A KINETIC ANALYSIS OF THE ENDOGENOUS RESPIRATION OF BAKERS' YEAST

By T. J. B. STIER and J. N. STANNARD

(From the Biological Laboratories, Harvard University, Cambridge)

(Accepted for publication, July 9, 1935)

I

We will discuss in this paper the kinetics of endogenous respiration of bakers' yeast; that is, the respiration of intact yeast cells suspended in non-nutrient media. In succeeding papers results growing from an examination of the nature of the metabolic processes involved will be presented. The purpose of this series is to demonstrate the necessity of describing the behavior of metabolic systems at their native loci by the use of *in vivo* procedures. By dealing with experimentally verifiable rate-controlling steps in the various metabolic systems we will show how these complex chains of reactions may behave as distinct functional units at one time and, depending upon the imposed experimental conditions, as interrelated but yet coordinated systems. Since the overall metabolic activity can be shown to be governed by these rate-controlling "loci,"[1] integrated action of the myriads of simultaneous reactions is achieved within a cell. It is felt that this method of investigation is a necessary procedure which should precede any final application of the results of *in vitro* studies of enzyme action to the dynamical organization of *in vivo* metabolic processes.

As a general rule in plant cells and tissues the rate of respiration under starvation conditions sooner or later decreases with time after the stored material has been reduced below a certain "critical concentra-

[1] Obviously in all but "zero order reactions" (for an example, *cf.* p. 471) the *instantaneous* rate of respiration may be a function of the concentration of reacting materials. The rate constant in these cases is then the appropriate constant describing the activity of the pace-setting locus; *e.g.*, a first order constant. We imply that the behavior of the descriptive constant for the rate of decomposition of a relatively large amount of substrate reflects the physical and chemical characteristics of this locus, whatever its real nature may be.

tion." This has been shown in *Aspergillus niger* by Kosinski (1902) and in yeast most recently by Geiger-Huber (1934). It is assumed that bacteria maintain themselves in non-nutrient solutions by decomposition of their internal stores (Rahn, 1932).

The term "autofermentation" has been applied to the decomposition of internally stored carbohydrates or their products of hydrolysis in yeast juices, dried yeast, pressed yeast, and intact yeast (*cf.* Harden, 1932; von Euler and Lindner, 1915). Harden and Young (1902) showed that glycogen is the principal carbohydrate reserve of yeast and it is generally held that the ultimate substrate in autofermentation is glycogen (*cf.* Harden, 1932;[2] Warburg, 1927;[3] Meyerhof, 1925; Warkany, 1924).

The tacit assumption has been that autofermentation proceeded by the same general mechanism as the respiration and fermentation of sugar added to the medium. The work which we are to describe emphasizes the necessity for exercising great care in the use of the term autofermentation. The metabolic utilization of reserve substances exhibits such differences in intact living yeast as compared with variously treated preparations, that the term autofermentation is not strictly applicable to the former. For reasons which will be given in another paper we prefer the term *endogenous respiration* and will reserve autofermentation for those cases in which the metabolism can be shown to be of a truly fermentative type as is evidently the case with yeast juice and pressed yeast.

II

EXPERIMENTAL PROCEDURES

Two strains of bakers' yeast, *Saccharomyces cerevisiae*, were employed; one was a pure strain grown in the laboratory, the other a pure strain grown in large quantities under commercial conditions and obtained from the Fleischmann Company through the kindness of Dr. Charles N. Frey. This latter strain is essentially commercial bakers' yeast, but is handled under such conditions as to exclude almost all possible contaminants. The time at which each lot was separated from its culture medium was noted on each package; the yeast was

[2] Pp. 33, 34, 40, 143, 161, and 186.
[3] P. 364.

kept under refrigeration en route from the factory to our Laboratory. This strain will be referred to as GM yeast. The pure strain which we cultivated was one already employed in connection with several studies made in this Laboratory (Richards, 1928; Oster, 1934–35). It was *Saccharomyces cerevisiae* Hansen, obtainable from the American Type Culture Collection, Chicago; formerly No. 2335, and now renumbered No. 4360. We will refer to this strain as Strain 4360.

Stock cultures of Strain 4360 were maintained at low temperature (7°C.) on agar slants made up with 1.5 per cent malt extract broth (Difco) and 2 per cent agar, sterilized 20 minutes at 15 lbs. pressure. At least 2 weeks prior to use the yeast was transferred to Williams' medium (*cf.* Williams, 1920; Williams, Wilson, and von der Ahe, 1927) and sub-cultures made every 2–3 days. Pure culture methods were used at all times. During any one period of experimentation stock cultures were maintained in Williams' medium.

The actual experimental material was incubated for the desired time in glass towers 30 × 3.5 cm. at 25–26°C. These had a tube sealed in at the top reaching to within ½ cm. of the bottom to allow aeration from a purified compressed air supply. Aeration is necessary, for, as shown by Stier, Arnold, and Stannard (1933–34) large "clumps" of yeast interfere with proper measurement of the turbidity of the suspension by the photoelectric densitometer, and probably somewhat with measurement of the metabolism as well. The aeration was sufficiently vigorous to reduce the number of clumps containing over 4 cells to less than 5 per cent of the total number of cells.

In the earlier portion of the work we used transfers from the original stock culture obtained from the American Type Culture Collection. At a later date single cells from this culture were isolated by a dilution technique employing Petri dishes and malt extract agar. The loci of single cells were marked after examination under the binocular microscope and several colonies arising from such single cells were separately inoculated onto agar slants and then later into Williams' medium. The most successful one of these was selected for future work. We repeated several experiments on this newly isolated "strain" and compared its behavior with that of our original pure line. No differences in respiratory activity or in its metabolic behavior could be detected.

The yeast was centrifuged away from the culture medium at approximately 3000 R.P.M. for 3–5 minutes. Fresh, sterile M/15 KH_2PO_4 solution (pH 4.5), or rarely Sørenson phosphate buffer at a series of pH values, was added; the cells were washed, recentrifuged, and resuspended at the proper concentration in the new medium. One washing was sufficient to remove the last traces of the culture medium, but not enough to wash out appreciably any of the soluble components of the cell.

The GM yeast was stored in a refrigerator at 7°C. and was used within 24–48 hours after its arrival. For each experiment a portion was removed from the

center of the package in a sterile transfer cabinet and made up to the desired concentration with sterile M/15 KH_2PO_4, usually after one centrifugal washing.

The photoelectric densitometer (Stier, Arnold, and Stannard, 1933–34) was used as a quick and accurate method of obtaining yeast suspensions of uniform and known concentration for any series of experiments. The densitometer was calibrated in terms of dry weight of yeast. The density of the suspensions used for the experiments here reported was made high since the rate of endogenous respiration of intact yeast is only 0.4–0.05 of that of yeast suspended in 2 per cent dextrose-phosphate solution (*cf.* Warburg, 1927; Meyerhof and Iwasaki, 1930).

The oxygen consumption and aerobic CO_2 production were measured in Warburg respirometers. The CO_2 production was measured by the two vessel method discussed by French, Kohn, and Tang (1934–35). The shaker speed necessary to insure freedom from the limits set by diffusion was *ca.* 120 complete oscillations per minute with an amplitude of 8 cm. As the rate of respiration declined the time between readings was increased so that the experimental error of reading manometer deflections was held to within ±2.5 per cent. Inaccuracies due to the retention of CO_2 by Na_2HPO_4 when this substance is used in preparing buffer solutions for manometric measurements of metabolism were reduced to a minimum by using only KH_2PO_4, as recommended by Krebs (1928). Addition of acid at the end of an experiment was thus unnecessary.

The growth of the yeast respiring in sterile M/15 KH_2PO_4 solution was determined by means of the densitometer. Two samples were prepared, one at the original concentration of yeast and another diluted with sterile primary phosphate solution to a concentration sufficiently low to eliminate any growth inhibition due to crowding (*cf.* Stephenson, 1930[4]). The samples were kept in densitometer tubes which were immersed in a thermostat. No growth was ever observed in pure phosphate solutions. The number of cells taking up methylene blue at the beginning and end of the experiment was found to be low and constant (*cf.* Richards, 1932, for details regarding the significance of this method of estimating the number of "dead" cells). Care was exercised to maintain reasonable sterility during all phases of an experiment. Microscopic examination after Gram staining revealed no bacterial growth even in experiments lasting 48 hours.

All (or representative) samples from the Warburg vessels were tested for change in pH by two methods, indicator and quinhydrone electrode with calomel half-cell, and found to remain constant within 0.1 pH unit during 24 hours time.

III

The Rate of Endogenous Respiration as a Function of Time in a Non-Nutrient Medium

Few studies of yeast under starvation conditions do more than state that the rate of O_2 consumption or CO_2 production declines with time.

[4] P. 26.

Geiger-Huber (1934) in a recent paper figures both differential and integral curves for the oxygen consumption of Delft beer-yeast suspended in M/15 KH_2PO_4 solution showing an initial rapid decline in rate to a constant level which persisted for the duration of the experiment, but he does not give a kinetic analysis of his data.

If bakers' yeast is suspended in a pure phosphate buffer medium the subsequent rate of respiratory exchange is a function of both the initial age of the cells and the time in the medium. If a young (24 hour) culture is used, the rate of respiration declines with time from

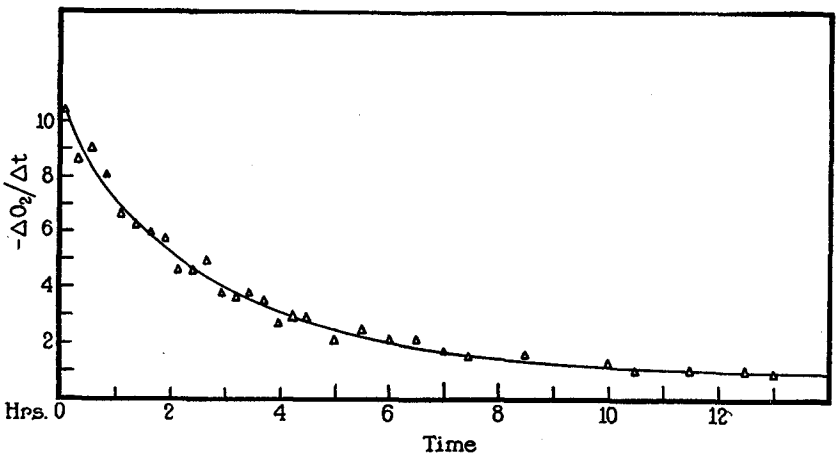

FIG. 1. The rate of O_2 consumption (c.mm./10 min.) as a function of time for a 16 hour culture suspended in M/15 KH_2PO_4 solution at pH 4.5. (Experiment at 25°C.; Strain 4360 cultured at 25°C.)

the first point while in older cultures a varying period of constant rate precedes the decline, which is somewhat slower in this case. For convenience we have termed these rate-time relations the "dissimilation curve." The events occurring in young cultures will be discussed first, followed by a short account of the influence of the age of the cell on the course of the dissimilation curve.

Young Cultures

Fig. 1 illustrates the relation of O_2 consumption to time in a 16 hour culture. The experiment shown in this figure was continued to 48 hours total time. The rate merely approached zero asymptotically.

At the close of the experiment only a slight increase was detected in the number of cells taking up methylene blue and 0.05 cc. seeded into fresh Williams' medium grew prolifically.

The KOH in the inset of the Warburg vessel was renewed at intervals with no consequent change in rate, and the addition of filter paper to the well to present greater surface for absorption of the CO_2 produced (Dixon and Elliott, 1930) was without effect. The partial pressure of oxygen was maintained well above the critical value throughout the experiment (Tang, 1933; Tang and French, 1933; French, 1934) by frequently opening the vessels to the air, removing the side arm stoppers, and drawing air through (*cf.* French, 1934). It is probably safe to assume the critical pO_2 for this endogenous reaction to be at least as low as that for the respiration of added sugar. (Stier (1935–36) shows that this assumption is probably justified.)

We feel that the decline in rate of respiration has real significance and is not due to the death of any significant percentage of the cells or to technical artifacts.

The Order of the Reaction

The shapes of both the differential and integral curves (*cf.* Figs. 1 and 2) for the declining rate type of curve were regular enough to suggest kinetic analysis. We assume that a single reaction is involved, *viz.* the decomposition of some substrate, designated for the present as *S*. (*Cf.* French, Kohn, and Tang, 1934–35, for an illustrative case where more than one reaction is involved.) The total amount of O_2 consumed or of CO_2 produced was assumed a direct measure of the amount of internally stored substrate utilized. Since the R.Q. (CO_2/O_2) equals 1 throughout the course of the reaction (*cf.* Fig. 2) the products of the reaction are designated provisionally as CO_2 and H_2O. We may then write the decomposition of *S* as:

$$(1) \qquad S \xrightarrow[O_2]{\text{Enzymes}} CO_2 + H_2O.$$

This predicts that the concentration of substrate might limit the rate of O_2 consumption and of CO_2 production and that the reaction is first order with respect to time as long as neither the partial pressure of O_2 nor the enzyme concentration is limiting. That the rate of autofermentation of living yeast is limited by the concentration of

substrate has been suggested many times (Harden and Paine, 1912, Harden, 1932) and most recently by Belitzer (1934). Our evidence verifies this suggestion.

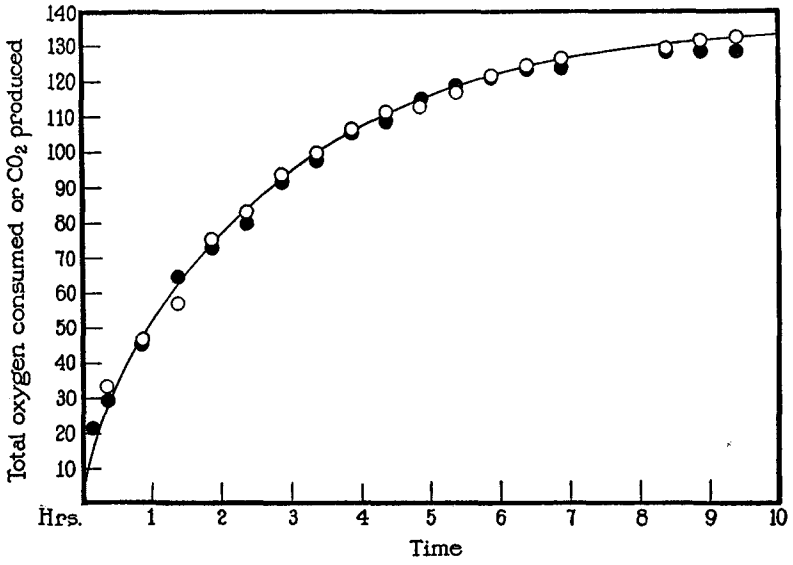

FIG. 2. Integral curve showing the total amounts (c.mm.) of O_2 consumed and CO_2 produced as a function of time at 23°C. in M/15 KH_2PO_4. The open circles represent O_2 consumption and the solid circles CO_2 production. The R.Q. = 1 throughout. (Strain 4360 cultured at 25°C.)

Data obtained in 15 experiments were tested by the following method:

Let

y = c.mm. O_2 consumed in a time, t
A = total c.mm. O_2 consumed or CO_2 produced; *i.e.*, the asymptotic value
a = original concentration of substrate, S
x = amount of substrate consumed in t minutes
k = velocity constant for decomposition of S
k' = velocity constant for oxygen consumption and CO_2 production

If the process is first order, *i.e.* pseudo-unimolecular,

$$\frac{dx}{dt} = K(a - x) \tag{2}$$

which gives on integration the usual expression:

$$k = \frac{1}{t} \ln \frac{a}{(a - x)} \tag{3}$$

A, the asymptotic value for the O_2 consumed or CO_2 produced, obtained from the integral curves, may be taken as proportional to the total amount of substrate S, and y, the amount of gas exchanged in the time t may be taken as proportional to x. In other words:

Let

$$y = cx$$

$$A = ca$$

Then:

$$\frac{dy}{dt} = \frac{k}{c}(A - y) = k'(A - y), \tag{4}$$

and

$$y = A(1 - e^{-k't}), \tag{5}$$

whence we obtain the expression

$$\ln\left(1 - \frac{y}{A}\right) = -k't. \tag{6}$$

Plotting $\ln\left(1 - \frac{y}{A}\right)$ against time should then give a straight line with slope $-k'$. Fig. 3 is an example of the fit obtained when data for the endogenous respiration of young cells are plotted in this manner. The velocity constant $-k'$ was calculated as 2.303 times the slope of the line since logarithms to the base 10 were used. The data used in plotting Fig. 3 are shown in Table I. Log rate against time also gives a linear relation, but we preferred the method shown in Table I and Fig. 3 in order to obtain the first order velocity constant.

A further test for the first order character of such reactions has been suggested in another connection by French, Kohn, and Tang (1934–35) for *Chlorella pyrenoidosa*, *viz.* plotting the rate at any time, dt, against the *total* amount of oxygen consumed in any total time, t. This method makes it unnecessary in the case of a single reaction to

determine the asymptotic value for y (*i.e.* A), and is useful when velocity constants are not desired as a quick test for the order of the reaction. It has further application when a constant basal rate obscures the order of a superimposed reaction.

The validity of the assumption that the concentration of substrate S is the rate-limiting concentration when oxygen consumption or CO_2 production is measured has been subjected to further tests. It has been shown already that pO_2 or concentration of oxygen in the cell is probably not rate-limiting. Following the data presented by Rubner (1913) and discussed by Rahn (1932)[5], showing

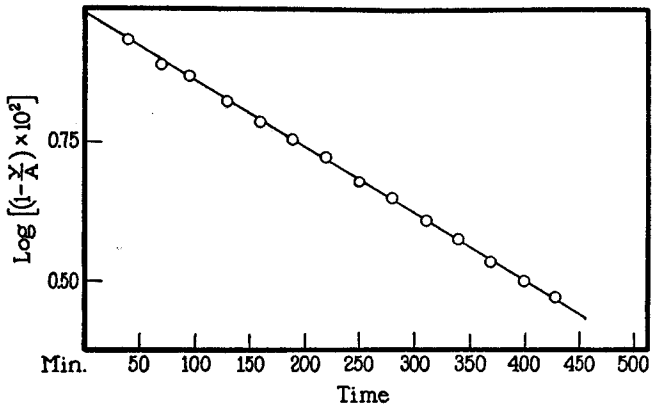

FIG. 3. Test for the first order character of the declining rate portion of the "dissimilation curve." The points plotted are those calculated in Table II. The value of the first order velocity constant, $-k' = 0.0029$. (Experiment at 20°C.; GM yeast used.)

that yeast suspended in non-nitrogenous media continually loses organic nitrogen to the medium, it might be supposed that the decline in rate is a reflection of the gradual deterioration of the enzymes involved, signifying a first order decomposition of the catalysts.

This possibility was tested by adding a few crystals of dextrose to the vessels at the end of a long experiment. Within 5 minutes the constant high rate of O_2 consumption and CO_2 production characteristic of yeast in the presence of a plentiful supply of dextrose was reached, and was found to agree quantitatively with that normally obtained with a fresh sample of yeast. A similar experiment is reported by Belitzer (1934). Unless we make the unwarranted assumption that the enzyme or enzymes concerned with endogenous respiration are both inde-

[5] P. 15.

pendent of and more easily decomposed than those involved in exogenous sugar metabolism, we must conclude that enzyme concentration does not limit the rate of endogenous respiration during the declining portion of the dissimilation curve. This constancy of the power to utilize exogenous sugar was shown by Buchner and Mitscherlich (1904) and by Iwanowski and Brzezinski (1934).

The velocity constant for endogenous respiration has a high temperature coefficient (*cf.* later papers), thus eliminating possible physical limitations on the rate; *e.g.*, diffusion.

TABLE I

Sample Calculation for Determining the First Order Velocity Constant

Temperature = 20°C.*		A = 130† ± c.mm. oxygen (or CO_2)		
Time	y	$\frac{y}{A}$	$1-\frac{y}{A}$	$\text{Log}\left[\left(1-\frac{y}{A}\right)\times 10^2\right]$
min.	*c.mm.*			
40	15.8	0.122	0.878	1.9435
70	27.0	0.208	0.792	1.8987
95	34.0	0.262	0.738	1.8681
130	43.0	0.331	0.669	1.8254
160	50.8	0.391	0.609	1.7846
190	55.5	0.427	0.573	1.7582
220	63.5	0.488	0.512	1.7093
250	67.5	0.519	0.481	1.6822
280	71.7	0.552	0.448	1.6513
310	76.5	0.588	0.412	1.6149
340	80.8	0.622	0.378	1.5775
370	84.5	0.650	0.350	1.5441
400	88.0	0.677	0.323	1.5092
430	90.8	0.698	0.302	1.4800

* Most of the experiments were performed at 25°C., but the shape of the curve is more clearly discernible when plotted from data obtained at the lower temperature.

† Characteristic asymptotic value for fresh *GM* yeast.

The asymptotic value, A, varied considerably from culture to culture as well as with the strain of yeast used. It was always higher for the 4360 strain which had just been removed from its culture medium than for the GM yeast. In the case of the latter there is good evidence that some of its carbohydrate reserves were utilized during transit and storage. The characteristic values of A were 400–200 c.mm. O_2 for Strain 4360 and 225–75 c.mm. O_2 for GM yeast. If the cultured cells of Strain 4360 were not washed completely an initial

fraction of the O_2 consumed is, of course, referable to the utilization of the last traces of hexose sugar. This is easily detected as a high initial rate of respiration followed by a sudden drop and a sharp bend on the first order plots. The amount of this fraction must be subtracted from

TABLE II

Endogenous Respiration Showing the Presence of "Residual" Hexose

Time	Rate O_2 uptake	Time	Rate O_2 uptake
min.	*c.mm./10 min.*	*min.*	*c.mm./10 min.*
25	35.7	125	7.5
45	36.8	145	5.3
65	16.8	185	5.5
85	9.2	225	5.1
110	8.9	265	4.7
		305	3.7
		345	3.1

GM yeast in M/15 KH_2PO_4 (pH 4.5); experiment at 25°C.

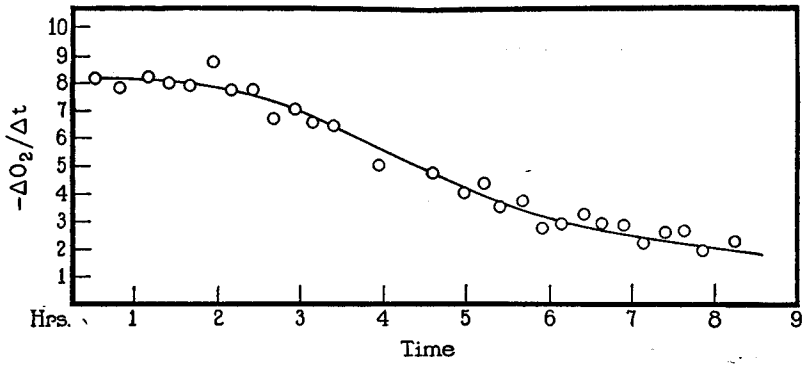

FIG. 4. The relation of the rate of O_2 consumption to time for a 4 day culture. Note that the initial rate is lower than the initial rate for younger cultures (*cf.* Fig. 1), and remains constant for almost 2 hours. The succeeding period of declining rate is only approximately first order. (Experiment at 25°C.; M/15 KH_2PO_4; Strain 4360 cultured at 25°C.)

the total amount of O_2 in determining A. Such a case is illustrated in Table II.

Older Cultures

The relation of the rate of dissimilation to time in a non-nutrient medium seen in either strain of yeast grown for more than *ca.* 24 hours

in Williams' medium at 25–26°C. differs from that seen in younger cultures. The initial rate of O_2 consumption and CO_2 production is lower, and remains constant for a period of time varying with the age of the culture, finally falling off slowly towards zero (*cf.* legend, Fig. 5). Figs. 4 and 5 are differential curves for the time-rate relation in 4 and 10 day old cultures respectively. It will be noted that the value of the y intercept decreases with the age of the culture. A gradual fall in the initial rate of O_2 consumption when yeast metabolizes added dextrose was also observed as a function of age of the culture. These phenomena will be discussed in a separate account.

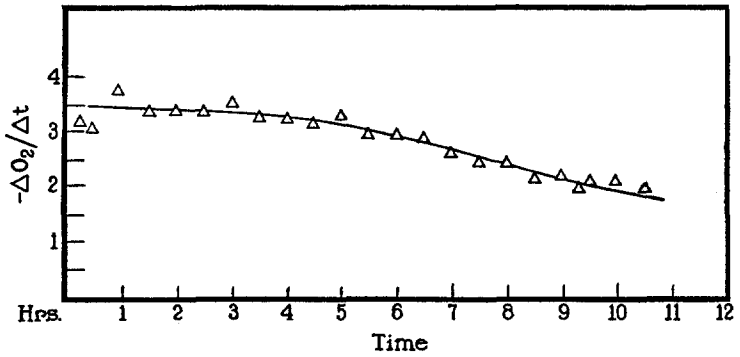

FIG. 5. The relation of the rate of O_2 consumption to time for a 10 day culture. Comparison with Figs. 1 and 4 will show that the initial rate is lower than in either of the previous cases and remains constant for a longer time (4–5 hours). Only a part of the declining portion of the rate curve is shown. It is characteristic that this portion of the curve often becomes less like a first order decline as the culture age increases. This awaits further investigation. See text. (Experiment at 25°C.; M/15 KH_2PO_4; Strain 4360 cultured at 25°C.)

The R.Q. is equal to 1 for the older cultures as well as for the younger cultures, and the characteristics of the endogenous metabolism (considered in the following paper) indicate that the two portions of the dissimilation curve are definitely aspects of the operation of a single system. The instantaneous rate of respiration in one phase is controlled by the substrate concentration and in the other presumably by the enzyme concentration. In this connection attention is called to the recent work of Hopkins and Roberts (1935) on the kinetics of alcoholic fermentation in the presence of glucose. At low sugar concentrations and high yeast concentrations a first order relation was

found between the rate of fermentation and time. They show that the various phases seen in the kinetics of alcoholic fermentation are

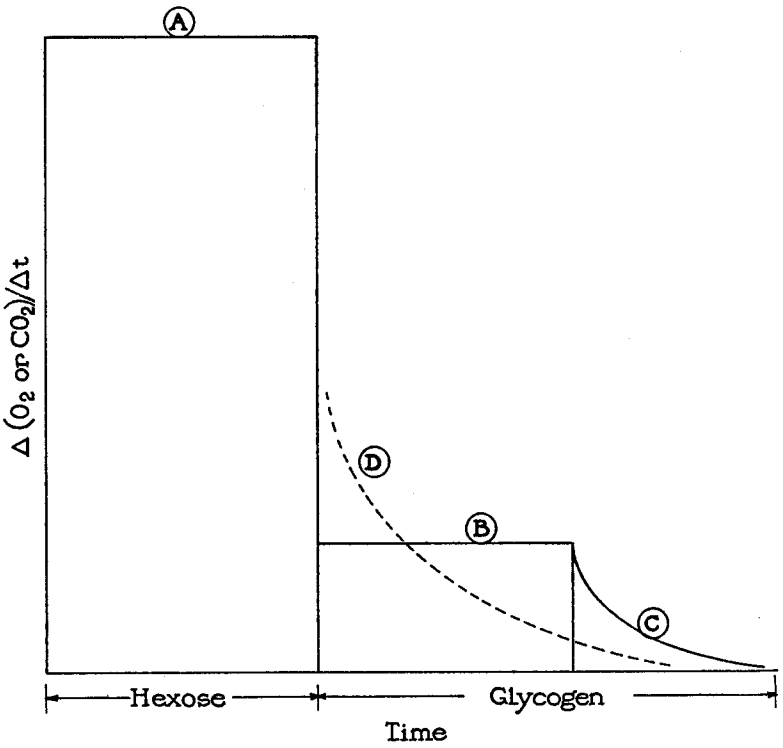

FIG. 6. Diagrammatic summary of the types of respiration seen in bakers' yeast. Part *A* represents the relation seen when bakers' yeast is suspended in dextrose-buffer solution or when the yeast, suspended in pure phosphate buffer, dissimilates the last traces of dextrose remaining within the cell (or adsorbed on its surface, *cf.* Wertheimer, 1934). In the former case the high rate of respiration is maintained for several hours; in the latter, the rate falls rapidly to the lower levels shown. Usually the rate of CO_2 production for part *A* is higher than the rate of O_2 consumption; *i.e.*, the R.Q. is greater than 1. Parts *B* and *C* refer to the dissimilation curve as seen for cultures more than 24 hours old—a period of constant rate followed by a declining rate. Part *D* refers to the first order decline characteristic of young cells in phosphate buffer. The R.Q. = 1 for parts *B*, *C*, and *D*.

but special cases of a general kinetic relationship between the concentration of added glucose and "enzyme" (yeast concentration).

A diagrammatic summary of the types of respiration seen in bakers' yeast is given in Fig. 6.

It was almost always possible by proper choice of asymptote to obtain first order constants and satisfactory linear relations, except in a few isolated cases (4 out of 19 experiments) where the rate suddenly assumed a constant low value for several hours instead of approaching zero. The R.Q. remained = 1 throughout the entire experiment in two of these cases; the temperature coefficient was low, indicating a peculiar phase in the mobilization of stored substances in which some physical process, possibly diffusion of the substrate from the storage "depots" to the active enzyme surfaces, became rate-limiting. These cases were both obtained with young cultures having higher stores than normal. However,

TABLE III

Change of Respiratory Quotient with Time

Time	$-\Delta O_2/\Delta t$	$\Delta CO_2/\Delta t$	R.Q.
min.	*c.mm. O_2/10 min.*	*c.mm. CO_2/10 min.*	
20	2.5	2.6	1.04
45	2.1	2.0	0.95
215	0.9	0.86	0.95
325	0.95	0.85	0.90
385	0.56	0.51	0.91
470	0.58	—	—
1070	0.61	0.44	0.72
1170	0.57	0.42	0.74

The rates of respiration are averages from two vessels (average deviation as per cent of the mean = ±3; maximum deviation = 6 per cent). From integral curves of these data, R.Q. = 0.89 to 0.90 between 470 and 1170 minutes. (Experiment at 25°C.; M/15 KH_2PO_4; GM yeast.)

other young cultures with high concentrations of stored material did not behave in this way.

In two other experiments the R.Q. gradually lowered to values as low as 0.72 and the rates of respiration were not fitted by a first order equation (*cf.* Table III). This was seen when cells having very low concentrations of stored material were employed, *e.g.* GM yeast stored 2 days at 20°C. or several days at 7°C.; it is possibly associated with the utilization of non-carbohydrate materials (*cf.* Meyerhof, 1925; and Geiger-Huber, 1934).

IV

The analysis presented above indicates that the rate of endogenous respiration is sooner or later limited by the concentration of some

substrate which we designated as *S*. Evidence from the literature, already given, identifies substance *S* as a carbohydrate, probably glycogen. Additional evidence is afforded by the following statements.

Careful chemical analyses of GM yeast performed at the Fleischmann Research Laboratories by Dr. Charles N. Frey (private communication) gave the following results.

1. The amount of CO_2 produced in the absence of added sugar was roughly proportional to the glycogen content of the cells.

2. The relative per cent total nitrogen, in milligrams per gram dry weight, *increases* with time in the absence of added sugar, becoming constant when the glycogen had completely disappeared. This rise corresponded *quantitatively* with the value expected if the stored carbohydrate were the only significant substrate disappearing during the experimental period.

Since analyses for the glycogen content of yeast are laborious and apt to be uncertain, this becomes independent evidence of a corroborative sort that the main substrate during the dissimilation of living yeast cells is not protein or other nitrogenous material. Through the kindness of Dr. P. R. Gast of the Harvard Forest, we obtained some preliminary data for the total nitrogen content of our No. 4360 strain of yeast before and after dissimilation. Our results agree with those obtained by Frey for GM yeast.

We have also made microscopic examinations of the glycogen content of yeast before and after dissimilation, using the iodine staining technique employed by Harden and Rowland (1901) and by Wager and Peniston (1910). A progressive decrease in the amount of red-brown staining material was observed during the course of the dissimilation curve (*cf.* Wager and Peniston, 1910, for further details). However, more direct evidence is necessary before it is decided unconditionally that the substrate concerned is uniformly glycogen and no other carbohydrate.

A reexamination of the enzyme systems involved in the endogenous metabolism of bakers' yeast is presented in the following paper.

SUMMARY

The process of endogenous respiration of two strains of bakers' yeast, *Saccharomyces cerevisiae*, was examined kinetically. The rate of respiration with respect to time in a non-nutrient medium was found to exhibit two phases: (*a*) a period of constant rate of O_2 consumption and CO_2 production (R.Q. = 1) characteristic of cells with ample con-

centrations of stored material; (*b*) a first order decline in rate of respiration with respect to time, where the rate was proportional to the concentration of some substrate, *S*. (R.Q. = 1 throughout second phase.) The nature of this substrate was reexamined and the evidence summarized confirms the notion that it is a carbohydrate, probably glycogen. These phases of endogenous respiration were shown to depend upon the age of the culture and the amount of substrate available.

CITATIONS

Belitzer, W. A., 1934, *Protoplasma*, **22,** 17.

Buchner, E., and Mitscherlich, S., 1904, *Z. physiol. Chem.*, **42,** 554.

Dixon, M., and Elliott, K. A., 1930, *Biochem. J.*, London, **24,** 820.

von Euler, H., and Lindner, P., Chemie der Hefe und der alkoholischen Gärung, Leipsic, Akademische Verlagsgesellschaft, 1915, 141.

French, C. S., 1934, The rates of respiratory processes in *Chlorella pyrenoidosa* as functions of temperature, Thesis, Harvard University.

French, C. S., Kohn, H. I., and Tang, P. S., 1934–35, *J. Gen. Physiol.*, **18,** 193.

Geiger-Huber, M., 1934, *Jahrb. wissensch. Bot.*, **81,** 1.

Harden, A., Alcoholic fermentation, London, Longmans, Green and Co., 1932.

Harden, A., and Paine, S. G., 1912, *Proc. Roy. Soc. London, Series B*, **84,** 448.

Harden, A., and Rowland, S., 1901, *J. Chem. Soc.*, **79,** 1227.

Harden, A., and Young, W. J., 1902, *J. Chem. Soc.*, **81,** 1224.

Hopkins, R. H., and Roberts, R. H., 1935, *Biochem. J.*, London, **29,** 919.

Iwanowski, W., and Brzezinski, H., 1934, *Ann. brass. et dist.*, **32,** 257; through *J. Inst. Brewing*, 1934, **40,** 430.

Kosinski, I., 1902, *Jahrb. wissensch. Bot.*, **37,** 137.

Krebs, H. A., Stoffwechsel der Zellen und Gewebe, in Péterfi, T., Methodik der wissenschaftlichen Biologie, Berlin, Julius Springer, 1928, **2,** 1048.

Meyerhof, O., 1925, *Biochem. Z.*, Berlin, **162,** 43.

Meyerhof, O., and Iwasaki, K., 1930, *Biochem. Z.*, Berlin, **226,** 16.

Oster, R. H., 1934–35, *J. Gen. Physiol.*, **18,** 243.

Rahn, O., Physiology of bacteria, Philadelphia, P. Blakiston's Son and Co., Inc., 1932, 385, 389.

Richards, O. W., 1928, *J. Phys. Chem.*, **32,** 1865. 1932, *Arch. Protistenk.*, **78,** 263.

Rubner, M., 1913, *Arch. Anat. u. Physiol., Physiol. Abt.*, suppl., 1913, 1.

Stephenson, M., Bacterial metabolism, London, Longmans, Green and Co., 1930, 26.

Stier, T. J. B., 1935–36, *J. Gen. Physiol.*, **19,** 339.

Stier, T. J. B., Arnold, W., and Stannard, J. N., 1933–34, *J. Gen. Physiol.*, **17,** 383.

Tang, P. S., 1933, *Quart. Rev. Biol.*, **8,** 260.

Tang, P. S., and French, C. S., 1933, *Chinese J. Physiol.*, **7,** 353.
Wager, H., and Peniston, A., 1910, *Ann. Bot.*, **24,** 45.
Warburg, O., 1927, *Biochem. Z.*, Berlin, **189,** 354.
Warkany, J., 1924, *Biochem. Z.*, Berlin, **150,** 271.
Wertheimer, E., 1934, *Protoplasma*, **21,** 522.
Williams, R. J., 1920, *J. Biol. Chem.*, **42,** 259.
Williams, R. J., Wilson, J. L., and von der Ahe, F. H., 1927, *J. Am. Chem. Soc.*, **49,** 227.