting from treatment with low temperatures or with anesthetic substances such as chloral. Nuclei smaller than half the normal size never gave rise to long successions of progeny but always died out; while double nuclei continued for many cellular generations. Frequently, however, the nuclear material separated into normal-sized nuclear masses and each mass was isolated into a separate cell. This process of adjustment could not be traced either to nuclear or to cytoplasmic factors. Cells with nuclear masses larger than double the normal did not reproduce their sizes for any large series of generations, but regulated back to smaller numbers of nuclei by multiple fissions.

What is the meaning of the constancy of the ratio between nuclear volume and cell surface in *Spirogyra*? In this type of cell the cytoplasm proper is spread in a thin layer over the cell surface. If the thickness of the layer were uniform for all cells, then a double volume of cytoplasm would give a doubling of cell surface. This would mean that the volume of cell sap is carefully controlled, so as to keep the thickness of the cytoplasmic layer constant. Or, the surface may con-

trol the amount of materials, through the fact that assimilation is limited by the area of chlorophyll exposed in the spiral bands.

It has been customary to regard the stiff wall of a plant cell as controlling cell size; yet it is the cytoplasm that determines where the cell wall shall lie, and, as growth of the cell proceeds how much the wall shall enlarge. We know nothing about the question whether the size of the nucleus or of the cytoplasm or of the cell sap or of the cell wall comes first. All are to be accounted for, and the account is hardly complete as long as they are put merely in terms of one another.

Equality of fission. The second fundamental study of size which has been carried out satisfactorily only upon *Spirogyra* cells is this: When a cell divides into two cells, how accurately is its substance distributed to the daughters? It might have been preferable to start this book with this question instead of ending with it. A very few data upon the sizes of sister individuals at their formation from the parent were obtained by Simpson ('02), Jennings ('08b) and Popoff ('09) upon *Paramecium*, but their number is insufficient to treat statistically.

Gerassimow ('01, page 192) compared the lengths of 29 pairs of daughter cells. In no case was the total difference between them more than 14 per cent of their mean length; the coefficient of variation was 3.9 per cent.

A much larger series was measured by VanWisselingh ('20). His individual frequencies are plotted in figure 66. With these the liberty has been taken to average each five groups. The average frequencies then fit exactly a logarithmic curve which is a half of a normal frequency curve. VanWisselingh fitted a hyperbola to the data; this curve agreed poorly, and was of a type rarely found in statistical distributions.

The coefficient of variation of this series of cells is 9.7 per cent.

A smaller series of measurements of size eccentricities between daughter cells was made by Baas-Becking and Baker ('26). In their 52 pairs of cells the coefficient of variation was only 2.1 per cent.

The accuracy with which two daughter cells are

Figure 66. Differences in length between sister cells of *Spirogyra maxima* at the time of fission. Circles represent the actual frequency at each per cent of difference; black squares represent the same data averaged for each five per cent. The line as drawn represents a probability curve, whose coefficient of variation is 9.8 per cent. (Data of Van Wisselingh, '20).

equally endowed with substance has, therefore, a standard error of two to ten per cent. Sister cells are unlike in size at their very formation; at the beginning of their independent lives. Not all size diversities are

due to variability of growth and to differences in environments. To evaluate each of these groups of factors it would be necessary to measure the diversities once at cell division, and again at the end of life when ready for the next cell division.

BIBLIOGRAPHY

- ACKERT, J. E. 1916 On the effect of selection in *Paramecium*. *Genetics*, 1, 387-405.
- ADOLPH, E. F. 1926 The metabolism of water in *Ameba* as measured in the contractile vacuole. *Jour. Exp. Zool.*, 44, 355-381.
- ADOLPH, E. F. 1929 The regulation of adult body size in the protozoan *Colpoda*. *Jour. Exp. Zool.*, 53, 269-311.
- ALLESCHER, M. 1912 Ueber den Einfluss der Gestalt des Kernes auf die Grössenabnahme hungernder Infusorien. *Arch. Prot.*, 27, 129-171.
- ALLISON, R. V. 1924 The density of unicellular organisms. *Ann. Appl. Biol.*, 11, 153-164.
- ANGERER, K. 1919a Ueber die Arbeitsleistung eigenbeweglicher Bakterien. *Arch. Hyg.*, 88, 139-145.
- ANGERER, K. 1919b Ueber die Oberfläche der Mikroorganismen. *Arch. Hyg.*, 88, 274-283.
- AUSTIN, M. L. 1927 Studies on *Uroleptus mobilis*. *Jour. Exp. Zool.*, 49, 149-216.
- BAAS-BECKING, L. G. M., and BAKER, L. S. 1926 Studies on growth. *Stanford Univ. Publ., Univ. Ser., Biol. Sci.*, 4, 61-135.
- BAITSELL, G. A. 1914 Experiments on the reproduction of the hypotrichous infusoria. II. A study of the so-called life cycle in *Oxytricha fallax* and *Pleurotricha lanceolata*. *Jour. Exp. Zool.*, 14, 211-235.
- BALBIANI, E. G. 1891 Sur la formation des monstres doubles chez les infusoires. *Jour. anat. physiol.*, 27, 169-196.
- BALBIANI, E. G. 1892 Nouvelles recherches expérimentales sur la mérotomie des infusoires ciliés. *Ann. microgr.*, 4, 369-407.
- BALBIANI, E. G. 1893 Nouvelles recherches expérimentales sur la mérotomie des infusoires ciliés. Deuxième partie. *Ann. microgr.*, 5, 113-137.
- BALL, G. H. 1925a Studies on *Paramecium*. I. Experiments on the action of various endocrine substances, of liver, and of glycogen on the division rate of *Paramecium*. *Univ. Calif. Publ. Zool.*, 26, 353-383.
- BALL, G. H. 1925b Studies on *Paramecium*. II. The behavior of a conjugating race of *Paramecium caudatum*. *Univ. Calif. Publ. Zool.*, 26, 385-433.
- BALLS, A. K., and BROWN, J. B. 1925 Studies in yeast metabolism. I. *Jour. Biol. Chem.*, 62, 789-821.
- BARBER, M. A. 1907 On heredity in certain micro-organisms. *Kansas Univ. Sci. Bull.*, 4, 3-48.

- BARBER, M. A. 1908 The rate of multiplication of *Bacillus coli* at different temperatures. *Jour. Infec. Dis.*, 5, 379-400.
- BARON, M. A. 1926 Ueber mitogenetische Strahlung bei Protisten. *Arch. Entw. Mech.*, 108, 611-633.
- BAUER, E. 1924 Ueber Förderung der Zellteilung mittels der Verminderung der Oberflächenspannung des umgebenden Mediums. *Arch. Entw. Mech.*, 101, 541-552.
- BEERS, C. D. 1926 The life-cycle in the ciliate *Didinium nasutum* with reference to encystment. *Jour. Morph. Physiol.*, 42, 1-21.
- BEERS, C. D. 1928a The relation of dietary insufficiency to vitality in the ciliate *Didinium nasutum*. *Jour. Exp. Zool.*, 51, 121-133.
- BEERS, C. D. 1928b Rhythms in infusoria, with special reference to *Didinium nasutum*. *Jour. Exp. Zool.*, 51, 485-493.
- BELAR, K. 1921 Untersuchungen über Thecamöben der Chlamy-dophrys-Gruppe. *Arch. Prot.*, 43, 287-354.
- BELAR, K. 1922 Untersuchungen an *Actinophrys sol* Ehrenberg. I. Die Morphologie des Formwechsels. *Arch. Prot.*, 46, 1-96.
- BELAR, K. 1922 Untersuchungen an *Actinophrys sol* Ehrenberg. II. Beiträge zur Physiologie des Formwechsels. *Arch. Prot.*, 48, 371-435.
- BELAR, K. 1928 Untersuchung der Protozoen. *Methodik wiss. Biol.*, 1, 735-826.
- BERTHOLD, G. 1886 Studien über Protoplasmamechanik. Leipzig.
- BLEDOWSKI, R., and ZWEIBAUM, J. 1915 Experiments on absorption of oxygen in *Colpidium colpoda*. *C. R. soc. sci. Varsovie*, Cl. 3, 8, 251-261.
- BLOCHMANN, F. 1888 Zur Kenntnis der Fortpflanzung von *Euglypha alveolata* Duj. *Morph. Jahrb.*, 13, 173-183.
- BOROWSKY, W. M. 1910 Untersuchungen über *Actinosphaerium eichhorni*. *Arch. Prot.*, 19, 255-288.
- BOTSFORD, E. F. 1926 Studies on the contractile vacuole of *Amoeba proteus*. *Jour. Exp. Zool.*, 45, 95-139.
- BOVERI, T. 1902 Ueber mehrpolige Mitosen als Mittel zur Analyse des Zellkerns. *Verh. phys.-med. Ges. Würzburg*, 35, 67-90.
- BOYD, G. H. 1929 Experimental modification of the reproductive activity of *Plasmodium cathemerium*. *Jour. Exp. Zool.*, 54, 111-126.
- BRANDT, K. 1877 Ueber *Actinosphaerium eichhornii*. Inaug., Diss., Halle.
- BRANDT, K. 1895 Ueber die Ursache des geringen spezifischen Gewichtes der Vacuolenflüssigkeit bei Meerestieren. *Biol. Cbl.*, 15, 855-859.
- BRESSLAU, E. 1913 Ueber das spezifische Gewicht des Protoplasmas und die Wimperkraft der Turbellarien und Infusorien. *Verhandl. deutsch. zool. Ges.*, 23, 226-232.
- BREUER, R. 1917 Fortpflanzung und biologische Erscheinungen

- einer Chlamydothrys-Form auf Agarkulturen. Arch. Prot., 37, 65-92.
- BRUCE, D. 1911 The morphology of *Trypanosoma evansi* (Steel). Proc. Roy. Soc., 84 B, 181-187.
- BRUCE, D., HARVEY, D., HAMERTON, A. E., DAVEY, J. B., and BRUCE, LADY. 1912 The morphology of *Trypanosoma simiae*, sp. nov. Proc. Roy. Soc., 85 B, 477-481.
- BRUCE, D., HARVEY, D., HAMERTON, A. E., DAVEY, J. B., and BRUCE, LADY. 1913 Trypanosome diseases of domestic animals in Nyasaland. II. *Trypanosoma caprae* (Kleine). Proc. Roy. Soc., 86 B, 278-284.
- BRUCK, W. F. 1908 Beiträge zur Physiologie der Mycetozoen. I. Teil: Verschmelzungsvorgänge, Entwicklungsänderungen. Zsch. allg. Physiol., 7, 505-558.
- BUCHANAN, R. E., and FULMER, E. I. 1928 Physiology and biochemistry of bacteria. Volume I. Baltimore. 516 pp.
- BUNTING, M. 1926 Studies of the life-cycle of *Tetramitus rostratus* Perty. Jour. Morph. Physiol., 42, 23-81.
- BUNTING, M., and WENRICH, D. H. 1929 Binary fission in the amoeboid and flagellate phases of *Tetramitus rostratus* (Protozoa). Jour. Morph. Physiol., 47, 37-87.
- BURNSIDE, L. H. 1929 Relation of body size to nuclear size in *Stentor caeruleus*. Jour. Exp. Zool., 54, 473-483.
- BÜTSCHLI, O. 1876 Studien über die ersten Entwicklungsvorgänge der Eizelle, die Zelltheilung und die Conjugation der Infusorien. Abhandl. Senck. naturf. Ges., 10, 213-464.
- BUTTERFIELD, C. T. 1929 Experimental studies of natural purification in polluted waters. III. A note on the relation between food concentration in liquid media and bacterial growth. Publ. Health Rep., 44, 2865-2872.
- CALKINS, G. N. 1902 Studies on the life history of the protozoa. I. The life-cycle of *Paramecium caudatum*. Arch. Entw. Mech., 15, 139-186.
- CALKINS, G. N. 1911a Regeneration and cell division in *Urodon*. Jour. Exp. Zool., 10, 95-116.
- CALKINS, G. N. 1911b Effects produced by cutting *Paramecium* cells. Biol. Bull., 21, 36-72.
- CALKINS, G. N. 1921 *Urodon mobilis* Engelm. II. Renewal of vitality through conjugation. Jour. Exp. Zool., 29, 121-156.
- CALKINS, G. N. 1921 *Urodon mobilis* Engelm. IV. Effect of cutting during conjugation. Jour. Exp. Zool., 34, 449-470.
- CALKINS, G. N. 1929 *Urodon halseyi*, n. sp. I. The effect of ultraviolet rays. Biol. Bull., 57, 59-68.
- CALKINS, G. N., and GREGORY, L. H. 1913 Variations in the prog-

- eny of a single ex-conjugant of *Paramecium caudatum*. Jour. Exp. Zool., 15, 467-525.
- CALLOW, A. B. 1924 The oxygen uptake of bacteria. Biochem. Jour., 18, 507-518.
- CARLSON, T. 1913 Ueber Geschwindigkeit und Grösse der Hefevermehrung in Würze. Biochem. Zsch., 57, 313-334.
- CHAINSKEY, A. 1906 Observations physiologiques sur les Paramécies. Trav. Lab. Zoot. Varsovie, 35, 1-101.
- CHALKLEY, H. W. 1927 Changes in the total volume, and in the volumetric ratio between plasmagel and plasmasol in *Amœba proteus*. Anat. Rec., 37, 146.
- CHALKLEY, H. W. 1929 Changes in water content in *Amoeba* in relation to changes in its protoplasmic structure. Physiol. Zool., 2, 535-574.
- CHAMBERS, R., and REZNIKOFF, P. 1926 Micrurgical studies in cell physiology. I. The action of the chlorides of Na, K, Ca, and Mg on the protoplasm of *Amœba proteus*. Jour. Gen. Physiol., 8, 369-401.
- CHATTON, E. 1921 Réversion de la scission chez les ciliés. Réalisation d'individus distomes et polyénergides de *Glaucoma scintillans* se multipliant indéfiniment par scissiparité. C. R., 173, 393-395.
- CHATTON, E., et CHATTON, M. 1923 La sexualité provoquée expérimentalement chez un infusoire: *Glaucoma scintillans*. Prédominance des conditions du milieu dans son déterminisme. C.R., 176, 1091-1093.
- CHATTON, E., et CHATTON, M. 1925 L'action des facteurs externes sur les infusoires. Le déterminisme de la formation des chaînes (dystomie) chez les *Colpidium*. C. R., 180, 1225-1227.
- CHESNEY, A. M. 1916 The latent period in the growth of bacteria. Jour. Exp. Med., 24, 387-418.
- CHILD, C. M., and DEVINEY, E. 1926 Contributions to the physiology of *Paramecium caudatum*. Jour. Exp. Zool., 43, 257-312.
- CIENKOWSKI, L. 1863 Zur Entwicklungsgeschichte der *Myxomycten*. Jahrb. wiss. Bot., 3, 325-337.
- CIENKOWSKI, L. 1865 Beiträge zur Kenntniss der Monaden. Arch. mikr. Anat., 1, 201-232.
- CLARK, N. A. 1922 The rate of formation and the yield of yeast in wort. Jour. Phys. Chem., 26, 42-60.
- CLARK, P. F., and RUEHL, W. H. 1919 Morphological changes during the growth of bacteria. Jour. Bact., 4, 615-629.
- COLLIN, B. 1909 La conjugaison d'*Anoplophrya branchiarum* (Stein). Arch. zool. exp. gén., [5], 1, 345-388.
- COPLANS, M. 1910 Influences affecting the growth of microorganisms—latency: inhibition: mass action. Jour. Path., 14, 1-27.
- COPPING, A. M. 1929 The effect of "bios" on the growth and metabolism of certain yeasts. Biochem. Jour., 23, 1050-1063.

- CRAMPTON, G. C. 1912 Experiments performed upon Protozoa confined in capillary tubes. *Arch. Prot.*, 27, 9-15.
- CUTLER, D. W., and CRUMP, L. M. 1923 The rate of reproduction in artificial culture of *Colpidium colpoda*. *Biochem. Jour.*, 17, 174-186 and 878-886.
- CUTLER, D. W., and CRUMP, L. M. 1923 The rate of reproduction in artificial culture of *Colpidium colpoda*. Part III. *Biochem. Jour.* 18, 905-912.
- CUTLER, D. W., and CRUMP, L. M. 1927 The qualitative and quantitative effects of food on the growth of a soil amoeba (*Hartmannella hyalina*). *Brit. Jour. Exp. Biol.*, 5, 155-165.
- DALLINGER, W. H. 1887 The president's address. *Jour. Roy. Micr. Soc.*, 1, 185-199.
- DALLINGER, W. H., and DRYSDALE, J. 1874 Further research into the life history of the monads. *Mo. Micr. Jour.*, 11, 7-10.
- DAWSON, A. I. 1919 Bacterial variations induced by changes in the composition of culture media. *Jour. Bact.*, 4, 133-148.
- DAWSON, J. A. 1919 An experimental study of an amiconucleate *Oxytricha*. I. Study of the normal animal, with an account of cannibalism. *Jour. Exp. Zool.*, 29, 473-513.
- DAWSON, J. A. 1920 An experimental study of an amiconucleate *Oxytricha*. II. The formation of double animals or 'twins'. *Jour. Exp. Zool.*, 30, 129-157.
- DAWSON, J. A. 1928. A comparison of the life-'cycles' of certain ciliates. *Jour. Exp. Zool.*, 51, 199-208.
- DEBARY, A. 1864 *Die Mycetozoen*. 2. Aufl. Leipzig.
- DEGARIS, C. F. 1927 A genetic study of *Paramecium caudatum* in pure lines through an interval of experimentally induced monster formation. *Jour. Exp. Zool.*, 49, 133-147.
- DEGARIS, C. F. 1928 The effects of anterior and posterior selections on fission rate in pure lines of *Paramecium caudatum*. *Jour. Exp. Zool.*, 50, 1-14.
- DEGEN, A. 1905 Untersuchungen über die kontraktile Vakuole und die Wabenstruktur des Protoplasmas. *Bot. Ztg.*, 63, 160-226.
- DEMBOWSKA, W. S. 1925 Studien über die Regeneration von *Stylonychia mytilus*. *Arch. Entw. Mech.*, 104, 185-209.
- DOBELL, C., and JEPPE, M. W. 1918 A study of the diverse races of *Entamoeba histolytica* distinguishable from one another by the dimensions of their cysts. *Parasitol.*, 10, 320-361.
- DOFLEIN, F. 1907 Beobachtungen und Ideen über die Konjugation der Infusorien. *Sitzungsb. Ges. Morph. Physiol. München*, 23, 107-114.
- DOFLEIN, F. 1911 *Lehrbuch der Protozoenkunde*. Jena. 3. Aufl., 1043 S., S. 609.

- DOGIEL, V. 1925 Die Geschlechtsprozesse bei Infusorien (speziell bei den Ophryoscoleciden). Arch. Prot., 50, 283-442.
- DRABBLE, E., DRABBLE, H., and SCOTT, D. G. 1907 On the size of the cells of *Pleurococcus* and *Saccharomyces* in solutions of a neutral salt. Biochem. Jour., 2, 221-229.
- DRIESCH, H. 1898 Von der Beendigung morphogener Elementarprocesse. Arch. Entw. Mech., 6, 198-227.
- EISENBERG, E. 1926 Recherches sur le fonctionnement de la vésicule pulsatile des infusoires (*Paramecium caudatum* Stein) dans les conditions normales et sous l'action de certains agents expérimentaux (pression osmotique et électrolytes). Arch. de Biol., 35, 441-464.
- ELPATIEWSKY, W. 1907 Zur Fortpflanzung von *Arcella vulgaris* Ehrbg. Arch. Prot., 10, 441-466.
- EMERSON, R. 1929 Measurements of the metabolism of two protozoans. Jour. Gen. Physiol., 13, 153-158.
- ENRIQUES, P. 1902 Ricerche osmotiche sugli Infusori. Atti R. Accad. Lincei, [5], Rend. Cl. Sci., 11, i, 240-347, 392-397.
- ENRIQUES, P. 1907 La coniugazione e il differenziamento sessuale negli Infusori. Arch. Prot., 9, 195-296.
- ENRIQUES, P. 1908 Die Conjugation und sexuelle Differenzierung der Infusorien. Zweite Abhandlung: Wiederconjugante und Hemisexe bei *Chilodon*. Arch. Prot., 12, 213-276.
- ENRIQUES, P. 1909 La teoria di Spencer sulla divisione cellulare studiata con ricerche biometriche negli infusori. Arch. di Fisiol., 7, 113-136.
- ENRIQUES, P. 1910 La coniugazione e il differenziamento sessuale negli infusori. III. Azione dei sali sulle epidemie di coniugazione nel *Cryptochilum nigricans*. Mem. R. Accad. Sci. Istit. Bologna, [6], 6, 463-500.
- ENRIQUES, P. 1916a Duemila cinquecento generazioni in un Infusorio, senza coniugazione nè partenogenesi, nè depressioni. Rend. R. Accad. Sci. Istit. Bologna, Cl. Sci. Fis., 20, 67-76.
- ENRIQUES, P. 1916b La coniugazione e il differenziamento sessuale negli infusori. VI. Condizioni che determinano la coniugazione ripetuta nel *Chilodon uncinatus*. Mem. R. Accad. Sci. Istit. Bologna, [7], 3, 49-54.
- EPHRUSSI, B., et LWOFF, A. 1923 Sur la double périodicité cyclique de la zone de division chez un cilié, *Colpidium colpoda*, mise en évidence par la réversion de la scission. C. R., 176, 930-932.
- ERDMANN, R. 1920 Endomixis and size variations in pure bred lines of *Paramecium aurelia*. Arch. Entw. Mech., 46, 85-148.
- ERDMANN, R., and WOODRUFF, L. L. 1916 The periodic reorganization process in *Paramecium caudatum*. Jour. Exp., Zool., 20, 59-95.

- ERRERA, L. 1903 Sur la limite de petitesse des organismes. *Revue sci.*, [4], 19, 169-172.
- ESTABROOK, A. H. 1910 Effect of chemicals on growth in *Paramecium*. *Jour. Exp. Zool.*, 8, 489-534.
- EVERTS, E. 1873 Untersuchungen an *Vorticella nebulifera*. *Zsch. wiss. Zool.*, 23, 592-622.
- FERMOR, X. 1913 Die Bedeutung der Encystierung bei *Stylonychia pustulata* Ehrbg. *Zool. Anz.*, 42, 380-384.
- FETTER, D. 1926 Determination of the protoplasmic viscosity of *Paramecium* by the centrifuge method. *Jour. Exp. Zool.*, 44, 279-283.
- FORTNER, H. 1925 Ueber die Gesetzmässigkeit der Wirkungen des osmotischen Druckes physiologisch indifferenter Lösungen auf einzellige, tierische Organismen. *Biol. Cbl.*, 45, 417-446.
- GALILEI, G. 1638 *Dialogues concerning two new sciences*. Translated by Crew and DeSalvio, New York, 1914. p. 89.
- GELEI, J. 1925 Ueber den Kannibalismus der Stentoren. *Arch. Prot.*, 52, 404-417.
- GERASSIMOW, J. J. 1901 Ueber den Einfluss des Kerns auf das Wachstum der Zelle. *Bull. Soc. Imper. Natur. Moscou*, 1901, 185-220.
- GERASSIMOW, J. J. 1902 Die Abhängigkeit der Grösse der Zelle von der Menge ihrer Kernmasse. *Zsch. allg. Physiol.*, 1, 220-258.
- GERASSIMOW, J. J. 1904a Zur Physiologie der Zelle. *Bull. Soc. Imper. Natur. Moscou*, 1904, 1-134.
- GERRASSIMOW, J. J. 1904b Ueber die Grösse des Zellkerns. *Bot. Cbl.*, Beih., 18, 45-118.
- GOETTE, A. 1917 Ueber den Lebenscyclus von *Diffugia lobostoma*. *Arch. Prot.*, 37, 93-137.
- GREENLEAF, W. E. 1926 The influence of volume of culture medium and cell proximity on the rate of reproduction of Infusoria. *Jour. Exp. Zool.*, 46, 143-167.
- GREFF, F. 1867 Ueber *Actinophrys Eichhornii* und einen neuen Süsswasserrhizopoden, besonders in Rücksicht auf Theilbarkeit derselben resp. Vermehrung durch künstliche Theilung. *Arch. mikr. Anat.*, 3, 396-403.
- GREGORY, L. H. 1909 Observations on the life history of *Tillina magna*. *Jour. Exp. Zool.*, 6, 383-431.
- GROBICKA, J., et WASILEWSKA, J. 1925 Essai d'analyse chimique quantitative de l'infusoire *Paramecium caudatum* St. *Trav. Inst. Nencki (Varsovie)*, Lab. Physiol., 3, i, 1-23.
- GRUBER, A. 1883a Untersuchungen über einige Protozoen. *Zsch. wiss. Zool.*, 38, 45-70.

- GRUBER, A. 1883b Ueber Kerntheilungsvorgänge bei einigen Protozoen. Zsch. wiss. Zool., 38, 372-391.
- GRUBER, A. 1887 Weitere Beobachtungen an vielkernigen Infusorien. Ber. naturf. Ges. Freiburg, 3, 57-69.
- GRUBER, K. 1912 Biologische und experimentelle Untersuchungen an *Amoeba proteus*. Arch. Prot., 25, 316-376.
- GUTMANOWNA, L. 1915 Ueber das Verhalten der gelösten Gase in den Infusorienkulturen. C. R. soc. sci. Varsovie, Cl. 3, 8, 73-79.
- HAIME, J. 1853. Observations sur les métamorphoses et sur l'organisation de la *Trichoda lynceus*. Ann. sci. nat., zool., [3], 19, 109-134.
- HAMMETT, F. S. 1929 The chemical stimulus essential for growth by increase in cell number. Protopl., 7, 297-322.
- HANCE, R. T. 1917 Studies on a race of *Paramoecium* possessing extra contractile vacuoles. I. An account of the morphology, physiology, genetics and cytology of this new race. Jour. Exp. Zool., 23, 287-333.
- HARDY, W. B., and HARVEY, H. W. 1911 Note on the surface electric charges of living cells. Proc. Roy. Soc., 84 B, 217-226.
- HARTMAN, E. 1927 Certain interrelations between *Plasmodium praecox* and its host. Amer. Jour. Hyg., 7, 407-432.
- HARTMANN, M. 1917 Untersuchungen über die Morphologie und Physiologie des Formwechsels (Entwicklung, Fortpflanzung, Befruchtung und Vererbung) der Phytomonaden (Volvocales). II. Mitteilung. Ueber die dauernde rein agame Züchtung von *Eudorina elegans* und ihre Bedeutung für das Befruchtungs- und Todproblem. Sitzungsab. Akad. Wiss. Berlin, phys.-math. Kl., 52, 760-776.
- HARTMANN, M. 1921 Untersuchungen über die Morphologie und Physiologie des Formwechsels der Phytomonaden (Volvocales). III. Mitt.: Die dauernd agame Zucht von *Eudorina elegans*, experimentelle Beiträge zum Befruchtungs- und Todproblem. Arch. Prot., 43, 223-286.
- HARTMANN, M. 1922 Ueber den dauernden Ersatz der ungeschlechtlichen Fortpflanzung durch fortgesetzte Regenerationen. Biol. Cbl., 42, 364-381.
- HARTMANN, M. 1924a Ueber die Veränderung der Koloniebildung von *Eudorina elegans* und *Gonium pectorale* unter dem Einfluss äusserer Bedingungen. IV. Mitt. der Untersuchungen über die Morphologie und Physiologie des Formwechsels der Phytomonaden (Volvocales). Arch. Prot., 49, 375-395.
- HARTMANN, M. 1924b Der Ersatz der Fortpflanzung von Amöben durch fortgesetzte Regenerationen. Weitere Versuche zum Todproblem. Arch. Prot., 49, 447-464.

- HARTMANN, M. 1926 Ueber experimentelle Unsterblichkeit von Protozoen-Individuen. *Naturwiss.*, 14, 433-435.
- HARTMANN, M. 1928 Ueber experimentelle Unsterblichkeit von Protozoen-Individuen. *Zool. Jahrb., Abt. Zool.*, 45, 973-987.
- HARTOG, M. M. 1891 Some problems of reproduction. *Quart Jour. Micr. Sci.*, 33, 1-79.
- HARVEY, E. B., 1917 A physiological study of specific gravity and of luminescence in *Noctiluca*, with special reference to anesthesia. *Carn. Inst. Wash., Publ. No. 251*, 235-253.
- HARVEY, E. N. 1928 The oxygen consumption of luminous bacteria. *Jour. Gen. Physiol.*, 11, 469-475.
- HATA, S. 1908 Ueber die durch bestimmte anorganische Salze verursachten Degenerationsformen bestimmter Bakterienarten. *Cbl. Bakt.*, 46 i, 289-292.
- HEGNER, R. W. 1919a Heredity, variation, and the appearance of diversities during the vegetative reproduction of *Arcella dentata*. *Genetics*, 4, 95-150.
- HEGNER, R. W. 1919b The effects of environmental factors upon the heritable characteristics of *Arcella dentata* and *A. Polypora*. *Jour. Exp. Zool.*, 29, 427-441.
- HEGNER, R. W. 1920 The relations between nuclear number, chromatin mass, cytoplasmic mass, and shell characteristics in four species of the genus *Arcella*. *Jour. Exp. Zool.*, 30, 1-95.
- HEGNER, R. W. 1921 Measurements of *Trypanosoma diemyctyli* from different hosts and their relation to specific identification, heredity and environment. *Jour. Parasit.*, 7, 105-113.
- HEGNER, R. W. 1929 The infection of parasite-free chicks with intestinal protozoa from birds and other animals. *Amer. Jour. Hyg.*, 10, 33-62.
- HEGNER, R. W., and WU, H. F. 1921 An analysis of the relation between growth and nuclear division in a parasitic infusorian, *Opalina* sp. *Amer. Nat.*, 55, 335-346.
- HEHEWERTH, F. H. 1901 Die mikroskopische Zählungsmethode der Bakterien von Alex. Klein und einige Anwendungen derselben. *Arch. Hyg.*, 39, 321-389.
- HEILBRUNN, L. V. 1923 The colloid chemistry of protoplasm. I and II. *Amer. Jour. Physiol.*, 64, 481-498.
- HEILBRUNN, L. V. 1927 The colloid chemistry of protoplasm. V. A preliminary study of the surface precipitation reactions of living cells. *Arch. exp. Zellf.*, 4, 246-263.
- HEILBRUNN, L. V. 1929 The absolute viscosity of amoeba protoplasm. *Protopl.*, 8, 65-69.
- HENRICI, A. T. 1923 A statistical study of the form and growth of *Bacterium coli*. *Proc. Soc. Exp. Biol. Med.*, 21, 215-217.
- HENRICI, A. T. 1924a Influence of age of parent culture on size of cells of *Bacillus megatherium*. *Proc. Soc. Exp. Biol. Med.*, 21, 343-345.

- HENRICI, A. T. 1924b Influence of concentration of nutrients on size of cells of *Bacillus megatherium*. *Proc. Soc. Exp. Biol. Med.*, *21*, 345-346.
- HENRICI, A. T. 1925a On cytomorphosis in bacteria. *Sci.*, *61*, 644-647.
- HENRICI, A. T. 1925b A statistical study of the form and growth of the cholera vibrio. *Jour. Infec. Dis.*, *37*, 75-81.
- HENRICI, A. T. 1926a Morphologic variations of bacteria in the lag phase. *Jour. Infec. Dis.*, *38*, 54-65.
- HENRICI, A. T. 1926b Involution forms of *Escherichia coli*. *Jour. Infec. Dis.*, *39*, 429-440.
- HENRICI, A. T. 1928 Morphologic variation and the rate of growth of bacteria. Springfield, Ill. 194 pp.
- HERFS, A. 1922 Die pulsierende Vakuole der Protozoen ein Schutzorgan gegen Aussüzung. Studien über Anpassungen der Organismen an das Leben im Süßwasser. *Arch. Prot.*, *44*, 227-260.
- HERTWIG, G. 1889 Ueber die Conjugation der Infusorien. *Abh. bayr. Akad. Wiss.*, II. Cl., *17*, 153-233.
- HERTWIG, R. 1898 Ueber Kernteilung, Richtungskörperbildung und Befruchtung von *Actinosphaerium Eichhorni*. *Abh. bayr. Akad. Wiss.*, II. Cl., *19*, 633-734.
- HERTWIG, R. 1900 Ueber physiologische Degeneration bei Protozoen. *Sitzungsb. Ges. Morph. Physiol. München*, *19*, 1-7.
- HERTWIG, R. 1902 Die Protozoen und die Zelltheorie. *Arch. Prot.*, *1*, 1-40.
- HERTWIG, R. 1903 Ueber Korrelation von Zell- und Kerngrösse und ihre Bedeutung für die geschlechtliche Differenzierung und die Teilung der Zelle. *Biol. Cbl.*, *23*, 49-62, 108-119.
- HERTWIG, R. 1904 Ueber physiologische Degeneration bei *Actinosphaerium Eichhorni*. *Fests. Haeckel, Jena.*, 303-354.
- HERTWIG, R. 1914 Ueber Parthenogenesis der Infusorien und die Depressionszustände der Protozoen. *Biol. Cbl.*, *34*, 557-581.
- HINRICHS, M. A. 1928 Ultra-violet radiation and division in *Paramecium caudatum*. *Physiol. Zool.*, *1*, 394-415.
- HOFMEISTER, W. 1863 Zusätze und Berichtigungen zu den 1851 veröffentlichten Untersuchungen der Entwicklung höherer Kryptogamen. *Jahrb. wiss. Bot.*, *3*, 259-293.
- HOFMEISTER, W. 1874 Ueber die Bewegungen der Fäden der *Spirogyra princeps*. (Vauch.) Link. *Württemb. naturw. Jahreshefte*, *30*, 211-226.
- HOPKINS, H. S. 1921 The conditions for conjugation in diverse races of *Paramecium*. *Jour. Exp. Zool.*, *34*, 339-384.
- HOWLAND, R. B. 1928 Grafting and reincorporation in *Actinosphaerium eichhornii* Ehr. *Biol. Bull.*, *54*, 279-288.
- HOWLAND, R. B., and POLLACK, H. 1927 Micrurgical studies on the contractile vacuole of *Amoeba dubia* (Schaeffer). *Jour. Exp. Zool.*, *48*, 441-458.

- IVANIC, M. 1927 Ueber den Kannibalismus bei *Amoeba verrucosa* (Ehrbg.), nebst Bemerkungen über den Kannibalismus bei Protozoan im allgemeinen. *Zool. Anz.*, 74, 313-321.
- JACOBS, M. H. 1922 The effects of carbon dioxide on the consistency of protoplasm. *Biol. Bull.*, 42, 14-30.
- JAHN, T. L. 1929 Studies on the physiology of the euglenoid flagellates. I. The relation of the density of population to the growth rate of *Euglena*. *Biol. Bull.*, 57, 81-106.
- JENNINGS, H. S. 1904 Contributions to the study of the behavior of lower organisms. *Carn. Inst. Wash.*, Publ. No. 16, Paper VI.
- JENNINGS, H. S. 1908a Heredity, variation and evolution in protozoa. I. The fate of new structural characters in *Paramecium*, in connection with the problem of the inheritance of acquired characters in unicellular organisms. *Jour. Exp. Zool.*, 5, 577-632.
- JENNINGS, H. S. 1908b Heredity, variation and evolution in Protozoa. II. Heredity and variation of size and form in *Paramecium*, with studies of growth, environmental action and selection. *Proc. Amer. Phil. Soc.*, 47, 393-546.
- JENNINGS, H. S. 1910 What conditions induce conjugation in *Paramecium*? *Jour. Exp. Zool.*, 9, 279-300.
- JENNINGS, H. S. 1911 Assortative mating, variability and inheritance of size, in the conjugation of *Paramecium*. *Jour. Exp. Zool.*, 11, 1-134.
- JENNINGS, H. S. 1916 Heredity, variation and the results of selection in the uniparental reproduction of *Diffugia corona*. *Genetics*, 1, 407-534.
- JENNINGS, H. S. 1920 Life and death, heredity and evolution in unicellular organisms. Boston. 233 pp.
- JENNINGS, H. S., and HARGITT, G. T. 1910 Characteristics of the diverse races of *Paramecium*. *Jour. Morph.*, 21, 495-561.
- JENNINGS, H. S., and LASHLEY, K. S. 1913a Biparental inheritance and the question of sexuality in *Paramecium*. *Jour. Exp. Zool.*, 14, 393-466.
- JENNINGS, H. S., and LASHLEY, K. S. 1913b Biparental inheritance of size in *Paramecium*. *Jour. Exp. Zool.*, 15, 193-199.
- JENSEN, P. 1893 Die absolute Kraft einer Flimmerzelle. *Arch. gesam. Physiol.*, 54, 537-551.
- JENSEN, P. 1895 Ueber individuelle physiologische Unterschiede zwischen Zellen der gleichen Art. *Arch. gesam. Physiol.*, 62, 172-200.
- JICKELI, C. F. 1902 Die Unvollkommenheit des Stoffwechsels als Veranlassung zur Vermehrung, Wachstum, Differenzierung, Rückbildung und Tod der Lebewesen im Kampf ums Dasein. Berlin. 353 S.

- JOHNSON, H. P. 1893 A contribution to the morphology and biology of the stentors. *Jour. Morph.*, 8, 467-562.
- JOHNSON, H. P. 1894 The plastogamy of *Actinosphaerium*. *Jour. Morph.*, 9, 269-276.
- JOLLOS, V. 1913 Experimentelle Untersuchungen an Infusorien (Vorläufige Mitteilung) *Biol. Cbl.*, 33, 222-236.
- JOLLOS, V. 1916 Die Fortpflanzung der Infusorien und die potentielle Unsterblichkeit der Einzelligen. *Biol. Cbl.*, 36, 497-514.
- JOLLOS, V. 1921 Experimentelle Protistenstudien. I. Untersuchungen über Variabilität und Vererbung bei Infusorien. *Arch. Prot.*, 43, 1-222.
- JOLLOS, V. 1924 Untersuchungen über Variabilität und Vererbung bei Arcellen. *Arch. Prot.*, 49, 307-374.
- JOUKOWSKY, D. 1898 Beiträge zur Frage nach den Bedingungen der Vermehrung und des Eintritts der Conjugation bei den Ciliaten. *Verh. natur.-med. Vereins Heidelberg*, 6, 17-42.
- KALMUS, H. 1928 Untersuchungen über die Atmung von *Paramecium caudatum*. *Zsch. vergl. Physiol.*, 7, 314-322.
- KALMUS, H. 1929 Versuche über die Teilung von *Paramecium caudatum* in der Kapillare nebst Bemerkungen über den sogenannten Raumfaktor. *Arch. Prot.*, 66, 402-408.
- KAZANZEFF, W. 1901 Experimentelle Untersuchungen über *Paramecium caudatum*. *Inaug. Diss.*, Zürich. 60 S.
- KEPNER, W. A., and REYNOLDS, B. D. 1923 Reactions of cell bodies and pseudopodial fragments of *Diffugia*. *Biol. Bull.*, 44, 22-46.
- KHAINSKY, A. 1911 Untersuchungen über Arcellen (Vorläufige Mitteilung). *Arch. Prot.*, 21, 165-185.
- KROGH, A. 1919a The rate of diffusion of gases through animal tissues, with some remarks on the coefficient of invasion. *Jour. Physiol.*, 52, 391-408.
- KROGH, A. 1919b The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue. *Jour. Physiol.*, 52, 409-415.
- KÜHN, A. 1916 Ueber die Beziehungen zwischen Plasmateilungen und Kernteilung bei Amöben. *Zool. Anz.*, 48, 193-203.
- KÜHN, A. 1930 Untersuchungen zur kausalen Analyse der Zellteilung. I. Teil: Zur Morphologie und Physiologie der Kernteilung von *Vahlkampfia bistadialis*. *Arch. Entw. Mech.*, 46, 259-327.
- KÜHNE, W. 1864 Untersuchungen über das Protoplasma und die Contractilität. Leipzig. 158 S.
- LANE-CLAYPON, J. E. 1909 Multiplication of bacteria and the influence of temperature and some other conditions thereon. *Jour. Hyg.*, 9, 239-248.
- LAPAGE, G. 1922 Cannibalism in *Amoeba vespertilo* (Penard). *Quart. Jour. Micr. Sci.*, 66, 669-710.

- LARSON, W. P. 1928 The effect of the surface tension of the medium upon bacteria and toxins. E. O. Jordan and I. S. Falk: The newer knowledge of bacteriology and immunology. Chicago. Pp. 179-187.
- LEIDY, J. 1879 Fresh-water Rhizopods of North America. Rept. U. S. Geol. Surv., 12, 324 pp.
- LEVY, J. 1924 Studies on reproduction in *Amoeba proteus*. Genetics, 9, 124-150.
- LEWIN, K. R. 1911 Nuclear relations of *Paramecium caudatum* during the asexual period. Proc. Camb. Phil. Soc., 16, 39-41.
- LILLIE, F. R. 1896 On the smallest parts of *Stentor* capable of regeneration; a contribution on the limits of divisibility of living matter. Jour. Morph., 12, 239-249.
- LIPSKA, I. 1910 Recherches sur l'inanition chez *Paramecium caudatum*. Revue suisse de zool., 18, 591-646.
- LÖHNIS, F. 1921 Studies upon the life cycles of the bacteria. Mem. Nat. Acad. Sci., 16, no. 2, 335 pp.
- LOOPER, J. B. 1928 Cytoplasmic fusion in *Actinophrys sol*, with special reference to the karyoplasmic ratio. Jour. Exp. Zool., 50, 31-49.
- LUDWIG, W. 1928a Der Betriebsstoffwechsel von *Paramecium caudatum* Ehrbg. Zugleich ein Beitrag zur Frage nach der Funktion der kontraktile Vakuolen. Arch. Prot., 62, 12-40.
- LUDWIG, W. 1928b Ueber den funktionellen Zusammenhang zwischen Populationsdichte, Nahrungsdichte und Teilungsrate bei Protisten und über die Zunahme der Bevölkerungsdichte überhaupt. Biol. Gen., 4, 351-376.
- LUDWIG, W. 1928c Permeabilität und Wasserwechsel bei *Noctiluca miliaris* Suriray. Zool. Anz., 76, 273-285.
- LUND, E. J. 1914 The relations of bursaria to food. I. Selection in feeding and in extrusion. Jour. Exp. Zool., 16, 1-52.
- MACDOUGALL, M. S. 1925 Cytological observations on gymnostomatous ciliata with a description of the maturation phenomena in diploid and tetraploid forms of *Chilodon uncinatus*. Quart. Jour. Micr. Sci., 69, 361-384.
- MACDOUGALL, M. S. 1929 Modifications in *Chilodon uncinatus* produced by ultra-violet radiation. Jour. Exp. Zool., 54, 95-109.
- MAST, S. O. 1909 The reactions of *Didinium nasutum* (Stein) with special reference to the feeding habits and the function of trichocysts. Biol. Bull., 16, 91-118.
- MAST, S. O. 1917 Conjugation and encystment in *Didinium nasutum* with especial reference to their significance. Jour. Exp. Zool., 23, 335-359.
- MAST, S. O. 1926 Structure, movement, locomotion and stimulation in *Amoeba*. Jour. Morph. Physiol., 41, 347-425.

- MAST, S. O. 1928 Factors involved in changes in form in *Amoeba*. Jour. Exp. Zool., 51, 97-120.
- MATTHEWS, J. R. 1929 A mensurative study of the cysts of *Entamoeba coli*. Ann. Trop. Med. Parasitol., 12, 259-272.
- MAUPAS, E. 1883 Contribution a l'étude morphologique et anatomique des infusoires ciliés. Arch. zool. exp. gén., [2], 1, 427-664.
- MAUPAS, E. 1888 Recherches expérimentales sur la multiplication des infusoires ciliés. Arch. zool. exp. gén., [2], 6, 165-277.
- MAUPAS, E. 1889 La rejeunissement karyogamique chez les ciliés. Arch. zool. exp. gén., [2], 7, 149-517.
- MAXWELL, J. C. 1875 Atom. Encyc. Brit., 9th ed., 3, 36-49.
- MCCLENDON, J. F. 1909 Protozoan studies. Jour. Exp. Zool., 6, 265-283.
- M'KENDRICK, A. G., and PAI, M. K. 1911 The rate of multiplication of microorganisms: a mathematical study. Proc. Roy. Soc. Edinb., 31, 649-655.
- MEDRKIEWICZOWNA, H. 1921 Le rôle de la surface libre du liquide dans le développement des cultures du *Colpidium colpoda* Ehr. C. R. soc. sci. Varsovie.
- METALNIKOW, S. 1922 Dix ans de culture des infusoires sans conjugaison. C. R., 175, 776-778.
- METZNER, P. 1927 Pulsierende Vakuolen. Tabulae Biologicae, 4, 490-496.
- MEYERHOF, O. 1925 Ueber den Einfluss des Sauerstoffs auf die alkoholische Gärung der Hefe. Biochem. Zsch. 162, 43-86.
- MIDDLETON, A. R. 1915 Heritable variations and the results of selection in the fission rate of *Stylonychia pustulata*. Jour. Exp. Zool., 19, 451-503.
- MIDDLETON, A. R. 1918 Heritable effects of temperature differences on the fission rate of *Stylonychia pustulata*. Genetics, 3, 534-572.
- MIDDLETON, A. R. 1928 Heritability of the effects of chemically different media upon the fission rate within the clone of *Paramecium caudatum*. Anat. Rec., 41, 50.
- MITCHELL, W. H., JR. 1929 The division rate of *Paramecium* in relation to temperature. Jour. Exp. Zool., 54, 383-410.
- MIZUNO, F. 1927 Sur la croissance normale du *Paramecium caudatum*. Sci. Rep. Tohoku Imp. Univ., [4], 2, 367-381.
- MOORE, E. L. 1924 Regeneration at various phases in the life-history of *Spathidium spathula* and *Blepharisma undulans*. Jour. Exp. Zool., 39, 249-316.
- MORGAN, T. H. 1901 Regeneration of proportionate structures in *Stentor*. Biol. Bull., 2, 311-328.
- MÜLLER, A. 1911 Die Abhängigkeit des Verlaufs der Sauerstoffzehrung in natürlichen Wässern and künstlichen Nährlösungen vom Bakterienwachstum. Arb. a. d. kais. Gesundheitsamt, 38, 294-326.

- MÜLLER, J. 1840 Handbuch der Physiologie des Menschen. Coblenz. Bd. 2, Teil 3, S 601.
- MÜLLER, M. 1895 Über den Einfluss von Fiebertemperaturen auf die Wachstumsgeschwindigkeit und die Virulenz des Typhus bacillus. Zsch. Hyg., 20, 245-280.
- MYERS, E. C. 1927 Relation of density of population and certain other factors to survival and reproduction in different biotypes of *Paramecium caudatum*. Jour. Exp. Zool., 49, 1-43.
- NECHELES, H. 1924 Unpublished. Results quoted in: O. Kestner und R. Plaut: Physiologie des Stoffwechsels. Winterstein's Handb. d. Physiol. 2, ii, 901-1112.
- NÖLLER, W. 1913 Die Blutprotozoen des Wasserfrosches und ihre Uebertragung. Arch. Prot., 31, 169-240.
- NOWIKOFF, M. 1908 Ueber die Wirkung des Schilddrüsenextrakts und einiger anderer Organstoffe auf Ciliaten. Arch. Prot., 11, 309-325.
- OSTWALD, W. 1908 Ueber die zeitlichen Eigenschaften der Entwicklungsvorgänge. Roux's Vortr. u. Aufsätze, 5. 71S.
- PACKARD, C. 1926 The effect of sodium on the rate of cell division. Jour. Cancer Res., 10, 1-14.
- PAINE, S. G. 1911 The permeability of the yeast cell. Proc. Roy. Soc., 84 B, 289-307.
- PARKER, R. C. 1927 The effect of selection in pedigree lines of Infusoria. Jour. Exp. Zool., 49, 401-439.
- PASCHER, A. 1917 Fusionsplasmodien bei Flagellaten und ihre Bedeutung für die Ableitung der Rhizopoden von den Flagellaten. Arch. Prot., 37, 31-64.
- PASTEUR, L. 1861 Animalcules infusoires vivant sans gaz oxygène libre et déterminant des fermentations. C. R., 52, 344-347, 1260-1264.
- PASTEUR, L. 1876 Etudes sur la bière. Paris. 387 pp.
- PAULI, W. E., und HARTMANN, A. 1924 Ueber die Wirkung von Kathodenstrahlen auf lebendes Gewebe. Arch. Entw. Mech., 103, 95-167.
- PEARL, R. 1906 Variation in *Chilomonas* under favorable and unfavorable conditions. Biometrika, 5, 53-72.
- PEARL, R. 1907 A biometrical study of conjugation in *Paramecium*. Biometrika, 5, 213-297.
- PEARL, R., and DUNBAR, F. J. 1903 Variation and correlation in *Arcella*. Biometrika, 2, 321-337.
- PEARL, R., and DUNBAR, F. J. 1905 Some results of a study of variation in *Paramecium*. Seventh Ann. Rept. Mich. Acad. Sci., 77-86.
- PEARSON, K. 1914 On the probability that two independent distribu-

- tions of frequency are really samples of the same population, with special reference to recent work on the identity of Trypanosome strains. *Biometrika*, 10, 85-143.
- PEEBLES, F. 1912 Regeneration and regulation in *Paramecium caudatum*. *Biol. Bull.*, 23, 154-170.
- PENARD, E. 1889 Etude sur quelques Héliozoaires d'eau douce, I. *Arch. de Biol.*, 9, 123-183.
- PENARD, E. 1890 Etudes sur les Rhizopodes d'eau douce. *Mém. soc. phys. hist. nat. Genève*, 31, 1-230.
- PENARD, E. 1902 Faune rhizopodique du bassin du Léman. *Geneve*, 714 pp.
- PENFOLD, W. J. 1914 On the nature of bacterial lag. *Jour. Hyg.*, 14, 215-241.
- PENFOLD, W. J., and NORRIS, D. 1912 The relation of concentration of food supply to the generation time of bacteria. *Jour. Hyg.*, 12, 527-531.
- PESKETT, G. L. 1924 Allelocatalysis and the growth of yeast. *Biochem. Jour.* 18, 866-871.
- PESKETT, G. L. 1925 Studies on the growth of yeast. I. The influence of volume of culture medium employed. *Biochem. Jour.*, 19, 464-473.
- PESKETT, G. L. 1927 Studies on the growth of yeast. III. A further study on the influence of volume of medium employed. *Biochem. Jour.*, 21, 104-110.
- PETERS, A. W. 1904 Metabolism and division in protozoa. *Proc. Amer. Acad. Arts Sci.*, 39, 441-516.
- PETERS, R. A. 1929 Observations upon the oxygen consumption of "*Colpidium colpoda*". *Jour. Physiol.*, 68, ii-iii.
- PETERSEN, W. A. 1927 The axial gradient in *Paramecium*. *Sci.*, 66, 157-158.
- PETERSEN, W. A. 1929 The relation of density of population to rate of reproduction in *Paramecium caudatum*. *Physiol. Zool.*, 2, 221-254.
- PFITZNER, W. 1886 Zur Kenntnis der Kerntheilung bei den Protozoen. *Morphol. Jahrb.*, 11, 454-467.
- PHELPS, L. A. 1926 Experimental analysis of factors concerned in division in *Ameba*. *Trans. Amer. Micr. Soc.*, 45, 133-145.
- POLJANSKY, G. 1926 Die Conjugation von *Dogielia sphaerii* (Infusoria, Holotricha, Astomata). *Arch. Prot.*, 53, 407-434.
- POPOFF, M. 1907 Depression der Protozoenzelle und der Geschlechtszellen der Metazoen. *Arch. Prot.*, Suppl. 1, 43-82.
- POPOFF, M. 1908 Experimentelle Zellstudien. *Arch. Zellf.*, 1, 245-379.
- POPOFF, M. 1909 Experimentelle Zellstudien. II. Ueber die Zellgrösse, ihre Fixierung und Vererbung. *Arch. Zellf.*, 3, 124-180.

- PRANDTL, H. 1907 Die physiologische Degeneration der *Amoeba proteus*. Arch. Prot., 8, 281-293.
- PROWAZEK, S. 1901 Beiträge zur Protoplasmaphysiologie. Biol. Cbl., 21, 87-95, 144-155.
- PROWAZEK, S. 1904a Beitrag zur Kenntnis der Regeneration und Biologie der Protozoen. Arch. Prot., 3, 44-59.
- PROWAZEK, S. 1904b Degenerative Hyperregeneration bei den Protozoen. Arch. Prot., 3, 60-63.
- PROWAZEK, S. 1908 Einfluss von Säurelösungen niedrigster Konzentration auf die Zell- und Kernteilung. Arch. Entw. Mech., 25, 643-647.
- PROWAZEK, S. 1910 Studien zur Biologie der Protozoen. V. Arch. Prot., 20, 201-222.
- PRZIBRAM, H. 1913 Die Kammerprogression der Foraminiferen als Parallele zur Häutungsprogression der Mantiden. Arch. Entw. Mech., 36, 194-210.
- PÜTTER, A. 1905 Die Atmung der Protozoen. Zsch. allg. Physiol., 5, 566-612.
- PÜTTER, A. 1924 Die Atmung der Planktonbakterien. Arch. gesamt. Physiol., 204, 94-126.
- RAHN, O. 1906 Ueber den Einfluss der Stoffwechselproducte auf das Wachstum der Bakterien. Cbl. Bakt., 16, ii, 417-429, 609-617.
- RAUTMANN, H. 1909 Der Einfluss der Temperatur auf das Größenverhältnis des Protoplasmakörpers zum Kern. Experimentelle Untersuchungen an *Paramecium caudatum*. Arch. Zellf., 3, 44-80.
- REED, G., and ORR, J. H. 1923 The influence of H ion concentration upon structure. I. H. influenzae. Jour. Bact., 8, 103-110.
- REUKAUF, E. 1912. Zur Encystierung von *Euglypha alveolata*. Zool. Anz., 39, 372-375.
- REVIS, C. 1912 The production of variation in the physiological activity of *Bacillus coli* by the use of malachite-green. Proc. Roy. Soc., 85 B, 192-194.
- REYNOLDS, B. D. 1923 Inheritance of double characteristics in *Arcella polyopora* Penard. Genetics, 8, 477-493.
- REYNOLDS, B. D. 1924 Interactions of protoplasmic masses in relation to the study of heredity and environment in *Arcella polyopora*. Biol. Bull., 46, 106-142.
- REZNIKOFF, P. 1928 Microsurgical studies in cell physiology. V. The antagonism of cations in their actions on the protoplasm of *Amoeba dubia*. Jour. Gen. Physiol., 11, 221-232.
- RHUMBLER, L. 1895 Beiträge zur Kenntnis der Rhizopoden (Beitrag III, IV, und V). Zsch. wiss. Zool., 61, 38-110.
- RHUMBLER, L. 1898a Zelleib-, Schalen- und Kern-Verschmelzungen bei den Rhizopoden und deren wahrscheinliche Beziehungen zu

- phylogenetischen Vorstufen der Metazoënbefruchtung. *Biol. Cbl.*, 18, 21-26, 33-38, 69-86, 113-130.
- RHUMBLER, L. 1898b Physikalische Analyse von Lebenserscheinungen der Zelle. *Arch. Entw. Mech.*, 7, 103-350.
- RHUMBLER, L. 1902 Die Doppelschalen von Orbitolites und anderer Foraminiferen, vom entwicklungsmechanischen Standpunkt aus betrachtet. *Arch. Prot.*, 1, 193-296.
- RHUMBLER, L. 1909-11 Die Foraminiferen der Plankton-Expedition. *Ergeb. d. Plankton-Exped. d. Humboldt-Stiftung. Kiel u. Leipzig*, Bd. III, L, c, 175-183.
- RICHARDS, O. W. 1928a Potentially unlimited multiplication of yeast with constant environment, and the limiting of growth by changing environment. *Jour. Gen. Physiol.*, 11, 525-538.
- RICHARDS, O. W. 1928b Changes in sizes of yeast cells during multiplication. *Bot. Gaz.*, 86, 93-101.
- ROACH, B. M. B. 1926 On the relation of certain soil algae to some soluble carbon compounds. *Ann. Bot.*, 40, 149-201.
- ROBERTSON, T. B. 1921 Experimental studies on cellular multiplication. I and II. *Biochem. Jour.*, 15, 595-619.
- ROOT, F. M. 1918 Inheritance in the asexual reproduction of *Centropyxis aculeata*. *Genetics*, 3, 173-206.
- ROSSBACH, M. J. 1872 Die rhythmischen Bewegungserscheinungen der einfachsten Organismen und ihr Verhalten gegen physikalische Agentien und Arzneimittel. *Arb. zool.-zoot. Inst. Würzburg*, 1, 9-72.
- RUBNER, M. 1912 Die Ernährungsphysiologie der Hefezelle bei der alkoholischen Gärung. *Arch. Physiol.*, 1912, Suppl., 1-396.
- RUBNER, M. 1924 Die Beziehung des Kolloidalzustandes der Gewebe für den Ablauf des Wachstums. *Biochem. Zsch.*, 148, 187-221.
- RYDER, J. A. 1893a The correlations of the volumes and surfaces of organisms. *Contrib. Zool. Lab. Univ. Penna.*, 1, 1-36.
- RYDER, J. A. 1893b The growth of *Euglena viridis* when constrained principally to two dimensions of space. *Contrib. Zool. Lab. Univ. Penna.*, 1, 37-50.
- SCHAUDINN, F. 1895 Plastogamie bei Foraminiferen. *Sitzungsb. Ges. naturf. Freunde Berlin*, 1895, 179-190.
- SCHAUDINN, F. 1896 Ueber die Copulation von *Actinophrys sol* Ehrbg. *Sitzungsb. preus. Akad. Wiss. Berlin*, 1896, 83-89.
- SCHAUDINN, F. 1903 Untersuchungen über die Fortpflanzung einiger Rhizopoden. (Vorl. Mitt.) *Arb. a. d. kais. Gesundheitsamt*, 19, 547-576.
- SCHMALHAUSEN, I., und BORDZILOWSKAJA, N. 1926 Studien über Wachstum und Differenzierung. II. Die individuelle Wachstumskurve der Bakterien. *Arch. Entw. Mech.*, 107, 672-678.
- SCHMALHAUSEN, I., und SYNGAJEWSKAJA, E. 1925 Studien über

- Wachstum und Differenzierung. I. Die individuelle Wachstumskurve von *Paramecium caudatum*. Arch. Entw. Mech., 105, 711-717.
- SCHULTZE, M. 1863 Das Protoplasma der Rhizopoden und der Pflanzenzellen. Leipzig. 68S.
- SELIBER, G., und KATZNELSON, R. 1929 Der Einfluss der Zusammensetzung des Nährbodens auf das Gewicht und den osmotischen Wert der Hefezelle. Protopl., 7, 204-231.
- SHOUP, C. S. 1929 The respiration of luminous bacteria and the effect of oxygen tension upon oxygen consumption. Jour. Gen. Physiol., 13, 27-45.
- SIEBOLD, C. T. 1851 Ueber die Conjugation des Diplozoon paradoxum, nebst Bemerkungen über den Conjugations-Process der Protozoen. Zsch. wiss. Zool., 3, 62-68.
- SIMPSON, J. Y. 1901 Observations on binary fission in the life-history of ciliata. Proc. Roy. Soc. Edinb., 23, 401-421.
- SIMPSON, J. Y. 1902 The relation of binary fission to variation. Biometrika, 1, 400-407.
- SLATOR, A. 1913 The rate of fermentation by growing yeast cells. Biochem. Jour., 7, 197-203.
- SLATOR, A. 1921 Yeast crops and the factors which determine them. Jour. Chem. Soc., 119, i, 115-131.
- SMITH, G. 1903 Actinosphaerium Eichorni. A biometrical study in the mass relations of nucleus and cytoplasm. Biometrika, 2, 241-254.
- SOKOLOFF, B. 1924 Das Regenerationsproblem bei Protozoen. Arch. Prot., 47, 143-252.
- SONDHEIM, M. 1916 Ueber Actinophrys oculata Stein. Arch. Prot., 36, 55-65.
- SOSNOWSKI, J. 1899 Relations entre le noyau et le protoplasme chez les protozoaires. Trav. Lab. Zoot. Varsovie, 20, 1-47.
- SPALLANZANI, L. 1803 Mémoires sur la respiration. Traduits en Français par J. Senebier. Genève. 373 pp.
- SPEK, J. 1920 Experimentelle Beiträge zur Kolloidchemie der Zellteilung. Koll. Beih., 12, 1-91.
- SPENCER, HERBERT 1866 The principles of biology. 2 vols. New York.
- SPENCER, HOPE 1924 Studies on a pedigree culture of *Paramecium calkinsi*. Jour. Morph. Physiol., 39, 543-551.
- STEIN, F. 1854 Die Infusionsthier auf ihre Entwicklungsgeschichte untersucht. Leipzig.
- STERN, C. 1924 Untersuchungen über Acanthocystideen. Arch. Prot., 48, 436-491.
- STOCKING, R. J. 1915 Variation and inheritance in abnormalities occurring after conjugation in *Paramecium caudatum*. Jour. Exp. Zool., 19, 387-449.

- STOLC, A. 1906 Plasmodiogenie, eine Vermehrungsart der neidersten Protozoen. Nach den Untersuchungen an mehrkernigen Formen der *Amoeba proteus*. Arch. Entw. Mech., 21, 111-125.
- STOLC, A. 1910 Ueber kernlose Individuen und kernlose Teile von *Amoeba*. Arch. Entw. Mech., 29, 152-168.
- STOLTE, H. A. 1922 Der Einfluss der Umwelt auf Macronucleus und Plasma von *Stentor coeruleus* Ehrbg. Ein experimenteller Beitrag zur Frage der Kernplasmabeziehungen. Arch. Prot., 45, 344-389.
- STOLTE, H. A. 1924 Morphologische und physiologische Untersuchungen an *Blepharisma undulans* Stein. Arch. Prot., 48, 245-301.
- TALIAFERRO, L. G. 1925 Infection and resistance in bird malaria, with special reference to periodicity and rate of reproduction of the parasite. Amer. Jour. Hyg., 5, 742-789.
- TALIAFERRO, L. G. 1928 Return to normal of the asexual cycle in bird malaria after retardation by low temperatures in vitro. Jour. Prev. Med., 2, 525-541.
- TALIAFERRO, W. H. 1923 A study of size and variability, throughout the course of "pure line" infections, with *Trypanosoma lewisi*. Jour. Exp. Zool., 37, 127-168.
- TALIAFERRO, W. H. 1924 A reaction product in infections with *Trypanosoma lewisi* which inhibits the reproduction of the trypanosomes. Jour. Exp. Med., 39, 171-190.
- TANNER, F. W. 1928 Bacteriology, a text-book of microorganisms. New York. 548 pp. p. 50.
- THOMPSON, D. W. 1917 On Growth and Form. Cambridge. 793 pp.
- UGATA, T. 1926 Biochemical studies on the growth of *Paramecium*. II. The effect of splitting products of nucleic acid upon the division rate in *Paramecium caudatum*. Jour. Biochem., 6, 451-464.
- UJIHARA, K. 1914 Studien über die Amöbendysenterie. (I. Mitteilung) Zsch., Hyg., 77, 417-426.
- UNGER, W. B. 1926 The relationship of rhythms to nutrition and excretion in *Paramecium*. Jour. Exp. Zool., 43, 373-412.
- URBANOWICZ, K. 1927 Gurwitschs mitogenetische Strahlung an Paramazienteilungen geprüft. Arch. Entw. Mech., 110, 417-426.
- VANWISSELINGH, C. 1908 Zur Physiologie der Spirogyrazelle. Bot. Cbl., Beih., 24, 133-210.
- VANWISSELINGH, C. 1914 Ueber den Nachweis des Gerbstoffes in der Pflanze und über seine physiologische Bedeutung. Bot. Cbl. Beih., 32, 155-217.
- VANWISSELINGH, C. 1920 Ueber Variabilität and Erbllichkeit. Zsch. ind. Abst. Vererb., 22, 65-126.
- VAY, F. 1910 Studien über die Strukturverhältnisse von Bakterien mit Hilfe von farbehaltigen Nährboden. Cbl. Bakt., 55, i, 193-208.

- VERNON, H. M. 1895 The respiratory exchange of the lower marine invertebrates. *Jour. Physiol.*, 19, 18-70.
- VERWORN, M. 1891 Die physiologische Bedeutung des Zellkerns. *Arch. gesam. Physiol.*, 51, 1-118.
- VERWORN, M. 1900 Biologische Protisten-Studien. II. *Zsch. wiss. Zool.*, 50, 443-468.
- VIEWEGER, T. 1923 Les rapports entre le développement des bactéries et des protozoaires. *Jour. de physiol.*, 21, 700-703.
- VIEWEGER, J. 1925 Recherches sur l'inanition de Colpidium Colpoda Ehrbg. *Arch. de biol.*, 34, 479-506.
- WACHENDORFF, T. 1912 Der Gaswechsel von Colpidium colpoda. *Zsch. allg. Physiol.*, 13, 105-110.
- WALLENGREN, H. 1902 Inanitionsercheinungen der Zelle. Untersuchungen an Protozoen. *Zsch. allg. Physiol.*, 1, 67-126.
- WARBURG, O. 1923 Versuche an überlebenden Carcinomgewebe (Methoden). *Biochem. Zsch.*, 142, 317-333.
- WARD, H. M. 1895 On the biology of Bacillus ramosus (Fraenkel). a Schizomycete of the River Thames. *Proc. Roy. Soc.*, 58, 265-468.
- WATTERS, F. A. 1912 Size relationships between conjugants and non-conjugants in Blepharisma undulans. *Biol. Bull.*, 23, 195-212.
- WENDELSTADT und FELLMER, T. 1910 Einwirkung von Kaltblüterspassagen auf Nagana- und Lewisi- Trypanosomen. II. Mitteilung. *Zsch. Immunitätsf.*, 5, 337-348.
- WETZEL, A. 1925 Zur Morphologie und Biologie von Raphidocystis infestans n. sp., einem temporär auf Ciliaten parasitierenden Heliozoon. *Arch. Prot.*, 53, 135-182.
- WILDIERS, E. 1901 Nouvelle substance indispensable au développement de la levûre. *Cellule*, 18, 311-333.
- WILSON, W. J. 1906 Pleomorphism, as exhibited by bacteria grown on media containing urea. *Jour. Path.*, 11, 394-404.
- WOODRUFF, L. L. 1908 The life cycle of Paramecium when subjected to a varied environment. *Amer. Nat.*, 42, 520-526.
- WOODRUFF, L. L. 1911 The effect of excretion products of Paramecium on its rate of reproduction. *Jour. Exp. Zool.*, 10, 557-581.
- WOODRUFF, L. L. 1911 Two thousand generations of Paramæcium. *Arch. Prot.*, 21, 263-266.
- WOODRUFF, L. L. 1913 Cell size, nuclear size and the nucleo-cytoplasmic relation during the life of a pedigreed race of Oxytricha fallax. *Jour. Exp. Zool.*, 15, 1-22.
- WOODRUFF, L. L. 1917 The influence of general environmental conditions on the periodicity of endomixis in Paramecium aurelia. *Biol. Bull.*, 33, 437-462.
- WOODRUFF, L. L. 1921 The present status of the long-continued cul-

- ture of *Paramecium aurelia* at Yale University. *Proc. Nat. Acad. Sci.*, 7, 41-44.
- WOODRUFF, L. L. 1925 The physiological significance of conjugation and endomixis in the infusoria. *Amer. Nat.*, 59, 225-249.
- WOODRUFF, L. L. 1928 Further studies on the life history of *Blepharisma undulans*. *Proc. Soc. Exp. Biol. Med.*, 25, 683-684.
- WOODRUFF, L. L. 1929 Thirteen thousand generations of *Paramecium*. *Proc. Soc. Exp. Biol. Med.*, 26, 707-708.
- WOODRUFF, L. L., AND BAITSELL, G. A. 1911 The temperature coefficient of the rate of reproduction of *Paramæcium aurelia*. *Amer. Jour. Physiol.*, 29, 147-155.
- WOODRUFF, L. L., and ERDMANN, R. 1914 A normal periodic reorganization process without cell fusion in *Paramecium*. *Jour. Exp. Zool.*, 17, 425-516.
- YOCOM, H. B. 1928 The effect of the quantity of culture medium on the division rate of *Oxytricha*. *Biol. Bull.*, 54, 410-416.
- YOUNG, D. B. 1922 A contribution to the morphology and physiology of the genus *Uronychia*. *Jour. Exp. Zool.*, 36, 353-395.
- YOUNG, R. T. 1917 Experimental induction of endomixis in *Paramecium aurelia*. *Jour. Exp. Zool.*, 24, 35-53.
- YOUNG, R. T. 1918 The relation of rhythms and endomixis: their periodicity and synchronism in *Paramecium aurelia*. *Biol. Bull.*, 35, 38-48.
- ZOETHOUT, W. D. 1899 On some analogies between the physiological effects of high temperature, lack of oxygen, and certain poisons. *Amer. Jour. Physiol.*, 2, 220-242.
- ZWEIBAUM, J. 1912 La conjugaison et la différenciation sexuelle chez les Infusoires. V. Les conditions nécessaires et suffisantes pour la conjugaison du *Paramæcium caudatum*. *Arch. Prot.*, 26, 275-393.
- ZWEIBAUM, J. 1922 *Richerche sperimentali sulla coniugazione degli Infusori. I. Influenza della coniugazione sull' assorbimento dell' O₂ nel Paramæcium caudatum.* *Arch. Prot.*, 44, 99-114.
- ZUELZER, M. 1904 Beiträge zur Kenntnis von *Diffugia urceolata* Carter. *Arch. Prot.*, 4, 240-295.

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AS ILLUSTRATED IN
UNICELLULAR ORGANISMS

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