(3000 rev./min. for 20 min.) shows an interspace volume of 23 % with inulin as the test substance, 22 % with gelatin and 24 % with peptone, when expressed as a percentage of the total volume. The interspace fluid can be pressed out with a simple hand press, so that an amount representing only 0–5 % of the total cell volume is left.

2. If the space be measured with arabinose, galactose, or lactose, with allowance for the water abstracted from the cells, it appears as 33–34 %. The difference is due to the penetration of such substances into an outer cell region which is identified with the thick cell wall.

3. The water for solution in this outer region is 0.10–0.11 of the total cell volume. Into this part of the cell many substances penetrate readily, including sodium and potassium chloride; also succinic acid, glyceric acid, etc., but succinate ions are debarred or enter slowly.

4. The inner surface or membrane of the outer region is impermeable to chloride ions in the resting yeast. It is likewise impermeable or only very slowly permeable to succinic acid, glyceric acid and a number of other acids with hydroxyl or amino groups. On the other hand, it is very freely permeable to formic, acetic, propionic and butyric acids.

REFERENCES

- Beetlestone, H. C. J. (1930). *J. Inst. Brew.* **36**, 483.
Boyle, P. & Conway, E. J. (1941). *J. Physiol.* **100**, 1.
Conway, E. J. (1947). *Microdiffusion Analysis and Volumetric Error*, 2nd ed. London: Crosby Lockwood & Son.
Conway, E. J. & Brady, T. G. (1947). *Nature, Lond.*, **159**, 137.
Conway, E. J. & Downey, M. (1948). *Biochem. J.* **42**, lxiii.
Hevesey, G. & Nielsen, N. (1941). *Acta physiol. scand.* **2**, 347.
Just, F. (1940). *Biochem. Z.* **306**, 33.
Krebs, H. A., Smyth, E. H. & Evans, E. A. (1940). *Biochem. J.* **34**, 1041.
Malm, M. (1947). *Ark. Kemi Min. Geol.* **25** A, no. 1.
Montgomery, E. A. V. & White, J. (1945). *J. Inst. Brew.* **51**, 279.
Orskov, S. L. (1945). *Acta path. microbiol. scand.* **22**, 523.
Rapoport, M. (1937). *Biochem. Z.* **291**, 429.
Steinitz, R. (1938). *J. biol. Chem.* **126**, 589.
Weil-Malherbe, H. (1937). *Biochem. J.* **31**, 299.

pH Values of the Yeast Cell

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In the previous communication (Conway & Downey, 1950) it was shown that the yeast cell contained an outer metabolic region, identified as the thick cell wall, which had a content of water for free solution equivalent to about 0.10 of the whole cell volume or about 15 % of the total water of the cell.

In the resting cell the pH of this outer region appears to be very similar to that of the overall or total pH figure as determined by the different methods used here, but in the fermenting cell the overall pH increases markedly while the pH of the outer region decreases. In other words, during fermentation the inner part of the cell or cytoplasm becomes more alkaline while the cell wall itself becomes more acid. At an early stage in the study of yeast acidity (Conway & O'Malley, 1943) we found an increase of the total pH, but assigned it to changed combinations of ionizing groups. Brandt (1945) describes similar alkaline changes, but did not connect them with a corresponding production of acid.

Three methods are used here to determine the overall pH of the resting cell. In the first of these,

cells of the centrifuged yeast are frozen with liquid air or oxygen and then thawed rapidly, the pH being at once determined by the glass electrode when the mixture had reached room temperature (20°).

In the second method, the fact that acetic acid very rapidly diffuses throughout the cells (Conway & Downey, 1950) was used to determine, by its distribution, the pH inside the cell. As a third method, the carbonic acid system was used in essentially the same way as acetic acid. The advantage with acetic or carbonic acid lies in the cells remaining uninjured, but in fact all three methods give the same results within the sampling error.

Brandt (1945) has given a comprehensive account of the literature relating to this subject, and has also described a method for determining the pH which consists in rapidly heating a thick suspension, by driving it through a coiled copper tube immersed in boiling water. The cells in this way are rapidly destroyed. The results obtained by Brandt's method are higher than we have obtained for resting baker's yeast by the three methods mentioned above, and we have found that heating in such a way has the

effect also of giving high pH values with our yeast samples. We believe, therefore, that the heating method gives figures in excess of the true value. At the same time it is very serviceable for the study of pH changes.

We have endeavoured to obtain a figure for the resting pH of the outer region, or cell wall, by utilizing the fact that such an organic acid as glyceric acid does not enter the central region at an appreciable rate, and its distribution can be studied in a similar way to that of acetic acid for the whole cell.

For pH values during fermentation the freezing and carbon dioxide methods were used for overall values, and the distribution of the succinate system was used to determine relatively large falls in the pH of the outer region.

METHODS

Experimental

The yeast examined, from the Cork Yeast Factory, does not constitute a pure strain, but is made up of various strains of *Saccharomyces cerevisiae*.

Freezing method. Fresh yeast was washed a few times in tap water then pressed so as to extrude the interspace fluid, the interspace volume being reduced to 0–5% of the initial value. The yeast is then friable and has a dry appearance. It was packed into celloidin tubes which were immersed for at least 6 min. in liquid air or O₂, then thawed. In a proportion of cases a small volume of monoiodoacetate solution, to make an overall concentration of 0.005M, was incorporated into the yeast, just thawed, but still at about 0°. pH values were determined by the glass electrode on reaching room temperature.

Acetic acid method. Here 1 kg. of washed centrifuged yeast was suspended in 1 l. 0.02M-acetic acid, and after 15 min. the acetic acid was determined by electrometric titration. From the decrease in the acetic acid concentration the total acid which entered the cells may be calculated, and, knowing the external pH, that inside the cells may be determined as shown in the theoretical section.

The acetic acid removed metabolically under these conditions was likewise investigated with different strengths of acetic acid outside, the acetic acid being determined by a microdiffusion method, the details of which it is hoped to have published shortly (Conway & Downey, 1949). The rate of this disappearance, under the conditions examined, does not appreciably interfere with the method.

Carbon dioxide method. CO₂ at atmospheric pressure was bubbled through 20 ml. of a mixture of baker's yeast containing washed centrifuged yeast in the proportion of 1 kg. to 0.6 l. of tap water, which gives approximately the same volume of cells and external fluid, or alternatively into 0.1M-KCl contained in narrow gas wash bottles. After about 20 min. bubbling, samples were taken for total CO₂ determination (Conway, 1947).

Bicarbonate determination. This could be indirectly determined by subtracting from the total acid-labile CO₂ that due to physical solution in the water of the yeast cell. It was also determined more directly as follows: 1 ml. of the suspension from the cylinders was added to 10 ml. of 2M-dimethylamine

in ice-cold water, and another 1 ml. sample added to a similar volume of 2M-dimethylamine containing 3–4% of a saturated solution of BaCl₂. The insoluble Ba fraction of the CO₂ system was assumed to be bicarbonate. Changes in the soluble Ba fraction of the CO₂ system after treatment with dimethylamine were assumed to be acid-labile CO₂ other than free CO₂ plus bicarbonate, and would include changes in possible carbamino fraction. Bubbling water, or 0.1M-KCl solution, with CO₂ gas gave no insoluble Ba fraction with this dimethylamine technique.

From a knowledge of the bicarbonate and free CO₂ in the yeast cell water, the pH of the cell may be calculated.

Glyceric acid method for the pH of the outer region. Here washed and centrifuged yeast was mixed 1 : 1 with glyceric acid of various strengths, centrifuged after 15 min. and the glyceric acid in the centrifuged fluid determined by electrometric titration as described in the previous paper. In some experiments it was also determined by the colorimetric method of Rapoport (1937).

Succinic acid method for pH changes of the outer region during fermentation. Washed and centrifuged yeast was suspended in 0.6 parts of a 5% (w/v) glucose solution in tap water. After 30 min. samples of the whole mixture, and of the centrifuged fluid, were analysed for succinic acid using the oxidase method (Weil-Malherbe, 1937; Krebs, Smyth & Evans, 1940); pH values of the suspension of centrifuged fluid were taken by the glass electrode. Examination of the data obtained gives evidence for change in pH of the outer region.

Theoretical formulae for pH calculations

Acetic acid method. It is assumed that the free acid diffuses to equality of concentration, C_a . If C_m is the total concentration of the acetate system outside the cells after suspending the yeast in the acetic acid solution, then

$$C_m = C_a (1 + K'_m/h_m). \quad (1)$$

Similarly, if C_i is the total concentration within the cell

$$C_i = C_a (1 + K'_i/h_i). \quad (2)$$

Here h_m , h_i are the hydrogen ion concentrations outside and inside the cells, and K'_m and K'_i the adjusted ionization constant for acetic acid in the suspending fluid and inside the cells.

A useful value R has been previously defined (Conway & Downey, 1950) as the moles of a substance that have entered 1 kg. of washed cells divided by the external concentration at the time of observation. It may be written

$$R = C_i S / C_m, \quad (3)$$

where C_i is the intracellular concentration and S the solvent water in the cell as l./kg. cells. From Eqns. 1, 2 and 3

$$R = \frac{(1 + K'_i/h_i)}{(1 + K'_m/h_m)} \cdot S. \quad (4)$$

But it has been shown (Conway & Downey, 1950) that R may also be expressed as

$$R = (C_i/C_m - 1.22 - w)/0.78, \quad (5)$$

when 1 kg. of washed centrifuged yeast is suspended in 1 l. of fluid. Here C_o is the concentration in the suspending fluid before mixing, w being the water abstracted osmotically from the cells, and if 0.02M solutions are used it may be neglected.

From Eqns. 4 and 5, h_i may be calculated, since C_o , C_m , h_m , S , K'_m and K'_i are determinable. Values obtained for the last two were 4.7 and 4.6, using simplified Debye-Hückel calculations, assuming the ionic strength inside the cell to be about 0.15–0.25. For the washed yeast the value of S has been determined as 0.70 l./kg.

The pH so determined must in turn be corrected (to obtain the resting yeast) value for the acidification within the cell caused by the accumulation of acetic acid. When the latter is known the correction may be determined either by direct observations of pH changes when acetic acid is added to frozen and thawed yeast, or it may be calculated from the average buffering of the yeast cell over such pH range and dividing this into the concentration of acetic acid anions. The buffering was found to be 63 m-equiv./l. of cell water.

Carbonic acid method. From a knowledge of the free CO_2 and the bicarbonate ion content of the yeast cell water, the Henderson-Hasselbalch equation in the form

$$\text{pH} = 6.2 + \log [\text{HCO}_3]/42.1 \quad (6)$$

was applied where 6.2 is the adjusted first apparent pK value of carbonic acid at 18°, the temperature of the observations.

RESULTS

Resting yeast cells

Freezing method. The mean value for eighteen determinations after freezing with liquid oxygen, then rapidly thawing to room temperature, was 5.80 ± 0.2 (giving S.D. of mean). The presence of 0.005M-sodium monoiodoacetate did not affect this value, within the sampling error, the mean of fourteen observations being 5.81 ± 0.02 .

If, without monoiodoacetate, the thawed suspension is allowed to stand, the pH falls progressively. Thus a sample immediately after thawing to room temperature gave a pH value of 5.80. After 2 hr. at the same temperature it gave a value of 5.58, and 5.59 after 4 hr.

Acetic acid method. Table 1 shows the results of six experiments in which 1 kg. of washed centrifuged yeast was suspended in 1 l. of 0.02M-acetic acid. The mean value of pH in the presence of 0.02M-acetic acid, determined in accordance with the previous theoretical section, was 5.44 ± 0.05 . The mean correction to be added to this for the acetic acid in the cell is calculated as follows.

The mean value of C_m after suspending is 4.66 m-equiv./l., or outside 0.78 kg. cells, the amount in 1 kg. of centrifuged yeast, there are $4.66 \times 1.22 = 5.15$ m-equiv. so that $20 - 5.15$ are present in the cells, the water of which is $0.78 \times 0.70 = 0.546$ l. The concentration of total acetic acid in the cell water is thus $14.85/0.546 = 27.2$ m-equiv./l. At a pH of 5.44 and pK value of 4.6, acetic acid is 87% ionized, so that 0.87×27.2 or 23.6 m-equiv. acetate ions have been formed. Dividing this by the buffering, 63 m-equiv./l. gives the correction as 0.37 pH.

The resting pH of the washed cells should then be $5.44 + 0.37 = 5.81$. Where the correction is applied to each experiment the result is the same and the pH may be expressed as 5.81 ± 0.06 (S.D. of mean; here the variability is a little higher than that obtained for the uncorrected pH which is 5.44 ± 0.05).

The possibility that the metabolic disappearance of acetic acid might influence the results obtained, necessitated the following control measurements. The centrifuged yeast was immersed in the proportion of 1 kg. to 1 l. of 0.2, 0.05 and 0.02M-acetic acid made up in tap water. At various times, samples of the suspension were taken, centrifuged and the acetic acid, as volatile acid other than carbon dioxide, determined. In addition, experiments were carried out where tap water was used to suspend the yeast instead of the acetic acid solutions. The results are shown in Table 2. It will be seen that there is very little disappearance of the acetic acid after the initial entrance, which occurs very rapidly. With the 0.2M solutions we have followed the change of pH on mixing as an index of the entrance rate, but the diffusion appeared to be practically complete in less than 1 min.

It will be seen that after 24 hr. there is a marked relative increase of the external acetic acid when using 0.02M-acid for suspending. This is related to a corresponding increase in acetic acid using water only as the suspending fluid. It is not certain that the acid determined in the latter case is acetic, but, for the present purpose, this has no practical significance.

Carbonic acid method. Twenty-six determinations of the total acid-labile carbon dioxide were carried out with a mixture of the proportions 1 kg. of centrifuged yeast to 0.6 l. of tap water, the mixture being

Table 1. *Intracellular pH of yeast as determined by the acetic acid method*

(Washed centrifuged yeast was suspended in 0.02M-acetic acid (1 kg. to 1 l.) at room temperature. After 15 min. the suspension was centrifuged and the supernatant fluid examined for its acetic acid content. $C_o = 20$ mmol./l.)

C_m (mmol./l.)	$R = (C_o/C_m - 1.22)/0.78$	R/S ($S = 0.70$)	pH (extracellular)	pH (intracellular calc.)	pH correction for acetic effect	pH (intracellular corr.)
6.20	2.57	3.69	4.29	5.22	+0.30	5.52
4.16	4.61	6.59	4.15	5.47	+0.40	5.87
4.07	4.73	6.76	4.20	5.50	+0.40	5.90
4.94	3.62	5.17	3.99	5.36	+0.33	5.69
3.87	5.06	7.23	4.12	5.52	+0.40	5.92
4.72	3.86	5.51	4.61	5.55	+0.38	5.93

Table 2. *Changes of acetic acid concentration in yeast suspensions*

(1 kg. washed centrifuged yeast to 1 l. of acetic acid solution in tap water.)

Suspension fluid	Acetic acid 200 mmol./l. (one series)	Acetic acid 50 mmol./l. (one series)	Acetic acid 20 mmol./l. (two series)	Tap water (two series)
Time (min.)	Acetic acid concentrations in fluid outside cells (mmol./l.)			
3	76.2	15.8	6.9	1.3
15	76.2	15.1	5.8	—
30	75.2	14.6	5.4	1.8
60	72.0	13.8	5.1	—
120	72.0	13.3	5.2	—
180	73.4	12.3	5.9	2.5
1440	76.0	12.5	9.4	4.2

Table 3. *Mean pH values for resting and fermenting yeast*

Method	No. of determinations	Mean pH found	Mean pH in the washed cells
RESTING YEAST			
<i>Overall pH</i>			
Freezing method:			
(a) without moniodoacetate	18	5.80 ± 0.02	5.80 ± 0.02
(b) with moniodoacetate	14	5.81 ± 0.02	5.81 ± 0.02
Acetic acid method	6	5.54 ± 0.05	5.81 ± 0.065
Carbonic acid method:			
(a) without dimethylamine	26	5.62 ± 0.05	5.85 ± 0.05
(b) with dimethylamine	9	5.66 ± 0.05	5.85 ± 0.05
<i>pH of outer region</i>			
Glyceric acid method	4	5.48 (extra-cellular glyceric concn. 0.0039M)	5.95 (deduced from 10 exps.)
FERMENTING YEAST			
<i>Overall pH</i>			
Freezing method	5	6.35 ± 0.05	6.35 ± 0.05
<i>pH of outer region</i>			
Succinic acid method	3	<4.2	<4.2

bubbled with carbon dioxide at atmospheric pressure as described under the Methods section, the same stream of carbon dioxide being passed through the suspending water without yeast. The mean acid-labile carbon dioxide in 1 l. of mixture was 41.5 ± 1.2 mmol. (S.D. of mean).

Centrifuged yeast (1 kg.) contains 0.78 kg. cells and 0.22 l. interspace water. The specific gravity of the yeast cells was determined as 1.077, so that 1 kg. centrifuged yeast contained 0.72 l. of cells and 0.22 l. interspace water. When added to 0.6 l. water there was altogether present in 1 l. of the mixture 0.51 kg. cells (or 0.47 l.) and 0.53 l. interspace water. In 0.51 kg. cells there is 0.36 l. of cell water (an average of 0.70 kg. water/kg. yeast cells was found after washing). From such data it will appear that in 0.36 l. of cell fluid there are 4.0 mmol. acid-labile CO_2 ($41.5 - 42.1 \times 0.89$) not present as dissolved gas ($0.89 = \text{water/l.}$).

Assuming this to be entirely or almost entirely present as bicarbonate ion, then per litre of cell water there are present 11.0 ± 1.2 m-equiv. HCO_3^- and 42.1 m-equiv. free carbon dioxide. From the Eqn. 6 and pK of 6.2 the pH of the cell appears as 5.62 ± 0.05 .

Direct determination of HCO_3^- from the dimethylamine and barium chloride procedure. Using this method the direct determination of the HCO_3^- gave a mean value of 11.8 ± 1.2 for nine determinations. The difference from the previous figure, however, falls well within the sampling error.

From such determinations of the HCO_3^- ion the pH appears from Eqn. 6 as 5.66 ± 0.05 .

The effect of the carbon dioxide in lowering the pH of the cell must now be taken into account. From the direct measurement of the bicarbonate and assuming normally a negligible bicarbonate content the correction is $11.8/63 - 0.19$. Hence the pH of the resting cell without carbon dioxide may be represented as 5.85 ± 0.05 . The mean pH figures for the different methods are listed in Table 3.

Effect of fermentation on the overall pH

When washed centrifuged yeast was suspended for 30 min. in the proportion of 1 kg. to 1 l. of 5% (w/v) glucose and 0.1M-potassium chloride, the pH of the cell, determined by the freezing method already

given, was found to be much increased. In using the method the mixture was rapidly evacuated of gas before the glass electrode observations. From such experiments the pH was found to be 6.35 ± 0.05 with range from 6.12 to 6.55. Similar determinations made on four out of these five yeast samples in the resting state showed that fermentation resulted in a mean increase in pH of $+0.47$ (range of $+0.29$ to $+0.70$).

pH values of the outer cell region

Resting value for washed centrifuged yeast. As discussed in the previous paper (Conway & Downey, 1950), the outer cell region may be identified with the thick cell wall, the external surface of which has a different permeability from the inner. The water of this region comprises 10–11 % of the whole cell, or 15 % of its water content.

We have used glyceric acid distribution, after suspending washed centrifuged yeast in glyceric acid solutions (1 : 1) of different strengths, to obtain data for the resting pH of this outer region.

Glyceric acid does not enter the central region to any appreciable extent over many hours. With the existence of some buffering in the outer region, as the external glyceric acid is decreased a relatively greater proportion of glyceric acid should enter this part of the cell and, from the distribution, pH values may be deduced. Glyceric acid was mainly determined by electrometric titration (allowing for blank values without glyceric acid) between two pH levels, the colorimetric method being found not very satisfactory, but giving results which were, on the whole, similar to the titration method.

substance used in the fluid after suspending. Here with 1 kg. of yeast having 0.96 kg. of cells instead of 0.78 as for pressed yeast, $w = 0.26M$. Applying Eqn. 4 above where S is taken as 0.09 l./kg. cells (the R value approached as the glyceric acid strength is increased) and K' , and K'_m as $10^{-3.5}$ and $10^{-3.6}$, pH values have been calculated for external glyceric acid concentrations of 0.133M or less (Table 4).

A correction for the effect of the acid on the pH value cannot be applied here as the buffering of the outer region is not directly known. It will appear, however, that if on suspending in an external solution of 0.01M-glyceric acid the pH is 5.4 and if in 0.02M it is 4.98, then on suspending in water it would probably be in the region of 5.7–6.0 so that the resting pH of the outer part of the cell is at, or near to, the resting value for the whole cell.

Change of pH of the outer region during fermentation. With the permeability of the outer surface of the cell wall as described, it would follow that with a low pH outside the cells, a low pH could be expected in the outer region. Evidence of this appears in the succinic acid distribution. After 40 min. fermentation with centrifuged yeast suspended in 0.6 vol. of 5 % (w/v) glucose, there was found an average of 2.0 m-equiv. (0.6–3.7) succinic acid per kg. in the cells and 26 m-equiv./l. externally (range of 19 to 30). With the solvent water in the outer region equal to 0.1 of the whole cell volume, it will be seen that even if the whole succinate system in the cell was then present in the outer region its total concentration would be practically the same as that outside. This implies that the pH is well below the level of 4.2, for at this pH, which corresponds to the

Table 4. Data with glyceric acid and pressed yeast, containing 4 % interspace, for pH calculations of the outer cell region

(1 kg. pressed yeast to 1 l. of glyceric acid. For the water abstracted from the cells the formula $w = 0.26M$ was used, where M is the molarity outside cells after suspending, determined experimentally. R determined from the equation $R = (C_o/C_m - 1.04 - w)/0.96$. Numbers in brackets in second column give number of experiments.)

Glyceric acid concn. outside cells after suspending C_m (mol./l.)	C_m/C_o	w water abstracted (l./kg. yeast)	R	pH _o	pH of outer region (calc.)
0.516	0.78 (3)	0.139	0.09	1.9	—
0.325	0.83 (2)	0.088	0.08	2.2	—
0.133	0.85 (5)	0.036	0.10	2.5	3.60
0.020	0.76 (6)	0.0054	0.26	3.6	4.16
0.0084	0.64 (6)	0.0020	0.51	4.2	4.94
0.0039	0.59 (4)	0.0011	0.64	4.7	5.48

From the results obtained, the data in Table 4 were assembled. The equation used for determining R values was

$$R = (C_o/C_m - 1.04 - w)/0.96, \quad (7)$$

similar to Eqn. 3 of the previous paper. In the previous paper w , as the water abstracted from 1 kg. of washed centrifuged yeast was determined from the relation $w = 0.21M$, where M is the molarity of the

first ionization constant of succinic acid, the total concentration in the outer region would be twice the external value.

DISCUSSION

It is of interest in the present study that the acetic acid method, the carbonic acid method and the direct measurement with glass electrode after freezing and thawing, all give the same average

value of 5.8, within the sampling error, for the total pH of the yeast cell. This in turn provides evidence for the view that the adjusted pK values of 4.6 and 6.2 for acetic and carbonic acids are at least approximately correct for the *in vivo* conditions of the cell. After freezing in liquid air or oxygen and without monoiodoacetate, the thawing to room temperature should be done rapidly and the pH then measured without further delay, as it falls slowly with time.

The average value of 5.8 for the intracellular pH is somewhat subject to the previous history of the yeast. Thus we have found that after prolonged oxygenation (8–48 hr.) it is approximately 6.

The pH of the small outer region, or cell wall (Conway & Downey, 1950), seems in the resting state to be about the same as for the whole cell.

During fermentation marked pH changes occur in the yeast cell. There is a considerable rise of the total or overall pH (carbon dioxide being evacuated). This increase of the pH was shown with 0.1M-potassium chloride in the suspending fluid, but it also occurs without potassium chloride in the suspending fluid but to a somewhat lesser degree (shown in a further communication). Increase in intracellular pH during fermentation was also observed by Brandt (1945).

At the same time there is a marked fall in the pH value of the outer region or cell wall. Such changes have an important bearing on the mechanism of the

production of acidity by yeast, which in turn has a general biological application.

When yeast is suspended in acetic acid solution (1:1) the acid enters very rapidly, and when in 0.2M concentration would appear to have fully entered in less than 1 min. Under the given conditions there is but little metabolic disappearance of the acetic acid, and when suspended in water some volatile acid, other than carbon dioxide and possibly acetic, appears outside the cells.

SUMMARY

1. The overall intracellular pH of resting baker's yeast was determined by three methods, a glass electrode method after freezing with liquid air and thawing out, a carbon dioxide method and an acetic acid method. The mean values obtained were 5.80 ± 0.02 (eighteen determinations), 5.81 ± 0.05 (six determinations) and 5.81 ± 0.02 (twenty-six determinations).

2. The resting pH of the outer region (cell wall) was determined by the distribution of glyceric acid at various concentrations after suspending in glyceric acid solutions. The resting value appears to be the same or nearly the same as for the whole cell.

3. When yeast ferments glucose this outer region becomes markedly acidic and the inner part of the cell more alkaline than in its resting state.

REFERENCES

- Brandt, K. M. (1945). *Acta physiol. scand.* **10**, 30.
Conway, E. J. (1947). *Microdiffusion Analysis and Volumetric Error*, 2nd ed. London: Crosby Lockwood & Son.
Conway, E. J. & Downey, M. (1949). Unpublished observations.
Conway, E. J. & Downey, M. (1950). *Biochem. J.* **47**, 347.
Conway, E. J. & O'Malley, E. (1943). Unpublished observations.
Krebs, H. A., Smyth, E. H. & Evans, E. A. (1940). *Biochem. J.* **34**, 1041.
Rapoport, M. (1937). *Biochem. Z.* **291**, 429.
Weil-Malherbe, H. (1937). *Biochem. J.* **31**, 299.

Biological Production of Acid and Alkali

1. QUANTITATIVE RELATIONS OF SUCCINIC AND CARBONIC ACIDS TO THE POTASSIUM AND HYDROGEN ION EXCHANGE IN FERMENTING YEAST

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When Pulver & Verzár (1940) showed that during short period fermentation potassium was absorbed by yeast and released again towards the end of fermentation, the occurrence appeared to be readily interpretable in terms of the process of potassium accumulation in muscle and other animal cells (Conway & Boyle, 1939; Boyle & Conway, 1941; Conway, 1945; Conway, FitzGerald & MacDougald,

1946; Conway, 1947a). It was quickly found that this special form of potassium absorption in yeast was associated with a high degree of acid formation (Conway & O'Malley, 1942). Direct exchange of K^+ and H^+ ions was recognized, but not considered as the main process until somewhat later (Conway & O'Malley, 1943), when it was shown that a pH as low as 1.7 could be produced by adjusting the con-