Chapter IV

ON THE MECHANISM OF COMPETITION IN YEAST CELLS

(1) No mathematical theories can be accepted by biologists without a most careful experimental verification. We can but agree with the following remarks made in *Nature* (H. T. H. P. '31) concerning the mathematical theory of the struggle for existence developed by Vito Volterra: "This work is connected with Prof. Volterra's researches on integro-differential equations and their applications to mechanics. In view of the simplifying hypothesis adopted, the results are not likely to be accepted by biologists until they have been confirmed experimentally, but this work has as yet scarcely begun." First of all, very reasonable doubts may arise whether the equations of the struggle for existence given in the preceding chapter express the essence of the processes of competition, or whether they are merely empirical expressions. everybody remembers the attempt to study from a purely formalistic viewpoint the phenomena of heredity by calculating the likeness between ancestors and descendants. This method did not give the means of penetrating into the mechanism of the corresponding processes and was consequently entirely abandoned. In order to dissipate these doubts and to show that the above-given equations actually express the mechanism of competition, we shall now turn to an experimental analysis of a comparatively simple case. It has been possible to measure directly the factors regulating the struggle for existence in this case, and thus to verify some of the mathematical theories.

Generally speaking, biologists usually have to deal with empirical equations. The essence of such equations is admirably expressed in the following words of Raymond Pearl ('30): "The worker in practically any branch of science is more or less frequently confronted with this sort of problem: he has a series of observations in which there is clear evidence of a certain orderliness, on the one hand, and evident fluctuations from that order, on the other hand. What he obviously wishes to do . . . is to emphasize the orderliness and minimize the fluctuations about it.... He would like an expression, exact if possible, or, failing that, approximate, of the law if there be one. This means a mathematical expression of the functional relation between the variables....

"It should be made clear at the start that there is, unfortunately, no methods known to mathematics which will tell anyone in advance of the trial what is either the correct or even the best mathematical function with which to graduate a particular set of data. The choice of the proper mathematical function is essentially, at its very best, only a

combination of good judgment and good luck. In this realm, as in every other, good judgment depends in the main only upon extensive experience. What we call good luck in this sort of connection has also about the same basis. The experienced person in this branch of applied mathematics knows at a glance what general class of mathematical expression will take a course, when plotted, on the whole like that followed by the observations. He furthermore knows that by putting as many constants into his equation as there are observations in the data he can make his curve hit all the observed points exactly, but in so doing will have defeated the very purpose with which he started, which was to emphasize the law (if any) and minimize the fluctuations, because actually if he does what has been described he emphasizes the fluctuations and probably loses completely any chance of discovering a law.

"Of mathematical functions involving a small number of constants there are but relatively few.... In short, we live in a world which appears to be organized in accordance with relatively few and relatively simple mathematical functions. Which of these one will choose in starting off to fit empirically a group of observations depends fundamentally, as has been said, only on good judgment and experience. There is no higher guide" (pp. 407-408).

(2) We are now confronted by an entirely different problem which has often arisen in other domains of exact science and which represents the next step after establishing the first empirical relations with out any mathematical theory. The problem is that *from clearly formulated hypotheses which appear probable on the ground of collected experimental material certain mathematical consequences are deduced, connecting the experimental values in equations accessible to experimental verification*. As a result a mathematical theory of the phenomena observed in a given field of science is obtained. The equations of the struggle for existence are just such theoretical equations that have been deduced from hypotheses about potential coefficients of multiplication of species and the participation of these species in the utilization of a limited opportunity for growth. The verification of such a theoretical equation of the struggle for existence may be reduced to the following: (1) we must determine experimentally the potential coefficients of multiplication of the species; (2) by means of a direct study of the factors limiting growth we must evaluate the degree of influence of one species on the opportunity for growth of another, i.e., the coefficients of the struggle for existence; (3) by inserting all these values into a theoretical equation we must obtain a complete agreement with the experimental data, if our mathematical theory connects correctly the coefficients furnished by experimentation. It seems to us that these three steps of verifying our theoretical equations must be somewhat modified, taking

into account the complicated situation in the competition between two species for a common place in the microcosm. We proceed as follows: (1) having determined the potential coefficients of multiplication b_1,b_2 and the maximal biomasses K_1, K_2 we pass on at once to (3), i.e., on the basis of the experimental data, taking our equations as purely empirical expressions or, in other terms, considering that they must *describe* the values observed, we calculate those empirical coefficients of the struggle for existence with which the equations *actually describe* the experimental data. It is only then that we pass to (2), and *compare these empirically found coefficients of the struggle for existence with those which are to be expected from , direct study of the factors limiting growth. If the empirical coefficients coincide with the theoretical ones, the correctness of the mathematical theory will be proved*.

This mode of verification of the mathematical theory has been adopted by us because the coincidence of theoretical coefficients with the empirical ones is but rarely to be expected. Such a rare case representing, most likely, rather an exception than a rule is described in this chapter. This small probability of a coincidence of the coefficients is connected with the fact that usually the growth of populations depends on numerous factors, many of which (e.g., waste-products) we often cannot specify exactly, and the influence of one species on the opportunity of growth of another under these conditions is realized in a very complicated manner. Hence the empirical coefficients of the struggle for existence, calculated by an equation which in certain cases has already been verified, can serve as a guide for the study of the very mechanism of the influence of one species on the growth of another.

II

(1) To verify our differential equations of the struggle for existence we had recourse to populations of yeast cells. Yeast cells were cultivated in a liquid nutritive medium, where they were nourished by various substances dissolved in water and excreted certain waste products into the surrounding medium. Owing to the considerable practical importance of yeast for the food industry a great number of papers has been devoted to investigation of its growth, and although the majority deals with purely practical questions that do not at present interest us, nevertheless it is pretty well ascertained what substances yeast requires for its growth, and what is the chemical composition of the waste-products it excretes.

For the study of competition we took two species of yeast: (1) a pure line of common yeast, *Saccharomyces cerevisiae* stock XII, received from the Berliner Gahrungsinstitut, and (2) a pure line of the yeast *Schizosaccharomyces kephir,* cultivated in the Moscow Institute of the

Alcohol Industry and obtained from Dr. Pervozvansky.[1] Both these species can grow under anaerobic conditions as well as when oxygen is accessible. It is very well known that the processes of life activity are connected with a continuous consumption of energy which is supplied by certain chemical reactions. In the case when the growth of yeast proceeds in the absence of oxygen it is the decomposition of sugar into alcohol and carbon dioxide which furnishes the available energy, and in the nutritive medium there takes place a considerable accumulation of the waste product ethyl alcohol. If we alter the conditions of cultivation and allow a direct access of oxygen to the growing yeast cells, although fermentation will still continue, a part of the available energy (different for different species) will be furnished by oxidation of sugar into carbon dioxide. In the commercial utilization of yeast, when it is desirable to accumulate alcohol in the culture, yeast is grown nearly without oxygen. But if alcohol is not needed and the object is to obtain a great quantity of yeast cells themselves, an intensive aeration of the growing culture is carried on, which leads to an enormous increase of oxidation processes. The yeast *Saccharomyces cerevisiae* as well as *Schizosaccharomyces kephir* produces alcoholic fermentation, and both can obtain a part of the available energy by oxidation, but they differ from one another in the relative intensities of the oxidation and fermentation processes. Common yeast, *Saccharomyces cerevisiae,* develops well in the absence of oxygen as for it fermentation is a powerful source of energy. It continues mainly to ferment even in the presence of oxygen (when cultivated in Erlenmeyer Hasks without aeration) and utilizes the oxidation process only to a very small extent. As regards our species of *Schizosaccharomyces* it grows very slowly under anaerobic conditions. However, when oxygen is available it has recourse to this source of energy; its rapidity of growth increases and it approaches *Saccharomyces* in its properties. Hence, *Saccharomyces* represents a species with distinctly expressed fermentative capacities, whilst *Schizosacchuromyces is* a species of a more oxidizing type. By mixing these species we obtain a very interesting situation for studying the competition between species in different conditions of environment.

---

[1] We began our experiments with yeast in 1930. The first group of experiments on competition between species was made in September-December, 1931, and appeared in the *Journal of Experimental Biology* (Gause, '32b). These experiments were extended and repeated in September-December, 1932. Their results coincided completely with the data of 1931. Later it appeared that the yeast culture kept in the Museum of the Institute of the Alcohol Industry under the name of "*Schizosaccharomyces kephir*" and used under the same name in our experiments, has been incorrectly determined by the specialists of the Museum and that it belonged to another species. The culture consists of oval, budding yeast cells much more minute than *Saccharomyces cerevisiae* and producing an alcoholic fermentation. An exact systematic determination presented extreme difficulty and seemed not to be indispensable, as this culture is kept in the Museum and can be obtained thence under the name of "*Schizosaccharomyces kephir*".

---

(2) We cultivated yeast in a sterilized nutritive medium which was prepared in the following manner: 20 gr. of dry pressed beer-yeast were mixed with 1 liter of distilled water, boiled for half an hour in a Kochs boiler, and then filtered through infusorial earth. Five per cent of sugar was added to this mixture, and then the medium was sterilized in an autoclave. A medium of such a type is very favorable for the growth of yeast, because the decoction contains all the nutritive substances required. The only disadvantage is our ignorance of the exact chemical composition of this medium. Therefore each series of experiments must be made with a solution of the very same preparation. But on the whole this method enables one to have sufficiently standardized conditions for cultivation.

The nutritive medium was sterilized in a large flask and then aseptically poured into small vessels for cultivation. These vessels were previously sterilized by dry heat (by heating to 180° for three hours). This method has many advantages as compared with the direct sterilization of the nutritive medium in small culture vessels. The fact is that when a liquid is heated in glass vessels in an autoclave, even if the best kind of glass be used, the latter can somewhat alter the composition of the nutritive liquid. This produces a considerable variation in the initial conditions of separate microcosms. The vessels used for cultivation belonged to two types: (1) in experiments with the deficiency in oxygen we used common test tubes with a diameter of 13 mm. Ten cm^3 of nutritive medium were poured into such a tube, the depth of the liquid being about 80 mm. (2) To obtain better aeration, cultures were made in small Erlenmeyer flasks of about 50 mm in diameter, and when 10 cm^3 of nutritive medium were poured in, the liquid reached a depth of 7-8 mm. In these conditions the layer of the liquid was almost ten times thinner than in the test tubes (Fig. 8). The test tubes as well as Erlenmeyer flasks were closed by cotton wool stoppers. The experiments made in the flasks will be described in this book as "aerobic" and those in test tubes as "anaerobic."

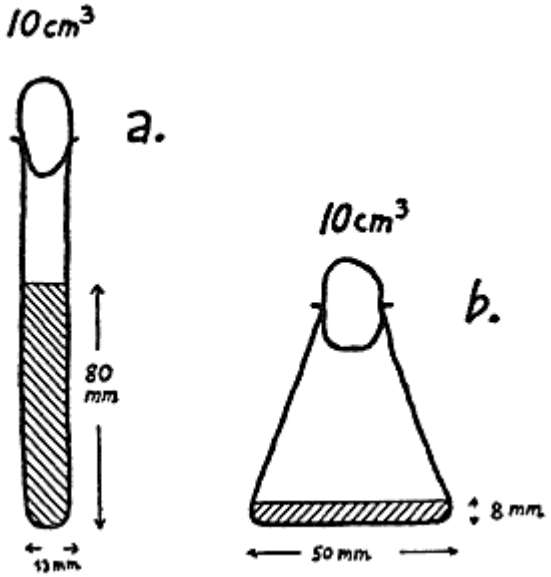

Fig. 8. The vessels for cultivation of yeast: (a) test tube, (b) Erlenmeyer's flask.

(3) An inoculation of yeast cells was made into the sterilized nutritive medium. Special attention was given to the standardization of the inoculating material, for in order to obtain exact and comparable results the inoculating cells had to be in a certain fixed physiological condition. Cells for inoculation were always taken from test tubes where the growth was just finished. For an anaerobic inoculation of *Saccharomyces* cultures 48 hours old (at 28°C) were used, whilst the slow-growing *Schizosaccharomyces* for an anaerobic inoculation was taken at the age of five days at 28°C. Before inoculation the contents of the test tube was shaken, and a fixed number of drops of the liquid was introduced into the nutritive medium by means of a sterilized pipette. It was also necessary that an equal initial quantity of each species or, in other words, equal initial masses should be inoculated. It was found that in anaerobic test tubes intended for inoculation a mass of yeast in a unit of volume of the nutritive liquid is two and a half times smaller in *Schizosaccharomyces* than in *Saccharomyces*. Therefore in order to inoculate an equal initial quantity two drops of uniform suspension of *Saccharomyces* and five drops of *Schizosaccharomyces* were always introduced. In the case of a mixed culture, two drops of the first species plus five drops of the second were taken.[2] We must prepare a perfectly uniform suspension of seed-yeast and the inoculation itself must be carried out rapidly so as to avoid possible errors from a settling of yeast cells in the inoculating pipette. This circumstance was pointed out by Richards ('32) and Klem ('33). All the experiments were carried out in a thermostat at a temperature of 28°C.

[2] A very strict equality of the masses of two species sown is not absolutely necessary. It is only important that the very same quantity of each species should be introduced into the mixed population and into the separately grown culture. This is very easy to do with our mode of inoculation.

(4) After inoculation it was necessary to study the growth of number and mass of yeast cells, and on the other hand to trace and to evaluate the changes in the factors of the medium. The counting of the number of yeast cells per unit of volume does not present any difficulty and for this purpose the Thoma counting chamber is usually employed. In our experiments three test tubes (or flasks) of the same age were taken and a uniform suspension of yeast was made by shaking. One cm^3 of liquid was taken by a pipette from every tube and poured into another clean tube, where the three cm^3 obtained from three tubes were fixed by 3 cm^3 of 20 per cent solution of H_2SO_4. Individual fluctuations of separate cultures were thus neutralized, and a certain "average suspension" from three test tubes was obtained. The material fixed was more or less diluted with water, and then the number of cells per unit of volume was counted in the Thoma chamber. Quite recently Richards ('32) in his interesting paper describes in detail the methods of studying the growth of yeast, there he points out that the counting of the number of yeast cells is a very satisfactory method. As regards the possible sources of error, he indicates the following: (1) the sample placed in the counting chamber is not truly representative of the population sampled; (2) the cells do not settle evenly in the counting chamber. To eliminate these errors it is necessary to take several sample groups from the "average suspension," and to count a great number of squares in the chamber. In our experiments the fixed suspension was carefully mixed before the taking of the sample, a few drops were taken with a pipette, placed in the chamber, and ten squares were counted. Six such sample groups were successively taken, and the total number of counted squares amounted to sixty. Sometimes a lesser number of squares sufficed.

The average number of cells in one large square of a Thoma chamber at the dilution corresponding to the material fixed (i.e., twice thinner than the initial suspension) is given in our tables. It is understood that the counts sometimes were made with considerably stronger dilutions, and they were correspondingly reduced to the accepted standard. A few words must be added concerning the counting of cells in mixed cultures. After a certain amount of practice it is quite easy to distinguish the two species of yeast, as the cells of *Saccharomyces* are much larger than those of *Schizosaccharomyces* and their structure is different.

(5) The numbers of yeast cells belonging to two different species do not allow us to form an idea as to their masses. But it is just the masses of the species that are of particular importance in the processes of the struggle for life. This is because a unit of mass of a given species is usually connected by definite relations with the amount of food consumed or that of the waste-products excreted or generally speaking, with the factors limiting growth. *Therefore the equations of the struggle for existence ought to be expressed in terms of masses of the species concerned* and not in terms of the numbers of individuals, which are connected by more complex relations with the factors limiting growth.

In order to pass on from the number of yeast cells of the first and second species counted at a definite moment to the masses of these species, we must take into account that: (1) the cells of the first species differ in their average volume from those of the second, (2) this average volume of the cell in each species can change in the course of growth of the culture. (Richards ('28b) showed that the average size of a cell of *Saccharomyces cerevisiae* is different at different stages of growth), and (3) the species can be of different specific weight. Therefore, by multiplying the volume of all the cells of a definite species at a given moment of time by their specific weight, we shall obtain the weight of the given organisms enabling us to judge of their mass. Assuming for the sake of simplification that the cells of our yeast species are near to one another in their specific weight, we can measure the volumes occupied by each species of yeast cells in order to obtain an idea of the masses of these cells.

(6) The volume of yeast was determined by the method of centrifugation. The fluid from the test tubes or flasks with the counted number of yeast cells was centrifuged for one minute in a special tube placed in an electric centrifuge making 4000 revolutions per minute (usually in portions of 10 cm^3 each). The liquid was then poured off and the yeast cells that had settled on the bottom were shaken up with the small quantity of the remaining liquid. The mixture thus obtained was transferred by means of a pipette into a short graduate glass tube of 3.5 mm in diameter. The mixture in the graduated tube was again centrifuged for 1.5 minutes, and then the volume of the sediment was rapidly measured with the aid of a magnifying glass. To avoid errors connected with the different degree of compression of the yeast in different cases, the quantity of the mixture poured into the short graduated tube was always such that the sediment did not exceed ten divisions of the graduated tube and, if necessary, the secondary centrifugation was made by several doses. The volume of yeast occupying one division of the graduated tube was taken for a unit.

The centrifugation method may be criticized as, according to Richards

('32), even in employing the super-centrifuge of Harvey one can not succeed in obtaining a solid packing of the cells, and interstices remain between them. If we draw our attention to the fact that the size of the cells changes in the process of the growth of the culture, and that in mixed populations of the two species we have to deal with cells of different sizes then, theoretically, this must lead to a very different degree of packing of the cells in different cases, and the volume of the cells determined by centrifugation apparently does not yet allow us to judge of their mass. However, the measurements, some of which will be given further on, show that the errors which actually arise are small, and that the centrifugation method is perfectly reliable for our purposes.

In the study of the population growth of yeast it is difficult to carry on observations upon the very same culture, as it is urgent to strictly maintain the sterility of the medium and to avoid injury to the cells. For this reason a great number of test tubes were inoculated at the beginning of the experiment; at certain fixed moments determinations were made upon a group of test tubes which were then put aside and further determinations were made upon new tubes.

III

(1) Having examined the technical details of cultivation of yeast cells we can now pass to the problem which interests us first of all: how does the multiplication of the yeast proceed in a microcosm with a limited amount of energy, and what are the factors which check the growth of the population? Let us begin by examining the kinetics of growth under anaerobic conditions. Figure 9 represents the growth of volume of the yeast *Saccharomyces cerevisiae*, according to the data of one of our experiments in 1930. It is clearly seen that the volume increases slowly at first, then faster, and finally slows down on approaching a certain fixed value. The curve of growth is asymmetrical, i.e., its concave part does not represent a reverse reflection of the convex one (Richards, '28c, Gause, '32a). The first of them is somewhat steep but the second comparatively inclined. This asymmetry is, however, not sharply expressed, and it can be neglected if we analyze the growth in a first approximation to reality.

In experiments of this type immediately after the yeast cells are inoculated an intensive multiplication begins. There is scarcely any lag-period, or period of an extremely slow initial growth, while the cells adapt themselves to the medium. This is because we used for inoculation fresh yeast cells developed in a medium of an identical composition with those used in the experiment. This circumstance has been pointed out by Richards ('32).

(2) An investigation of the shape of the curve which represents the accumulation of the yeast volume in the population of yeast cells does not enable us to judge what factors control the growth of the population and limit the accumulation of the biomass. The fact that the growth curve is S-shaped and resembles the well-known autocatalytic curve does not prove at all that the phenomenon we are studying has anything in common with autocatalysis. The question of the basic nature of the yeast growth in a limited microcosm can be elucidated only by means of specially arranged experiments. Such experiments were recently carried out by Richards ('28a) and confirmed by Klem ('33).

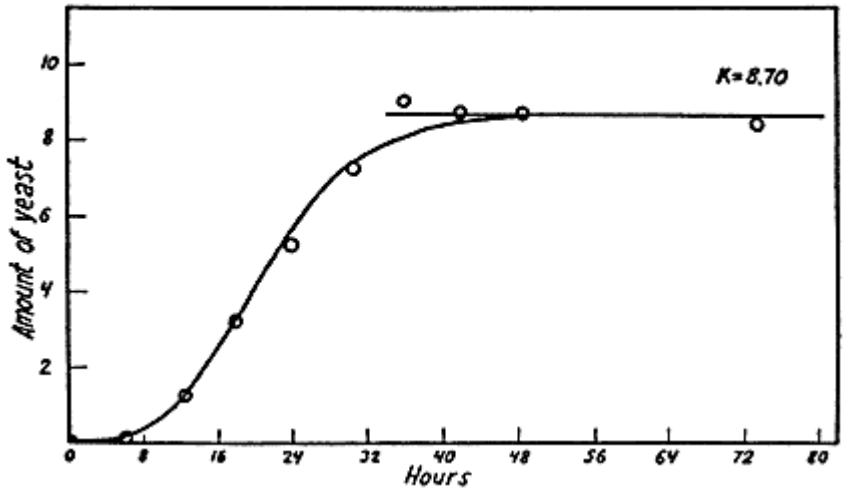

Fig. 9. Growth in volume of the yeast *Saccharomyces cerevisiae*. From Gause ('32a).

We have already mentioned that the process of multiplication of organisms is potentially unlimited. It follows the law of geometric increase, and limitations are here introduced only by the external forces. In the case of yeast this circumstance was noted by Slator ('13), and recently Richards carefully verified it in the following manner. A control culture after the inoculation of yeast was left to itself, and the growth of the number of cells in this culture followed a common S-shaped curve and then stopped. In an experimental culture a change of the medium was made at very short intervals of time (every 3 hours). Here the conditions were all the time maintained constant and favorable for growth. Under these conditions the multiplication of yeast followed the law of geometric increase: in every moment of time the increase of the population constituted a certain definite portion of the size of the population. The relative rate of growth (i.e., the rate of growth per unit of population) remained constant all the time, or in other words there was no autocatalysis here. Figure 10 represents the data of Richards. To the left are shown the growth curves of the number of cells per unit of volume: the S-shaped curve in the control

culture, and the exponentially increasing one with continuously renewed medium. One can in the following manner be easily convinced that the exponentially increasing curve corresponds to the geometric increase: if against the absolute values of time we plot the logarithms of cell numbers, a straight line will be obtained (see the right part of Figure 10 taken from Richards). As is well known this is a characteristic property of a geometric increase. Nearly the same results were recently obtained by Klem ('33).

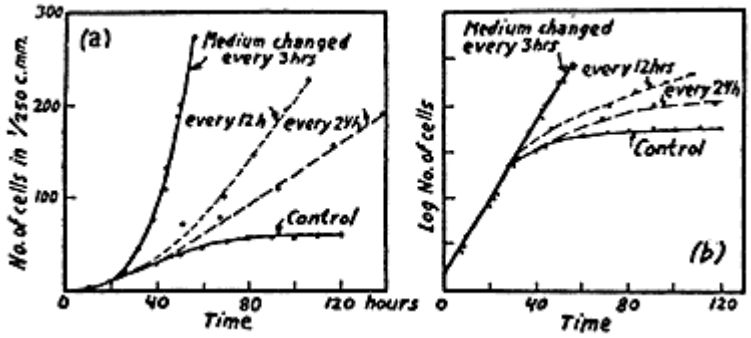

Fig. 10. Growth curves of the yeast *Saccharomyces cerevisiae*. (a) Growth of the number of cells. (b) The same, plotted on logarithmic scale. From Richards ('28a).

The experiments made by Richards show clearly that the growth of the yeast population is founded on a potential geometric multiplication of yeast cells (b_1N_1), but the latter can not be completely realized owing to the limited dimensions of the microcosm and consequently to the limited number of places (K). As a result the geometric increase becomes S-shaped. It is easy to see that the experimentation has led us to the very same assumptions that are at the bottom of Pearl's logistic equation of growth (see Chapter III, equations (8) and (9)). This equation is one that gives us the S-shaped curve starting from the point that growth depends on a certain potential geometric increase which at every moment of time is realized only in a certain degree depending on the unutilized opportunity for growth at that moment.

In the equation of Pearl the unutilized opportunity for growth is expressed in terms of the population itself, i.e., as the relative number of the still vacant places. This presents a great advantage as we shall see later on. The unutilized opportunity of growth often depends on various factors, and to translate the number of "still vacant places" into the language of these factors may become a very difficult task.

(3) Let us now analyze this problem. What is the nature of those factors of the environment which depress the growth of the yeast population and finally stop it? Of course they may be different in various cases, and we have in view only our conditions of cultivation.

The nature of the factors limiting growth in such an environment has been explained mainly by the investigations of Richards. When the growth of yeast ceases in a test tube under almost anaerobic conditions, there still exists in the nutritive medium a considerable amount of sugar and other substances necessary for growth. A simple experiment made by Richards ('28a) is convincing: if at the moment when the growth ceases in the microcosm yeast cells from young cultures are introduced, they will give a certain increment and the population will somewhat increase. Consequently, there is no lack of substances required for growth. The presence of a considerable quantity of sugar at the moment when the growth ceases has been chemically established, and in our experiments this is even more apparent than in those of Richards, as our initial concentration of sugar was 5 per cent and his only 2 per cent.

If the growth ceases before the reserves of food and energy have been exhausted we must evidently seek an explanation in some kind of changes in the environment. This question has been studied by Richards and led him to conclude that the decisive influence here is the accumulation of ethyl alcohol. As has already been mentioned, when yeast cells grow in test tubes under almost anaerobic conditions the decomposition of sugar into alcohol and carbon dioxide serves them as a source of energy. Sugar is almost entirely utilized to obtain the available energy, and serves as food only in a very slight degree. As result a considerable amount of alcohol accumulates in the nutritive medium, which corresponds pretty well to the amount of sugar consumed. Curves of such accumulation of alcohol, taken from the paper of Gause ('32b), are represented in Figure 11. Here are given the results of two experiments made in test tubes, but on a nutritive medium of somewhat different concentration. In both cases a certain time after the experiment was begun the accumulation of alcohol (and, consequently, the consumption of sugar) proceeds almost in proportion to the increase of the volume of yeast. In other terms, a proportionality exists between the metabolism of the yeast cells and the growth of their volume. Later on, conditions arise in which the growth of the yeast ceases, but alcohol continues to accumulate. Therefore, at the moment when the growth ceases there are still unutilized resources of sugar in the medium. The life activity of the yeast cells and the accumulation of alcohol continue after the biomass has ceased growing.

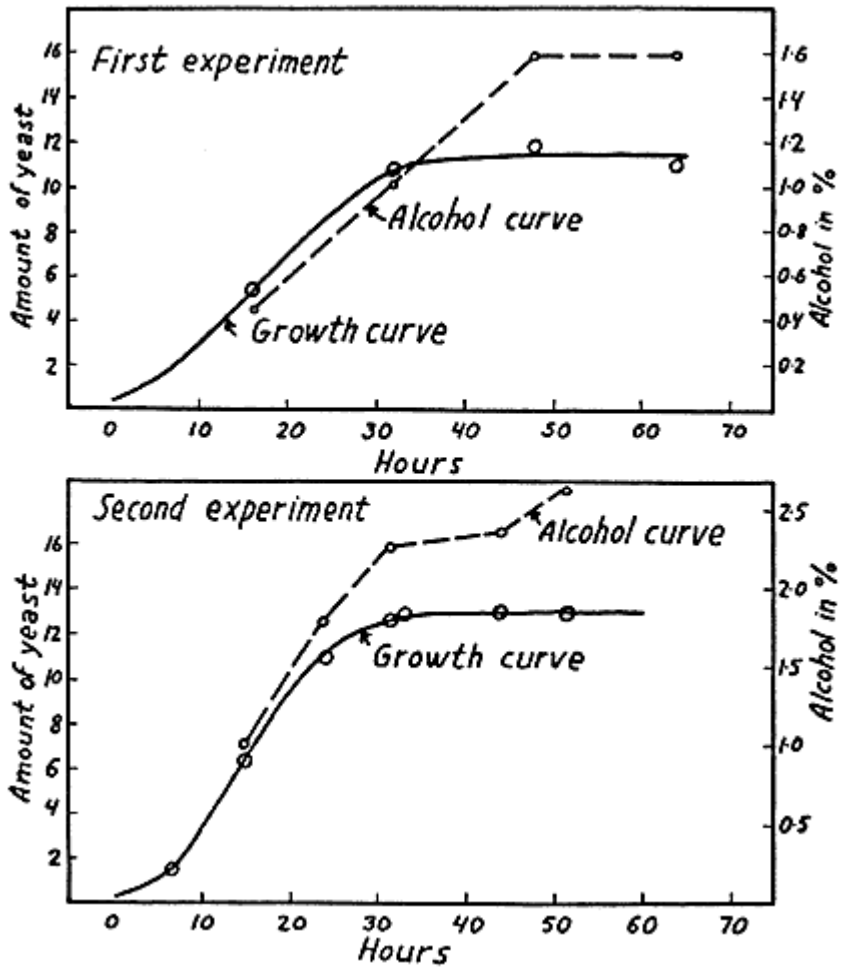

Fig. 11. The growth in volume and accumulation of alcohol in *Saccharomyces cerevisiae* in test tubes. From Gause ('32b).

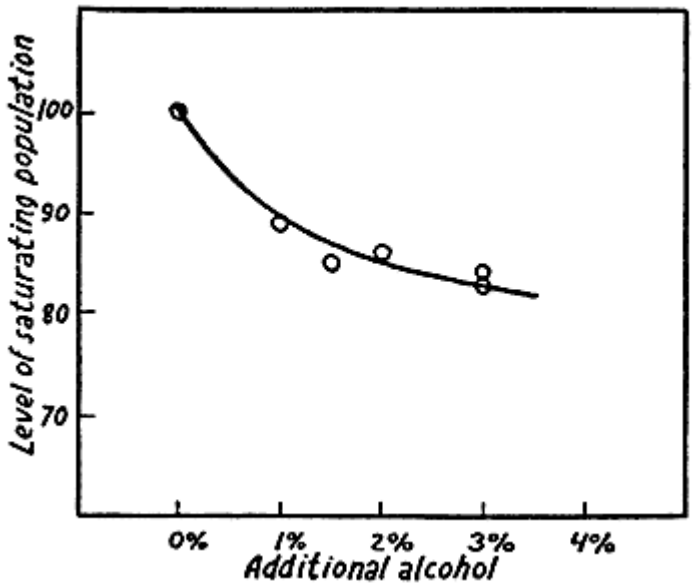

Fig. 12. The effect of additional alcohol upon the level of saturating population in *Saccharomyces cerevisiae* in test tubes.

The microscopical study of the population of yeast cells made by Richards at the moment when growth was ceasing, has shown the following facts. The yeast cells continue to bud actively, but as soon as a bud separates from the mother cell it perishes. In this way, unfavorable chemical changes in the medium destroy the most sensitive link in the population, and lead to a cessation of its growth. According to Richards ('28a) the accumulating ethyl alcohol is just the factor which kills the young buds and inhibits the growth of the population. He showed this experimentally: with an addition of 1.2 per cent of ethyl alcohol to the nutritive medium, the maximal yield of population was 65 per cent from that of the control population (acidity kept constant). Therefore, with the additional alcohol the critical concentration of waste products at which growth ceases was reached with a smaller quantity of accumulated yeast volume.

These data were criticized by Klem ('33) who carried out experiments with wort and not with William's synthetic medium, which Richards worked upon. Klem did not obtain any depression of growth by adding a small quantity of alcohol corresponding to the quantity which is usually accumulated in his cultures at the moment when the growth ceases. According to Klem, it is only at a concentration above 3 per cent that alcohol begins to depress growth, and only concentrations of about 7 per cent have a distinctly hindering influence. The experiments which I have made with yeast decoction and 5 per cent sugar confirm the data of Richards and not those of Klem. Figure 12 presents the results of several experiments. The level of the maximal population in the control was taken as 100, and the levels of the maximal populations in the cultures with this or that per cent of alcohol (added before the yeast was sown, all other conditions being equal) were expressed in per cent from the population level in the control. This figure shows that even 1 per cent of alcohol in our conditions lowers the maximal level of population considerably. As we have already seen (Fig. 11, bottom) at the moment the growth ceases in our cultures the concentration of alcohol is near to 2 per cent (with the usual composition of medium). This concentration is undoubtedly sufficiently high to be responsible for the cessation of growth.

Klem expressed an interesting idea, namely that the cessation of growth is connected with the reaching of a definite relation between the concentration of the waste-products and the nutritive substances, i.e., alcohol and sugar. In other terms, the critical concentration of alcohol checking growth is by no means of an absolute character. With a small concentration of sugar, a comparatively weak concentration of alcohol hinders growth. But if the quantity of sugar be increased, this concentration of alcohol will no longer be sufficient for checking growth which will continue. Klem's opinion is perfectly justified and many experimental data confirm it. But, as he himself remarks, the

ratio alcohol/sugar left at the moment growth ceases, also varies within rather wide limits. (A critical analysis of Figs. 53-54 on pp. 80-81 of his paper ('33) shows that even with concentrations of sugar from 1 to 5 per cent the ratio alcohol/sugar left does not remain constant, and that Klem's calculations are not quite exact.)

(4) All we have said may be resumed thus: under our conditions of cultivation the cessation of growth of the population of yeast cells begins before the exhaustion of the nutritive and energetic resources of the medium. The direct cause of this cessation is the accumulation of ethyl alcohol which kills the most sensitive members of the population—the young buds. This critical concentration of alcohol is not of an absolute character, and in a first approximation we can say that the cessation of growth is connected with the establishment of a definite ratio between the concentrations of waste-products (alcohol) and the nutritive substances (sugar). We now have to answer the question raised earlier: what factors will furnish us with the terminology for expressing the "number of vacant places" or "the unutilized opportunity for growth" in the population of yeast cells under our conditions of cultivation? Since the growth of population ceases with the establishment of a certain ratio alcohol/sugar a thought might appear that we ought to connect the unutilized opportunity for growth somehow with the ratio. However this would be a false deduction from correct premises. We can see at once that we have to deal here with two different things. (1) Should we wish to make a purely theoretical calculation of the level of saturating population m our microcosm, we would certainly be obliged to take into consideration the ratio between the concentrations of alcohol and sugar, and to try to calculate the moment when this ratio attains a definite value. But certainly we should at once have to introduce numerous corrections, as various other factors have also an influence here. (2) The conditions of the problem before us are quite different. *We know beforehand* at what level the population ceases to grow, and what is the corresponding value of different factors of the environment. We wish only for different moments of time preceding the cessation of growth to translate "the unutilized opportunity for growth" into terms of the limiting factor. Such limiting factor is always alcohol destroying the young buds. However considerably other factors of the environment and the condition of the cells themselves should alter the absolute value of the critical alcohol concentration, this does not essentially change the matter. Consequently *"the unutilized opportunity for growth" or "the number of still vacant places" can simply be determined by the difference between the critical concentration of alcohol at the moment of cessation of growth, which is characteristic for the given conditions and established experimentally in every case, and the concentration of alcohol at a given moment of time.*

The accumulation of the yeast volume at the moment of the cessation of growth is everywhere marked by K, and the amount of volume at a given moment is N. Alcohol production per unit of yeast volume is rather constant, and increases somewhat only before growth is checked (see Fig. 11). Taking the alcohol production per unit of yeast volume as a constant for the entire process of growth of the population as the very first approximation to reality, we can easily pass from the given (N) and maximal (K) amount of yeast, through multiplying them by certain coefficients, to the given and critical concentrations of alcohol.

(5) It is easy to see that, while we give up any attempt to discover a certain universal growth equation forecasting the level of the saturating population under any conditions, if we use the logistic equation we express rationally, very simply, and in complete agreement with experimental data, the mechanism of growth of a homogeneous population of yeast cells. The attempts to find universal equations will scarcely lead to satisfactory results, and in any case all this would be too complicated for a mathematical theory of the struggle for existence in a mixed population of two species. One of the leading ideas of this book is that all the quantitative theories of population growth must be only constructed for strictly determined cycles or epochs of growth, within which the same limiting factors dominate and a certain regulating mechanism remains invariable.

Experiments with yeast point also to a very important circumstance in the experimental analysis of populations. All the conditions of cultivation ought to be so arranged that the growth depends distinctly on only one limiting factor. In the case of yeast we must have a sufficiently high concentration of sugar and other necessary substances in the nutritive medium so that the alcohol can in full measure manifest its inhibitory action. As we shall see in the next chapter in experimenting with Protozoa, it is very easy to arrange experiments under such complicated conditions and with the interference of such a great number of various factors that the attempts to discover certain fundamental quantitative relations in the struggle for existence will never have any success.

IV

(1) Our study of the growth of homogeneous populations of yeast cells was only a preparation before we pass on to the investigation of the struggle for existence between two species in a mixed culture. The simplest way to do this is again to begin by an analysis of the kinetics of growth. Let us examine the experiments of 1931. In Table 1 (Appendix) data are given on the anaerobic growth of the volume and of the number of cells in the two species of yeast: *Saccharomyces* and *Schizosaccharomyces,* cultivated separately and in a mixed population

in two independent series of experiments. One hundred and eleven separate microcosms were studied in these two series, and every figure in Table 1 (Appendix) is founded on three observations. Figure 13 represents graphically the growth of the yeast volume. We can see that the growth of *Schizosaccharomyces* under anaerobic conditions is exceedingly slow. Let us note also that its population attains a much lower level than that of *Saccharomyces*. The volume of the mixed population is also smaller than the volume of the pure culture of *Saccharomyces*.

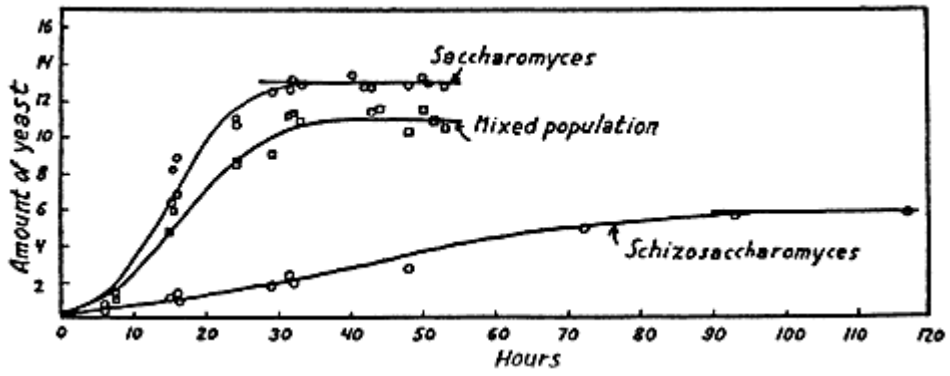

Fig. 13. The growth in volume of *Saccharomyces cerevisiae*, *Schizosaccharomyces kephir* and mixed population in two series of experiments. Anaerobic conditions. From Gause ('32b).

The parts taken up by each of the species in the yeast volume of a mixed culture have been evaluated in the following manner. First of all, a calculation was made of the average number of cells per unit of yeast volume for the separate growth of *Saccharomyces* and *Schizosaccharomyces* (see Appendix, Table 1). It appears that the mean number of cells occupying a unit of yeast volume varies in the course of the growth of the culture, as Richards has already established. However, these variations are not great, and for further calculations average values for the entire cycle of growth can be taken. According to the first series of experiments, in *Saccharomyces* 16.59 cells in a square of a Thoma counting chamber correspond to one unit of yeast volume; in the smaller species *Schizosaccharomyces* there are 57.70 cells in one unit of yeast volume. Starting from these averages, we have calculated the volumes occupied by each species in the mixed population at a given moment, according to the number of cells of each species observed in the mixed population. (In the experiments of 1932 which are given further on we did not use such general averages for our calculations, but started every time from the average number of cells observed at a given moment of time.)

The sum of the calculated volumes of both species in the mixed culture at a given moment should agree with the actual volume of mixed population at this moment determined by the method of centrifugation. In the first series the totals of calculated volumes are somewhat smaller than the volumes actually observed, and we know the causes of this disagreement. In the second series these causes have been eliminated, and the coincidence between the totals of the volumes calculated and the volumes actually observed is a satisfactory one.

(2) Figures 14 and 15 give the curves of the growth of the yeast volume in *Saccharomyces* and *Schizosaccharomyces* cultivated separately and in a mixed population. The curves of the separate growth of each species are expressed with the aid of simple logistic curves of the following type (the details of these calculations are to be found in the Appendix):

$$\frac{dN}{dt} = bN\frac{K-N}{K},$$

where N is yeast volume, t is time, b and K are constants. The fitting of the logistic curves has given us the following values of the parameters for the separate growth of our species (Sp. No. 1 is *Saccharomyces*, No. 2 is *Schizosaccharomyces):*

Maximal volumes: K_1=13.0; K_2 = 5.8

Coefficients of geometric increase:

b_1 = 0.21827; b_2= 0.06069

The calculated coefficients of geometric increase show that per unit of time (one hour) every unit of volume of *Saccharomyces* can potentially give an increase equal to 0.21827 of this unit, and in *Schizosaccharomyces* equal to only 0.06069.

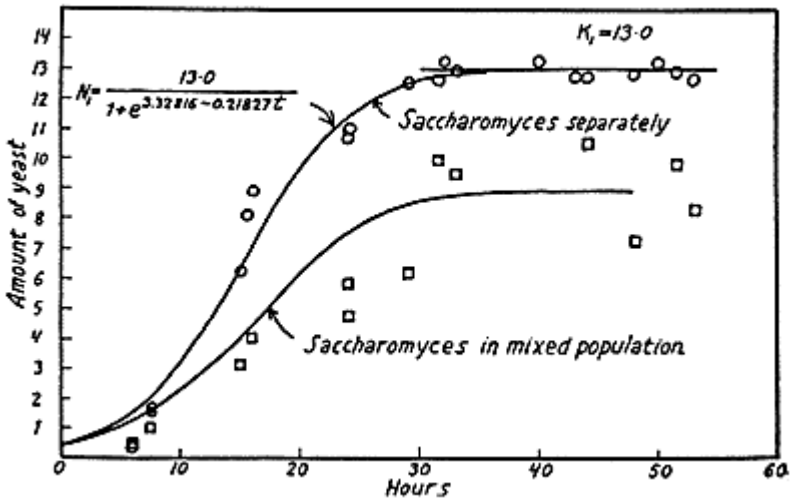

Fig. 14. The growth in volume of *Saccharomyces cerevisiae* cultivated separately and in the mixed population in two series of experiments. Anaerobic conditions. From Gause ('32b).

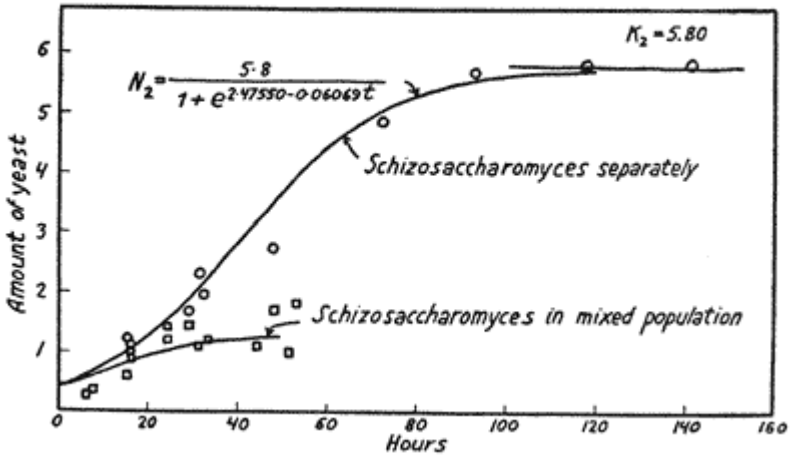

Fig. 15. The growth in volume of *Schizosaccaromyces kephir* cultivated separately and in the mixed population in two series of experiments. Anaerobic conditions. From Gause (32b).

Having obtained in this way the potential coefficients of multiplication of our species (or, which means the same, the coefficients of geometric increase) we must now according to the general plan given at the beginning of this chapter pass on to a calculation of the empirical coefficients of the struggle for existence. In this we start by assuming that the system of equations of competition (see Chap. 3, equations (11) and (12)):

$$\left.\begin{aligned}\frac{dN_1}{dt} &= b_1 N_1 \frac{K_1 - (N_1 + \alpha N_2)}{K_1} \\ \frac{dN_2}{dt} &= b_2 N_2 \frac{K_2 - (N_2 + \alpha N_1)}{K_2}\end{aligned}\right\}$$

actually describes the experimental data. All the values in these equations except the coefficients of the struggle for existence a and b, are known to us. To find the latter let us solve this system of two equations with two unknown values in respect to a and b . We obtain:

$$\alpha = \frac{K_1 - \frac{dN_1 / dt \cdot K_1}{b_1 N_1} - N_1}{N_2}; \quad \beta = \frac{K_2 - \frac{dN_2 / dt \cdot K_2}{b_2 N_2} - N_2}{N_1}$$

The values on the right side of both expressions can easily be calculated from experimental data. Thus in the case of the coefficient a : (1) b_1 and K_1 are known from the curve of separate growth of the first species, (2) N_1 and N_2 or the volumes of the first and second species in a mixed population at a given moment of time (t), can be taken from the graph by measuring the ordinates of the corresponding curves of growth, (3) $\frac{dN_1}{dt}$ represents the rate of growth of the first species in the mixed population, or the increase of volume per unit of time, and can also be easily determined from the graph. It will be sufficient for this to draw a tangent at a given point and to measure $\frac{dN_1}{dt}$ graphically or, better, to use a Richards-Roope ('30) tangent meter for graphical differentiation.[3] As a result we shall obtain the values of the coefficients of the struggle for existence (a and b) *for different points of the curve*, i.e., for different moments of growth: t_1, t_2, etc. The values of the coefficients calculated for different moments are subject to fluctuations, but by using the middle zone of growth sufficiently constant values will be obtained. Thus, the coefficient b in the experiments of 1931 was equal to: 0.501, 0.349, 0.467, with an average of 0.439. The fluctuations of the coefficient *a* were more considerable, but the experiments of 1932 give more constant values for a also: 3.11, 3.06, 2.85, etc.

---

[3] Made by Bausch and Lomb Optical Co.

The fluctuations in the values of the coefficients of the struggle for existence are due in this case in a considerable measure to an imperfect method of their calculation.[4] However, this is of no serious consequence, as we have a good method for verifying the average values of the coefficients of competition. This method consists in constructing a curve corresponding to the differential equation of competition (the details of this calculation are to be found in the Appendix). A close agreement of the calculated curve of growth of each species in a mixed population with experimental observations represents a good proof of the correctness of the numerical values of the coefficients of the struggle for existence. As regards the yeasts *Saccharomyces* and *Schizosaccharomyces* here concerned, their calculated curves of growth are given in Figures 14 and 15.

---

[4] In Chapter V we shall meet a more complicated situation.

In a mixed population of *Saccharomyces* and *Schizosaccharomyces* under anaerobic conditions the coefficients of the struggle for existence have the following values: a (showing the intensity of the influence of *Schizosaccharomyces* on *Saccharomyces)* = 3.15; b (intensity of the influence of *Saccharomyces* on *Schizosaccharomyces)* = 0.439. In other words, *one unit of volume of Schizosaccharomyces decreases the unutilized opportunity for growth of Saccharomyces 3.15 times as much as an equal unit of volume of Saccharomyces itself*. The species *Schizosaccharomyces* with its comparatively small volume takes up "a great number of places" in the microcosm. The reverse action of *Saccharomyces* on *Schizosaccharomyces is* comparatively weak. One unit of volume of *Saccharomyces* decreases the unutilized opportunity for growth of *Schizosaccharomyces* as much as 0.439 unit of the latter species' own volume.

(3) We now pass on to the most important part of this chapter, i.e., to the comparison of the empirically established coefficients of the struggle for existence with those which are to be expected on the basis of a direct study of the factors controlling growth. The values of the coefficients of the struggle for existence mentioned above are founded upon an analysis of the kinetics of growth of a mixed population. Let us at present leave them aside and endeavor to calculate the values of the coefficients of competition starting from the alcohol production. As mentioned above, the cessation of growth is connected with the reaching of a certain critical concentration of alcohol (characteristic for the given species under given conditions). Let us now assume that it is mainly alcohol that matters and that other byproducts of fermentation are but of subordinate importance. Consequently, every unit of volume in each species produces a determined amount of alcohol, and when

the latter reaches a certain threshold concentration the growth is checked. It follows that when a unit of volume of the first species produces an amount of alcohol considerably surpassing that produced by a unit of volume of the other species and the threshold values of alcohol in both are somewhat near to one another, the critical concentration of alcohol and the cessation of growth in the first species will be reached with a lower level of accumulated yeast volume. In Table V are given the data on the alcohol production in *Saccharomyces* and *Schizosaccharomyces* under anaerobic conditions. The determinations of the alcohol were made for the middle stages of growth, when its accumulation was almost strictly in proportion to the increase of the yeast volume. In *Saccharomyces* the alcohol production per unit of volume averages 0.113 per cent by weight, and in *Schizosaccharomyces* 0.247. These data show clearly that the latter species utilizes the medium unproductively and it occupies "a great number of places" by a comparatively small volume. At the same time this is an explanation of the low level of the accumulation of biomass in the separate cultures of *Schizosaccharomyces*, and the diminished volume of the mixed population in comparison with the volume of *Saccharomyces* cultivated separately.

We can now calculate approximately the critical concentrations of alcohol for the separate growth of each species of yeast if we multiply the maximal volumes of these species (*K*) by the alcohol production per unit of yeast volume. For *Saccharomyces* we shall have: 13.0 ´ 0.113 = 1.47, and for *Schizosaccharomyces:* 5.8 ´ 0.247= 1.43. In other words, the critical alcohol concentrations for both species are about equal.

TABLE V
Alcohol production in Saccharomyces cerevisiae and Schizosaccharomyces kephir
From Gause ('32b)

SACCHAROMYCES				SCHIZOSACCHAROMYCES			
Age in hours	Alcohol, per cent	Yeast volume in 10 c.c. of the medium	Alcohol per unit of yeast volume	Age in hours	Alcohol, per cent	Yeast volume in 10 c.c. of the medium	Alcohol per unit of yeast volume
16	1.100	10.20	0.108	48	0.728	3.08	0.236
16	0.480	5.33	0.090	72	1.425	5.51	0.259
24	1.690	12.22	0.138				

			Mean=0.113				Mean=0.247

$$\alpha_1 = \frac{0.247}{0.113} = 2.186$$

Let us now calculate the degree of influence of one species upon the unutilized opportunity for growth of another in a mixed population, or the coefficients of the struggle for existence. If we take as a unit the degree of decrease of the unutilized opportunity for growth of *Saccharomyces* by a unit of its own yeast volume, we have then to answer the following question: how much more or less does a unit of the yeast volume of *Schizosaccharomyces* decrease the unutilized opportunity for growth of *Saccharomyces* in the mixed population, in comparison with the effect of a unit of the volume of the latter species? Then, taking the ratio of the alcohol production per unit of yeast volume in *Schizosaccharomyces* to the alcohol production of *Saccharomyces* we shall find the coefficient of the struggle for

existence according to the alcohol production: $\alpha = \frac{0.247}{0.113} = 2.186$.

Correspondingly: $\beta = \frac{0.113}{0.247} = 0.457$.

(4) Comparing the results of the examination of the kinetics of growth of a mixed population with the data on the alcohol production, we observe a certain agreement in the general features. A very strong influence of *Schizosaccharomyces* upon *Saccharomyces* made apparent in the analysis of the kinetics of growth proved itself to be connected with the great alcohol production per unit of yeast volume in the former species. However, a strict coincidence of the data of these two independent methods of investigation does not occur here. Thus *Schizosaccharomyces* excretes a quantity of alcohol per unit of yeast volume 2.186 times as great as *Saccharomyces*, but influences the growth of the latter 3.15 times as much. Consequently, *Schizosaccharomyces* not only produces a greater amount of alcohol, but the alcohol produced by it is so to say "*more toxic*" for *Saccharomyces* than the alcohol produced by the latter itself. All this tends to imply that the situation is here complicated by the influence of certain other waste products getting into the surrounding medium in small quantities. The relations between species in these experiments are therefore not so simple as has been supposed at the beginning of this section

V

(1) The above described experiments of 1931 were repeated in 1932, and the new data confirmed all the observed regularities. In these new experiments the influence of oxygen upon the growth of a mixed population of the same two species of yeast was investigated, and this enabled us to further somewhat our understanding of the nature of the competitive process.

The experimental data given in the preceding section have to do with the growth of a yeast population under "anaerobic conditions," i.e., in test tubes. In order to study the influence of oxygen on the growth of the yeast population, together with experiments in test tubes we arranged other experiments under conditions of somewhat better aeration. The technique of such "aerobic" and "anaerobic" experiments has already been described at the beginning of this chapter. Here it must only be remarked that in the "aerobic" series the access of oxygen was very limited, and a part of the available energy was, as before, obtained by our species through alcoholic fermentation. As a result, a considerable amount of alcohol accumulated in the nutritive medium (as will be seen in the corresponding tables), and in its essential features the mechanism limiting the growth of the yeast population remained the same. The experiments of 1932 consisted of two aerobic and two anaerobic series. In them 168 separate microcosms were studied.

In all the experiments of 1932 nutritive medium of the same preparation was used. It was made according to the usual method, but the dry beer yeast was of another origin. As a result, the absolute values of growth were somewhat different. It must also be remarked that in all the new experiments the centrifuged volume of yeast was always reduced to 10 cm^3 of nutritive medium.

(2) Figure 16 represents the growth curves of *Saccharomyces, Schizosaccharomyces* and of the mixed population according to two series of experiments in conditions analogous to the former anaerobic ones. The general character of these curves coincides with that of Figure 13. A more careful comparison of the anaerobic series of 1932 with that of 1931 shows that the first is characterized by considerably smaller absolute values of growth (Table VI). At the same time *Schizosaccharomyces* grown separately attains a somewhat higher level in comparison with *Saccharomyces* than formerly. Thus, the volume of the saturating population of the separately growing *Schizosaccharomyces* represented in older experiments $\frac{5.8}{13.0} = 44.6$ per cent of that of *Saccharomyces* (1931), but in the new experiments it is $\frac{3.0}{6.25} = 48.0$ per cent (1932). In the experiments of 1932 the

relative volume of *Schizosaccharomyces* in the mixed population increased also. As a result the decrease of the volume of the mixed population in comparison with the volume of separately growing *Saccharomyces* is more pronounced in 1932 than in 1931.

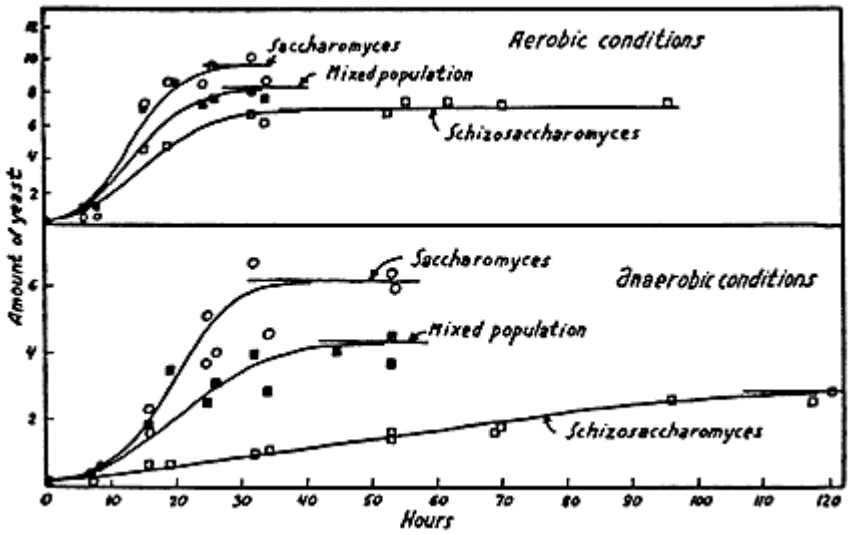

Fig. 16. The growth in volume *of Saccharomyces cerevisiae*, *Schizosaccharomyces kephir* and mixed population. Above: Aerobic conditions. Below: Anaerobic conditions (1932).

In spite of the alterations in the absolute values of growth and a certain change in the relative quantities of species, the coefficients of the struggle for existence which we had calculated for the anaerobic experiments of 1932 coincided almost completely with those of the year before. A similar coincidence exists in the ratio of the alcohol production of one species to that of another, which is to be found in Table VII. In this manner *the coefficients of the struggle for existence remain invariable under definite conditions in spite of the changing absolute values of growth.*

TABLE VI

Parameters of the logistic curves for separate growth of Saccharomyces cerevisiae and Schizosaccharomyces kephir under aerobic and anaerobic conditions (1932)

	K (Maximal volume)	*b* (Coefficient of geometric increase)	a (See Appendix II)	The volume of *N* at *t*=0
Saccharomyces anaerobic *Saccharomyces* aerobic	6.25 9.80	0.21529 0.28769	4.00652 4.16358	0.112 0.152

Schizosaccharomyces anaerobic *Schizosaccharomyces* aerobic	3.0 6.9	0.04375 0.18939	2.07234 2.78615	0.335 0.401

TABLE VII

Coefficients of the struggle for existence and the relative alcohol production under aerobic and anaerobic conditions

	Coeffiicients of the struggle for existence		Relative alcohol production	
	a	*b*	a_1	$\beta_1 = \frac{1}{\alpha_1}$
Anaerobic conditions (1931)	3.15	0.439	2.186	0.457
Anaerobic conditions (1932)	3.05	0.400	2.080	0.481
Aerobic conditions (1932)	1.25	0.850	1.25	0.80

(3) Let us now turn to the aerobic experiments (1932) and compare them to the anaerobic ones (1932). As might have been expected, in aerobic conditions the absolute values of growth of the yeast increase considerably (Fig. 16). What is especially striking is the behavior of *Schizosaccharomyces*. Though it is a slowly growing species under anaerobic conditions, with a low level of biomass, it begins to grow rapidly with an access of oxygen and in its properties approaches *Saccharomyces*. The maximal volumes and coefficients of geometric increase given in Table VI show these regularities in a quantitative form. When there is no oxygen and fermentation is the only source of available energy, the coefficient of geometric increase in *Schizosaccharomyces* is very low and equal to 0.04375. Under the influence of oxygen this coefficient increases 4.3 times and attains 0.18939, whereas in *Saccharomyces* the coefficient of geometric increase under the same conditions rises but slightly (from 0.21529 to 0.28769).

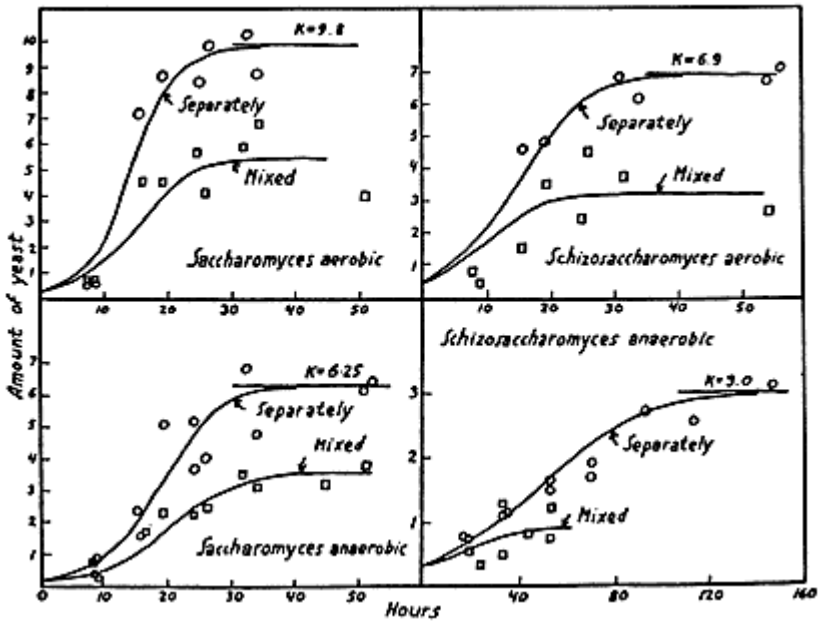

Fig. 17. The growth in volume of *Saccharomyces cerevisiae* and *Schizosaccharomyces kephir* cultivated separately and in the mixed population under aerobic and anaerobic conditions (1932). All curves are drawn according to equations.

The sharp changes in the properties of our species under aerobic conditions produce a completely new situation for the growth of a mixed population (see Fig. 17). As before, we have calculated the coefficients of the struggle for existence and Table VII shows that they differ considerably from the anaerobic ones. If in anaerobic experiments the coefficient a , which characterizes the intensity of influence of *Schizosaccharomyces* upon *Saccharomyces*, was equal to 3.05-3.15, than under aerobic conditions it is equal to 1.25. In other terms the influence of *Schizosaccharomyces* on *Saccharomyces* is no longer 3.05, but only 1.25 times as strong as the influence of the latter upon itself.

(4) Let us now examine the production of alcohol under aerobic conditions. The corresponding data are given in Table 2 (Appendix). As was to be expected, in aerobic conditions the amount of alcohol per unit of yeast volume is smaller than in anaerobic ones, because a part of the available energy is furnished by oxidation. It is interesting to compare the critical concentration of alcohol at which growth ceases, in aerobic and anaerobic conditions. Let us multiply as before the production of alcohol per unit of yeast volume by the maximal volume. For the anaerobic experiments of 1932 we shall obtain: *Saccharomyces*, 6.25 ´ 0.245 = 1.53; *Schizosaccharomyces*, 3.0 ´ 0.510 = 1.53. These threshold concentrations of alcohol coincide in both

species, and they are sufficiently near to those with which we have had to deal in the anaerobic experiments of 1931. As to the threshold concentrations of alcohol in aerobic conditions, they prove to be higher than in the anaerobic ones, and in *Saccharomyces* the threshold lies somewhat higher than in *Schizosaccharomyces: Saccharomyces,* 9.80 ´ 0.207 = 2.03; *Schizosaccharomyces,* 6.9 ´ 0.258 = 1.78.

If we now calculate for aerobic conditions the degree of influence of *Schizosaccharomyces* upon *Saccharomyces* starting from the production of alcohol per unit of yeast volume, we shall obtain:

$$\alpha_1 = \frac{0.258}{0.207} = 1.25.$$

Correspondingly the coefficient

$$\beta_1 = \frac{0.207}{0.258} = 0.80.$$

Comparing these results with the data of the kinetics of growth, we see (Table VII) that in aerobic conditions the degree of influence of one species upon another calculated according to the system of equations of the struggle for existence fully coincides with the coefficients of the relative alcohol production. Therefore, the process of competition between our species in aerobic conditions is entirely regulated by alcohol, and there is scarcely any interference of other factors.

(5) We can now appreciate from a more general viewpoint the results of the aerobic experiments as well as those of this chapter. It has been shown that under aerobic conditions the theoretical equation of competition between two species of yeast for a common place in the microcosm given for the first time by Vito Volterra is completely realized. In other words, *if we know the properties of two species growing separately,* i.e., their coefficients of geometric increase, their maximal volumes, and alcohol production per unit of volume when alcohol limits the growth, *then connecting these values into a theoretical equation of the struggle for existence we can calculate in what proportion a certain limited amount of energy will be distributed between the populations of two competing species*. This means that we can calculate theoretically the growth of species and their maximal volumes in a mixed population. The equation of the struggle for existence expresses the idea that a potential geometric increase of each species in every infinitesimal interval of time is only realized up to a certain degree depending on the unutilized opportunity for growth at that moment, and that the species possesses certain coefficients of seizing this unutilized opportunity. Such theoretical calculations agree

completely with the experimental data only under aerobic conditions, where the limitation of growth in both species depends almost completely on the ethyl alcohol. In the case of anaerobic conditions the situation becomes more complicated as a result of the influence of certain other waste products. This shows that extreme care is necessary in the investigation of biological systems, because various and often unexpected factors may participate in the process of interaction between two species.