CXIII. THE EFFECT OF "BIOS" ON THE GROWTH AND METABOLISM OF CERTAIN YEASTS.

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INTRODUCTION.

A CRITICAL survey of the literature shows that in spite of a great deal of experimentation, there remain many divergent opinions regarding "bios" and its existence as an essential substance for growth.

Bearing in mind the difference in the techniques employed by various investigators, it is not difficult to understand how the discrepancies between the published results have arisen. Artificial media of widely differing types have been used. In some cases great care has been taken to ensure the purity of the constituents of the artificial culture fluids; in other cases practically none. A number of the researches have been made with yeasts of uncertain history, and character; and, as Wallace and Tanner [1928] point out, it is probable that some of the work has been vitiated by bacterial contamination.

The present study was begun with the object of obtaining evidence of a definite character regarding the existence of "bios." Intimately related to this problem was that of selecting the most suitable artificial culture fluid for the growth of yeast. Furthermore, it was obviously necessary to study a number of yeasts representing the chief types. It has been found that different types of yeast in an artificial medium exhibit different requirements for "bios" and that some of the highly cultivated brewers' yeasts will not grow in a purified glucose-salt medium without the addition of small amounts of extracts containing an organic "bios." Following this confirmation of Wildiers's [1901] original claims, the investigation was extended with the object of ascertaining what rôle this substance plays in the growth and nutrition of those yeasts which demand it.

PART I.

Technique.

The salt solution for the medium was prepared with "organic-free" distilled water and pure salts of the "Analytical Reagent" quality supplied by British Drug Houses. Dextrose (A.R.) was only added to the medium immediately before use and the solution was then immediately transferred to the

culture flasks and sterilised by heating in a Koch steriliser for 30 minutes on three successive days. In view of the claim of Fulmer and Huesselmann [1927] that production of a yeast growth stimulant may occur during heating of dextrose under pressure, this process is preferable to autoclaving the flasks for 30 minutes at 20 lb. pressure.

All glass apparatus was cleaned with hot chromic acid solution, thoroughly rinsed with tap water and distilled water, and dried in an electric oven at 105°.

Inoculations were made from fresh (24–48 hour) cultures on beer-wort agar slopes. A small amount of culture was transferred on a platinum loop to a tube containing 10 cc. of medium. This suspension was shaken and any large clumps of cells allowed to settle to the bottom. The flasks were then inoculated with a Pasteur pipette, two or more drops being given according to the size of seeding required. The number of cells thus given was calculated by taking a cell count on the suspension, and by weighing the drops delivered by each pipette. The cell counts were made with a Thoma haemocytometer, the unit adopted being that used by many other workers, namely, 250,000 cells per cc.

Composition of the medium in relation to growth¹.

Workers who reported conflicting results have often used very dissimilar media. There is no uniformity as regards nature, concentration and purity of the ingredients of the various artificial media used. For example, Fulmer, Nelson and Sherwood [1921] described several media which, they claimed, provided for all the nutritional requirements of their yeasts, but their solutions all contained 10 % of sugar, while that evolved by Reader [1927] contained only 1 %. More significant were the variations in the purity of the components, so that it was possible that a growth-promoting substance might be present in the media. Thus before attempting to obtain definite data on the question of the need of a growth-promoting factor and its effects it was necessary to investigate the influence of the composition of the medium on growth.

Reader's [1927] medium, which has been extensively used in this laboratory and found satisfactory, was chosen as a control, and four others of varying composition were used for comparison. The composition of all the media is shown in Table I. Those selected are representative of the media used by four different groups of workers.

Wildiers's medium was that used in his original experiments, and Pringsheim's [1906] in experiments the results of which contradicted those of Wildiers. Robertson's [1924] medium was alleged not to support the growth of yeasts, though Devereux and Tanner [1927] used it successfully; while Fulmer's medium was one of a group which were claimed to allow continuous cultivation of yeast without any added growth stimulant.

¹ Shortly after this work was completed Wallace and Tanner [1928] published an account of a study of the growth of four yeasts in six different media. They used single-cell cultures and their results are in accord with those found in the present research.

The control medium was prepared in the manner described, and the other four according to the directions of their original users, as regards purity of constituents and method of sterilisation. In order to test the relative growth-

Table I. *Artificial media.*

Medium A (Reader)		Medium B (Wildiers)		Medium C (Pringsheim)	
Dextrose	1.0 %	Sucrose	10.0 %	Sugar (not specified)	5.0 %
(NH ₄) ₂ SO ₄	0.3	(table sugar)		(NH ₄) ₂ SO ₄	5.0
MgSO ₄ · 7H ₂ O	0.07	MgSO ₄	0.25	MgSO ₄	0.18
KH ₂ PO ₄	0.10	KCl	0.25	K ₂ HPO ₄	0.75
K ₂ HPO ₄	0.016	NH ₄ Cl	0.25		
NaCl	0.05	Na ₂ HPO ₄	0.25		
Ca(NO ₃) ₂	0.04	CaCO ₃	0.05		
Medium D (Robertson)		Medium E (Fulmer)			
Asparagine	0.34 %	Sucrose	8.0 %		
CaCl ₂	0.01	(purified)			
Dextrin (purity not defined)	2.0	CaCO ₃	0.04		
MgSO ₄	0.02	NH ₄ Cl	0.188		
K ₂ HPO ₄	0.1	K ₂ HPO ₄	0.1		
NaCl	0.5	CaCl ₂	0.1		

supporting powers of the five media, five typical yeasts were chosen from a group of twenty selected from the National Type Culture Collection at the Lister Institute. They were *Saccharomyces logos*, *Torula sphaerica*, *Saccharomyces cerevisiae* (bakery), *Saccharomyces cerevisiae* (brewery), *Saccharomyces cerevisiae* (Hansen). Two organisms which Fulmer, Nelson and Sherwood had isolated from Fleischmann's yeast cake were also included in the experiment. They were a *Mycoderma* and a *Saccharomyces cerevisiae* which, according to these workers, proved entirely satisfactory in continuous sub-culture in an artificial medium.

In preparing the cultures of these seven yeasts in the five media the only divergence from the standard method was in the use of tubes of sterile water instead of medium for making the initial suspension. By this arrangement the same suspension could be used to inoculate each medium and so the same seeding in all five media was ensured. The tests were made in 150 cc. conical flasks containing 25 cc. of culture fluid. Four flasks of each culture were prepared, so as to have duplicate flasks with and without "bios." The preparation of "bios" used was an autoclaved solution of marmite containing 14 mg. solid matter per cc. The dose was 0.5 cc. in 25 cc. of medium.

The original cell count was taken for each yeast and the flasks were incubated for 96 hours at 28°. At the end of this period growth was stopped by transferring the flasks to the cold room at 0°, after which counts were made as quickly as possible. The results are shown in Tables II, III and IV. In Tables II and III the figures opposite each medium denote the growth indices of the yeasts in that medium. These values were obtained by dividing the original count into the final count. Table IV indicates the numerical relation between growth with and without "bios," in cases where growth occurred under both conditions.

Table II. *Multiplication of yeasts without "bios."*

Organism	<i>Sacch. logos</i>	<i>Torula</i>	<i>Sacch. cerev. (bakery)</i>	<i>Sacch. cerev. (brewery)</i>	<i>Sacch. cerev. (Hansen)</i>	<i>Mycoderma (Fulmer)</i>	<i>Sacch. cerev. (Fulmer)</i>
Original cell count	1.04	0.77	0.74	0.58	0.55	0.80	0.98
Medium <i>A</i>	553.9	—	—	—	—	1120.0	1.6
„ <i>B</i>	369.2	10.4	1.1	—	—	88.0	3.3
„ <i>C</i>	407.7	259.7	8.6	—	—	470.0	5.7
„ <i>D</i>	223.0	—	3.2	—	—	20.0	3.3
„ <i>E</i>	126.9	332.5	32.4	—	—	23.0	14.7

Table III. *Multiplication of yeasts with "bios."*

Organism	<i>Sacch. logos</i>	<i>Torula</i>	<i>Sacch. cerev. (bakery)</i>	<i>Sacch. cerev. (brewery)</i>	<i>Sacch. cerev. (Hansen)</i>	<i>Mycoderma (Fulmer)</i>	<i>Sacch. cerev. (Fulmer)</i>
Original cell count	1.04	0.77	0.74	0.58	0.55	0.80	0.98
Medium <i>A</i>	346	280	229	40	4.4	500	69
„ <i>B</i>	346	634	110	14	11.6	92	82
„ <i>C</i>	400	457	86	18	8.7	64	41
„ <i>D</i>	500	50	22	8	7.3	65	15
„ <i>E</i>	192	62	50	15	10.2	128	42

Table IV. *Ratio of growths without and with "bios" obtained in different media.*

Organism	<i>Sacch. logos</i>	<i>Torula</i>	<i>Sacch. cerev. (bakery)</i>	<i>Sacch. cerev. (brewery)</i>	<i>Sacch. cerev. (Hansen)</i>	<i>Mycoderma (Fulmer)</i>	<i>Sacch. cerev. (Fulmer)</i>
Original cell count	1.04	0.77	0.74	0.58	0.55	0.80	0.98
Medium <i>A</i>	1.60	—	—	—	—	2.24	0.02
„ <i>B</i>	1.06	0.016	0.01	—	—	0.95	0.04
„ <i>C</i>	1.00	0.57	0.09	—	—	7.34	0.14
„ <i>D</i>	0.45	—	0.15	—	—	0.31	0.29
„ <i>E</i>	0.66	5.3	0.65	—	—	0.18	0.35

Though the results are somewhat irregular it is evident that the composition of the medium is a very important factor in the study of yeast growth, especially with regard to the comparison of observations of different workers. For instance, there is sufficient difference between growth in Wildiers's medium (*B*) and that in Pringsheim's medium (*C*) to account for Pringsheim's [1906] denial of the necessity of "bios." Of the two satisfactory media, namely *A* and *E*, *A* was the more suitable for use in testing work as it showed the "bios" effect more markedly than *E*. For further experiments therefore Reader's medium was the artificial culture fluid used.

Preliminary growth test.

Twenty types of yeasts (varying from wild yeasts to highly cultivated forms) were tested for their ability to grow in Reader's medium. A loopful of a 24-hour culture on beer-wort agar was inoculated into 25 cc. of medium in a 150 cc. conical flask, and incubated at 28°.

Even with so large an inoculation there was a marked difference in the rate and amount of growth of the various yeasts. On the basis of this test the yeasts were arranged in the order shown in Table V. The first five types showed rapid growth during the first 24 hours after inoculation; the next five

were slow in showing development but after 2 or 3 days there was appreciable growth. The rest showed little or no growth in 14 days.

Table V.

National Type Culture Collection No.		Growth of large inoculations in Reader's medium at 28°.
		Yeast type
474	<i>Willia anomala</i>	} Rapid growth in first 24 hours
475	<i>Willia Saturnus</i>	
819	<i>Mycoderma cerevisiae</i> (Harden)	
1848	<i>Mycoderma Bordetii</i>	
909	<i>Saccharomyces logos</i> (Ling)	
1302	<i>Torula sphaerica</i>	} Slow growth in 3 days
1303	<i>Torula cremoris</i>	
813	<i>Zygosaccharomyces Barkeri</i>	
467	<i>Saccharomyces ellipsoideus</i>	
743	<i>Saccharomyces monacensis</i>	
815	<i>Saccharomyces cerevisiae</i> (bakery)	} Little or no growth in 14 days
476	<i>Schwanniomyces occidentalis</i>	
790	<i>Saccharomyces thermantionum</i>	
902	<i>Schizosaccharomyces Pombe</i>	
381	<i>Saccharomyces cerevisiae</i> (brewery)	
466	<i>Saccharomyces cerevisiae</i> (Hansen)	
473	<i>Saccharomyces Ludwigi</i>	
499	<i>Pseudosaccharomyces Africanus</i>	
814	<i>Saccharomyces capsularis</i> (Harden)	
817	<i>Hansenia apiculata</i> (Reess)	

Testing "bios" requirements of yeasts. The preliminary growth test was repeated under controlled conditions with very much smaller inoculations. Except for the first five yeasts there was little growth in any of the flasks in 72-96 hours. Most of the yeasts having shown no signs of growth in 7 days, the effect of adding "bios" was studied. The dose was again 0.5 cc. of autoclaved yeast extract. The addition produced a good growth in the flasks within 24 hours.

From this experiment it appeared that those yeasts which were unable to grow in the artificial medium remained alive, and were promptly stimulated by the addition of "bios." In another set of experiments, in which the "bios" was given at the commencement, all the yeasts showed marked growth. In the case of *Pseudosaccharomyces Africanus*, *Saccharomyces capsularis* and *Hansenia apiculata*, however, it was not as rapid as in the case of other species.

Production of "bios" by certain rapidly proliferating yeasts. To investigate the production of "bios" by organisms capable of rapid growth in the synthetic medium alone, *Willia anomala*, *W. Saturnus*, *Mycoderma cerevisiae*, *M. Bordetii* and *Saccharomyces logos* were grown in Reader's medium for ten generations. The initial inoculation was small (count 0.25) and so were all sub-cultures, which were made every 48 hours. Growth was well maintained. The contents of the flasks in which the tenth generation had grown for 48 hours were centrifuged to remove the cells and the supernatant liquid was re-sterilised. The five different culture fluids were kept separate and each was tested for its growth-promoting power on three different yeasts. An arbitrary

amount of 1 cc. of the undiluted culture fluid was given as the "bios" dose to 25 cc. of medium.

Table VI gives the results of this experiment. It clearly indicates that the organisms which maintained good growth in this artificial medium, at the same time produced in the solution a substance which is capable of stimulating the growth of species which cannot grow in the artificial medium.

Table VI.

Extract		Test organism		Time for growth without "bios" hours	Time for growth with "bios" hours
909	<i>Saccharomyces logos</i>	1303	<i>Torula cremoris</i>	120	48
"	"	466	<i>Saccharomyces cerevisiae</i> (Hansen)	No growth	72
"	"	473	<i>Saccharomycodes</i>	"	120
474	<i>Willia anomala</i>	467	<i>Saccharomyces ellipsoideus</i>	96	48
"	"	815	<i>Saccharomyces cerevisiae</i> (bakery)	No growth	72
"	"	814	<i>Saccharomyces capsularis</i>	"	96
475	<i>Willia Saturnus</i>	743	<i>Saccharomyces monacensis</i>	144	72
"	"	476	<i>Schwanniomyces</i>	No growth	96
"	"	381	<i>Saccharomyces cerevisiae</i> (brewery)	"	96
1848	<i>Mycoderma Bordetii</i>	499	<i>Pseudosaccharomyces</i>	"	72
"	"	1302	<i>Torula sphaerica</i>	"	96
"	"	790	<i>Saccharomyces thermantitonus</i>	"	144
819	<i>Mycoderma cerevisiae</i>	817	<i>Hansenia apiculata</i>	"	96
"	"	902	<i>Schizosaccharomyces</i>	"	96
"	"	813	<i>Zygosaccharomyces</i>	"	(8 days)

CONCLUSIONS AS TO THE "BIOS" REQUIREMENTS OF YEASTS.

The results of these experiments show that yeasts vary considerably in their "bios" requirements. Two factors, the type of yeast and the composition of the medium, influence the demand for an added growth-promoting substance. Some organisms, such as *Saccharomyces logos* and *Mycoderma*, will grow under almost any conditions, while others such as *Saccharomyces cerevisiae* (Hansen) appear to be unable to grow except in the presence of added organic matter. The presence of "bios" in an artificial medium accelerates the growth of yeasts of the former type, but does not increase the final number of cells as this is very probably limited by the supply of nutriment in the medium.

PART II.

The results of the experiments reported in Part I showed that those species growing best in an artificial medium were not actively fermenting types. This seemed to indicate a possible connection between the "bios" requirements and the fermentative activity of different yeasts. The relation between the fermentative and other forms of metabolic activity of yeast has recently attracted rather wide attention. In particular Meyerhof [1925] has studied in some detail the changes which occur in the gaseous exchange under aerobic and anaerobic conditions.

By measuring the oxygen uptake, the fermentation CO_2 , and the alcohol production of a number of yeasts in dextrose-phosphate solutions, both aerobically and anaerobically, he showed that the effect of respired oxygen on the amount of fermentation was similar to the effect of oxidation on the degradation of sugar to lactic acid. Top-fermenting and bottom-fermenting yeasts, wine yeasts, a *Mycoderma* and a *Torula* were used. He found that distillery and bakery yeasts respired very little in phosphate solution, but the addition of dextrose increased the value eight- or ten-fold ($Q_{\text{O}_2} = 80\text{--}100$).

In nitrogen the fermentation $Q_{\text{CO}_2}^{\text{N}_2} = 250\text{--}300$, but in oxygen it falls to $Q_{\text{CO}_2}^{\text{O}_2} = 60\text{--}100^1$.

From consideration of Meyerhof's results it seemed that the determination of the Q_{O_2} and $Q_{\text{CO}_2}^{\text{O}_2}$ values, together with alcohol production and carbohydrate assimilation, might give some indication of the nature of the part played by "bios" in the metabolism of yeasts.

*Determination of oxygen uptake and carbon dioxide output of yeasts,
with and without "bios."*

As the work of Meyerhof had established the usefulness of Warburg's manometer in measuring the respiration of yeasts, a set of similar instruments was used for these determinations. Details of the apparatus are given by Warburg [1923, 1926].

The constant for each manometer was determined in the usual way. For measuring oxygen uptake of a yeast 0.4 cc. of *N* KOH solution was placed in the inner tube and 2 cc. of culture in the flask. As the experiments were carried out in air, the flasks could be prepared and put with their manometers directly into a thermostat at 28° . The vessels were shaken with the taps open for 10–15 minutes to bring them to equilibrium, then the manometer levels adjusted and the taps closed. Readings were usually taken every 15 minutes and the rate of shaking was kept constant by means of a rheostat adjustment. When the carbon dioxide output was to be measured, KOH was not placed in the inner tube and the manometer reading then indicated the excess of CO_2 output over O_2 uptake. This excess of CO_2 represents the fermentation CO_2 of the yeast and the value $Q_{\text{CO}_2}^{\text{O}_2}$ is calculated directly from it. In order to obtain the dry weight of yeast present in the culture used, 2 cc. were filtered through a Jena glass filter, which was dried in a vacuum desiccator for 24 hours and then weighed on a micro-balance. It was found that 2 cc. of a 24-hour old culture was a suitable quantity for measuring Q_{O_2} and $Q_{\text{CO}_2}^{\text{O}_2}$ of the yeasts.

¹ Q_{O_2} is the oxygen quotient and indicates mm.³ O_2 taken up per mg. of dry yeast per hour.

$Q_{\text{CO}_2}^{\text{N}_2}$ is the quotient for CO_2 under anaerobic conditions and is mm.³ CO_2 given off per mg. of dry yeast per hour.

$Q_{\text{CO}_2}^{\text{O}_2}$ is the quotient for CO_2 under aerobic conditions and is mm.³ CO_2 given off per mg. of dry yeast per hour.

In testing the "bios" effect it was thought possible that it might be shown by adding "bios" to the culture when it was in the manometer flask. A slight effect could be observed in the case of yeasts that were already growing, but for such as *Saccharomyces cerevisiae* (Hansen) no increase in either O_2 uptake or CO_2 output could be shown. Therefore "bios" was always added to the medium before the yeast inoculation. For simple measuring of the O_2 uptake and CO_2 output of yeasts 24-hour cultures were used. Remarkably constant results were obtained after some experience with the technique. In Table VII are given the average results of a number of determinations of the Q_{O_2} and $Q_{CO_2}^{O_2}$ of the seven types of yeasts whose "bios" requirements were being studied.

Table VII. O_2 uptake and CO_2 output of yeasts.

Organism	Without "bios"		With "bios"	
	Q_{O_2}	$Q_{CO_2}^{O_2}$	Q_{O_2}	$Q_{CO_2}^{O_2}$
<i>Saccharomyces logos</i>	50	<10	70	40
<i>Torula sphærica</i>	10	20	50	100
<i>Saccharomyces cerevisiae</i> (bakery)	Nil	30	30	100
<i>Saccharomyces cerevisiae</i> (brewery)	Nil	20	10	150
<i>Saccharomyces cerevisiae</i> (Hansen)	Nil	10	30	200
<i>Saccharomyces cerevisiae</i> (Fulmer)	Nil	50	50	Nil
<i>Mycoderma</i> (Fulmer)	100	<10	100	<10

These figures show that yeasts which do not respire are those that require "bios" in order to grow in a synthetic mineral medium. The presence of "bios" seems to cause a slight respiratory activity and a great increase in fermentation.

The correlation of respiratory and fermentative activity of yeasts.

It now remained to determine the dextrose consumption and alcohol production in parallel with the oxygen uptake and carbon dioxide output of the yeasts, with and without "bios." For this purpose suitable micro-methods of estimating dextrose and alcohol in the medium were used. Hanes [1929] published details of a modification of Hagedorn and Jensen's [1923] micro-method of estimating sugars. The range of the modified method was 0.20 mg. to 3.80 mg. of dextrose and by diluting the medium the dextrose content could be brought within this range. The method was tested on medium, cultures, and filtrates from cultures and found to be satisfactory. The dextrose content of a culture and its filtrate were always the same. The presence of yeast cells therefore did not affect the estimations.

The method of Cannan and Sulzer [1924] for the estimation of alcohol in blood was adapted to the requirements of the culture solutions and found reliable. In this method the alcohol is distilled under reduced pressure and the distillate collected in an absorption tube containing a mixture of 0.1 *N* potassium dichromate and an equal volume of strong sulphuric acid. It was found convenient to deliver 1 cc. or 2 cc. of undiluted culture on to a fairly

large amount of anhydrous sodium sulphate and to distil for 30 minutes. The contents of the absorption tube were then washed into a flask with sufficient water to dilute the sulphuric acid to less than 5 %, excess of 10 % potassium iodide solution was added and the liberated iodine titrated with 0.1 *N* sodium thiosulphate. A blank titration of the potassium dichromate was made daily. The difference between the titration after the absorption of alcohol and the dichromate blank gave the amount of dichromate required to oxidise the alcohol. The amount of alcohol was calculated from the factor 1 cc. 0.1 *N* $K_2Cr_2O_7$ = 1.15 mg. alcohol.

With these methods it was possible to make simultaneous estimations of dextrose consumption, alcohol production, oxygen respired and carbon dioxide produced in a yeast culture. For each yeast determinations were made with and without "bios" at 24, 48 and 72 hours after inoculation. Control flasks of medium were incubated with the cultures to allow for the slight alteration in dextrose concentration due to evaporation. Cell counts were also made so as to check growth against respiration and fermentation. The results are shown in Figs. 1-7.

Fig. 1. *Saccharomyces logos*.

DISCUSSION.

From the results of these experiments on the respiration and fermentation of yeasts with and without "bios" some definite conclusions may be formed as to the action of this factor. In the case of *Saccharomyces logos* the first action of "bios" is to accelerate growth and fermentation, but as soon as the

Fig. 2. *Torula sphaerica*.

Fig. 3. *Saccharomyces cerevisiae* (bakery).

Fig. 4. *Saccharomyces cerevisiae* (brewery). Fig. 5. *Saccharomyces cerevisiae* (Hansen).

Fig. 6. *Saccharomyces cerevisiae* (Fulmer).

Fig. 7. *Mycoderma* (Fulmer).

dextrose supply in the medium is exhausted the respiration falls to about the same value as in growth without "bios" and some of the alcohol formed disappears. For *Torula sphaerica* there is a very marked increase in cell count together with increased fermentation and respiration, and the $Q_{CO_2}^{O_2}$ falls considerably below the normal value when the combustion of the dextrose is complete.

In all four types of *Saccharomyces cerevisiae* the addition of "bios" markedly increases growth and seems to provide the organism with the power to respire oxygen. There is no Q_{O_2} in synthetic medium, but with "bios" there appears a Q_{O_2} and at first a raised $Q_{CO_2}^{O_2}$. As soon as the dextrose concentration of the medium falls, the Q_{O_2} seems to increase while the $Q_{CO_2}^{O_2}$ falls and some of the alcohol produced seems to be utilised to support growth. Without "bios" there is a very slight alcohol production by the *Saccharomyces cerevisiae* and it is always equivalent to the dextrose combustion. Thus "bios," while increasing the growth of these organisms, seems to alter their metabolism in some manner, providing them with the power of respiring oxygen and utilising their own fermentation products.

For the *Mycoderma* the action of "bios" is not very notable, except that it causes a slight increase in the alcohol production.

Apart from the fact of showing that "bios" is definitely a growth-promoting factor, little can be deduced from these results. The apparent change of respiratory function in *Saccharomyces cerevisiae* may simply be due to the fact that with very few cells present the Q_{O_2} would be immeasurably small, but as soon as growth increased the Q_{O_2} would be appreciable. A larger number of cells would account for the first rise of the $Q_{CO_2}^{O_2}$, but the subsequent fall of this quotient can only be accounted for by some change in the metabolism of the yeast.

SUMMARY.

The necessity for "bios" and the part it plays in the growth and metabolism of yeasts has been studied. The need for "bios" seems to depend on the type of yeast and on the composition of the medium; it is probably connected with fermentative activity of the yeast as is indicated by experiments showing increased oxygen uptake and carbon dioxide output in the presence of this factor. "Bios" appears to act as a growth stimulant, but from the above experiments it is not possible to determine whether the increased respiratory activity of the yeast is due to the greater number of cells present or to a change in their metabolic processes.

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