6. From these results it appears that compounds of the tricarboxylic acid cycle are synthesized from glycine by a scheme similar to that suggested for another species of *Pseudomonas* grown with glycollate as carbon source (Kornberg & Gotto, 1961*a*).

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The Total Intracellular Concentration of Solutes in Yeast and Other Plant Cells and the Distensibility of the Plant-Cell Wall

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It is primarily intended to determine in yeast and other plant cells the total intracellular molar concentration of free solutes, including ion species, which may be conveniently stated as total intracellular molarity, the molarity of any single solute being the moles/l. of intracellular water (or kg. of such water without appreciable relative error).

In determining total intracellular molarities a variant of the cryoscopic method after breakage of the cell walls by freezing in liquid oxygen and thawing has been used in the experiments described, attention being given to the volume of the extracellular fluid as well as to the possibility of autolytic changes increasing the total molarity in the frozen and thawed mixture. Such changes occur rapidly even at 0° in certain animal tissues (Conway, Geoghegan & McCormack, 1955).

The conversion of freezing-point depression so determined into total molecular concentrations involves the use of a conversion factor which is here taken as 1·86° per unit molarity. This, applied in general, is no doubt approximate but sufficiently accurate for present purposes.

It is also necessary to know the volume of the extracellular water. For centrifuged yeast this has been dealt with in some detail by Conway & Downey (1950), and similar considerations have been applied here to plant tissues in general as described under Methods.

The total intracellular molarity so determined for the resting yeast has been compared with that obtained by summing up the intracellular concentrations of the major constituents as analytically determined.

Measurements have also been made on yeast fermenting under a variety of conditions. Other measurements have been made on potatoes, carrots and sugar-beet, the last-named being of particular interest as an example of a plant tissue which contains, in addition to the usual content of metabolites and electrolytes, an exceptionally high concentration of sucrose inside the cells. The distensibility of plant-cell walls has also been investigated by using varying external concentrations of a non-penetrating solute, and a coefficient of distensibility has been defined. This is easy to determine and may prove useful for comparative investigations.

METHODS

Chemical methods

Amino acids. These were determined on protein-free yeast extracts by (*a*) a modified Folin colorimeter method (Walters, 1937) at wavelength 470 mμ, and (*b*) a micro-modification of the Pope & Stevens (1939) method in which the final titration was made from a Conway burette. Close agreement was obtained with the two methods, indicating that the sulphur-containing amino acids were either absent or present in very small amounts in these extracts.

Total ether-extractable acids. Portions (10 ml.) of yeast suspension (1 part in 6 of tap water) were added to 5 ml. of 10 N-H_2SO_4 in a Kutscher–Steudel type of extractor (Krebs, Smyth & Evans, 1940). The high concentration of acid was used to ensure that the yeast cells would be rapidly ruptured. The extraction was continued for 9 hr. after which the ether extract was dried over anhydrous Na_2SO_4 to remove any mineral acid that might have been carried over during the extraction. After very thorough washing of the Na_2SO_4 with successive portions of dry ether, the combined ether extracts were evaporated to dryness, and the residue was dissolved in 10 ml. of hot water, cooled and titrated electrometrically.

Succinic acid. After the titration the neutral solution was usually transferred to a 25 ml. volumetric flask and made up to volume, and the succinic acid content determined with succinic dehydrogenase (Weil-Malherbe, 1937; Krebs *et al.* 1940).

Hydroxy acids (ether-soluble). These were determined by subtracting succinic acid from the total of ether-extractable acids. The succinic acid amounted to about 70% of the total, the remainder being mainly lactic acid, with some other acids in addition.

Citric acid. This was determined colorimetrically on the protein-free extract by a modified pentabromoacetone method with vanadic acid as oxidant (Weil-Malherbe & Bone, 1949).

Keto acid. The method used was a modification of the colorimetric method developed by Lu (1939). Details were as given by Conway & Brady (1950).

Ortho- and meta-phosphate. A trichloroacetic acid extract of centrifuged yeast cells was first prepared in the manner described by Juni, Kamer, Reiner & Spiegelman (1948), who used it before the fractionation of acid-soluble phosphate.

Inorganic phosphate was precipitated by $Mg(NO_3)_2$ at an alkaline pH (the mixture standing in the refrigerator for 2–3 hr.). Separation of the inorganic phosphate into ortho- and meta-phosphate fractions was effected as described (Juni *et al.* 1948). The phosphate in both fractions was determined by the method of Berenblum & Chain (1938).

Hexose esters. These were determined by the method of Conway & Hingerty (1946), proceeding through a precipitation with neutral alcohol of a sample of the trichloroacetic acid solution after the removal of ATP with barium acetate.

Sodium and potassium. These were determined by flame photometry in a Beckman model DU spectrophotometer with flame attachment. Samples of twice-washed yeast were boiled in 10–20 vol. of distilled water and diluted to give a convenient photometer reading. Before reading, all samples were centrifuged.

Calcium. Twice-washed yeast (1 g.) was boiled for 5 min. with about 5–6 ml. of water. The suspension was then allowed to cool and 2 ml. of 20% trichloroacetic acid added. The total volume was adjusted to 10 ml. with distilled water, the suspension centrifuged, and 5 ml. of the supernatant taken. This was neutralized with dil. NH_3 and the Ca precipitated on addition of 2 ml. of 4% ammonium oxalate. This precipitate was centrifuged, washed with dilute NH_3 solution and dissolved in 2 ml. of N-H_2SO_4, and the oxalic acid liberated was titrated against standard $KMnO_4$ solution.

Magnesium. This was determined by the titan-yellow method of Young & Gill (1951).

Calculation of concentration in the yeast-cell water. The concentrations were expressed first per/kg. of washed and centrifuged yeast. As the amount of cell water in 1 kg. of such yeast is 0·49 l. the concentration/kg. of the centrifuged yeast is multiplied by 1/0·49 = 2·04.

Freezing-point depressions. These were determined by the micro-cryoscopic method of Conway & McCormack (1953). With the apparatus employed, 0·25 ml. or less of tissue fluid was required. The apparatus had a sensitivity of 0·002° and successive readings for a given sample did not normally differ by more than 0·01°.

Baker's yeast (*Saccharomyces cerevisiae*) as supplied by the Cork Yeast factory was used. [This was also the source of the yeast used in the experiments of Conway & Downey (1950).]

Yeast samples for freezing-point measurements were prepared as follows: After the cells had been washed twice in about 20 times their volume of tap water and centrifuged (the second centrifuging being continued for 20 min. at 3000 rev./min.), the supernatant fluid was poured off and the yeast, contained in stoppered plastic tubes, was plunged into liquid O_2 for 6–8 min. The tubes were then removed from the liquid O_2, their contents allowed to thaw, and then immediately reimmersed for a second period of 6–8 min. and allowed to thaw once more. This procedure gave a fluid mixture on which freezing-point measurements could be readily carried out.

The vegetable tissues were cut into thin slices (less than 1 mm. thick) and placed in perforated metal containers

that could be completely immersed in liquid O_2. They were then twice frozen and thawed in a similar manner to the yeast cells, the tissue slices in their containers being kept in a closed vessel during the thawing process in order to avoid excessive condensation on them of atmospheric moisture. Immediately after the second thawing the tissue slices were quickly broken up by grinding them in a small mortar, the resulting material was rapidly centrifuged and the supernatant fluid introduced into the small glass tubes used in the freezing-point apparatus.

All samples for freezing-point determinations were stored frozen in stoppered tubes and were allowed to thaw out immediately before being placed in the apparatus for determining the freezing point.

Measurement of the cell-water volume

First, the total water in vegetable tissues or centrifuged cell species was determined by dry-weight measurements of weighed fresh tissue samples or samples of centrifuged cells from which the supernatant water had been removed. The water between the cells plus that in the cell wall external to the cell membrane (this total being termed throughout here 'extracellular water') was then determined in a similar manner to that adopted for centrifuged yeast by Conway & Downey (1950). Weighed amounts of the finely sliced tissue were immersed in solutions containing various concentrations of a non-penetrating reducing sugar (D-galactose for potato and carrot tissue, and L-arabinose for sugar-beet). The volume of the immersion fluid used was 1 l./kg. of tissue. About 1 hr. was allowed for equilibrium to be established and the concentration of the reducing sugar in the external fluid was determined by the method of Bertrand (Bertho & Grassman, 1938), the ratio of the initial sugar concentration to its concentration at equilibrium only being required. Control suspensions in which the tissues were immersed in distilled water indicated that the amount of reducing sugars lost by the tissues under the conditions of these experiments was quite negligible.

From the data obtained the volume of the intracellular water and related quantities may be derived as shown in the following section, after the symbols used in the calculations have been defined.

Symbols used in the calculations

Where 1 kg. of tissue is mentioned here this may be taken as applicable also to 1 kg. of washed and centrifuged yeast. All volumes are expressed as litres, and volumes and concentrations refer to 1 kg. of the washed tissue before the 1 hr. immersion:

C_0 Molar concentration of the non-penetrating sugar in the immersion fluid used.

C_e Molar concentration of the same sugar in the extracellular fluid, after the immersion procedure described above.

C_i Total molar concentration of constituents within the cells, which are non-diffusible with respect to the membrane.

C_{i0} The same as C_i but with zero concentration in the extracellular fluid.

V_{e0} Volume of extracellular water before the immersion period.

V_{ec} Volume of water coming from cells as the result of immersion in the solution of non-penetrating solute.

V_i Volume of intracellular water in equilibrium with C_e.

V_{i0} Volume of intracellular water when C_e is zero.

Since each litre of immersion solution will be diluted by the addition of V_{e0} plus V_{ec} volumes of water, then:

$$C_e = C_0/(1 + V_{e0} + V_{ec}). \quad (1)$$

From this equation

$$\frac{C_0 - C_e}{C_e} = V_{e0} + V_{ec}. \quad (2)$$

With centrifuged yeast it has been shown (Fig. 2 of Conway & Downey, 1950) that on plotting $(C_0 - C_e)/C_e$ against C_e, a linear relation is obtained up to $C_e = 0{\cdot}7$ M. (The non-penetrating sugars used were L-arabinose and D-galactose and the symbol C_m then used has been changed here to C_e.) When extrapolated to zero external concentration, this line cuts the ordinate at a level indicating the volume of the extracellular fluid (V_{e0})/kg. of the original tissue or the washed and centrifuged yeast. The linear relation indicates that V_{ec} is proportional to the external concentration of the non-penetrating sugar (C_e).

Thus one may write:

$$\frac{V_{ec}}{C_e} = b, \quad (3)$$

where b is the slope of the line.

In this the water abstracted from 1 kg. of vegetable tissue or of centrifuged yeast is considered. For comparative purposes, it is more suitable to deal with the water abstracted from 1 l. of cell fluid, by different sugar concentrations outside, and, starting from the condition where the cells are at equilibrium with water only as the external fluid, one may write:

$$\frac{V_{ec}}{C_e V_{i0}} = \frac{b}{V_{i0}} = d, \quad (4)$$

where d is termed the 'coefficient of distensibility'. It may be defined as the volume of water abstracted from 1 l. of cell water, which had been in equilibrium with external water only, by unit concentration of a non-penetrating solute. Alternatively it may be defined as the gain of cell water per unit concentration of non-penetrating solute when this is replaced by water, the gain in volume being expressed per litre of final cell water.

The greater the value of d the greater the exchange of water in accordance with the above definitions, and hence the greater the distensibility of the cell wall. So far, d may be regarded as an empirical coefficient, but it is hoped to give elsewhere an extended account of its theoretical significance.

RESULTS

Freezing-point depressions of frozen and thawed yeast and total intracellular concentration. Twelve determinations by the method described above gave a mean value of $0{\cdot}680 \pm 0{\cdot}04°$ for the freezing-point depression of frozen and thawed resting yeast. Assuming a freezing-point depression of 1·86°/mole, this corresponds to a mean value of 0·366 M in the frozen and thawed mixture. From the data of Conway & Downey (1950) the total water in the centrifuged yeast is 0·79 l./kg. The total extracellular water (which includes the outer

region considered by Conway & Downey is 0·30 l./kg. The intracellular water (V_{i0}) is therefore 0·49 l./kg. and the molarity of the cell water (C_{i0}) is $0·366 \times 0·79/0·49 = 0·59$ M. It was considered that this value for the total intracellular concentration of resting yeast might differ from the true value owing to the presence, in the fluid obtained from yeast frozen in liquid oxygen and allowed to thaw, of high protein concentrations that might have effects similar to those described by Kahn & Majer (1955).

This possibility was investigated as follows. The effect of storage in the cold (1–2°) on the freezing-point depression of frozen and thawed yeast tissue was first determined. In contrast with results previously reported for certain mammalian tissues (Conway, Geoghegan & McCormack, 1955), the freezing-point depression of yeast increased only very slowly under these conditions and no appreciable change could be detected over a period of 4–5 hr. Samples (2 ml.) of the fluid material obtained after freezing and thawing were then dialysed in the cold against an equal volume of distilled water, the conditions being such that equilibrium was attained in a comparatively short time (2–3 hr.). The total intracellular concentration calculated from the freezing-point depression of the diffusate at equilibrium could then be compared with that obtained from direct measurements on the same sample of frozen and thawed yeast.

The results of four experiments of this kind are shown in Table 1. Taking for each yeast sample the percentage difference between the calculated intracellular molarities from mixture and diffusate the mean figure with the latter procedure was −1·2% less than for the mixture, the range being +1·8 to −3·4. Such differences come within the sampling error, and the results show that no appreciable effect on the freezing-point depression is produced by the protein concentration of the frozen and thawed mixture.

Effect of fermentation on the total intracellular concentration of yeast. In Table 2 the total intracellular concentrations of yeast that had been fermented under a variety of conditions are compared with the mean value found for the resting cells.

In the fermentation experiments 1 in 20 (w/v) of washed and centrifuged yeast was suspended in the external fluid. After fermentation for 5 hr. the suspension was centrifuged, and the yeast was washed twice with distilled water and centrifuged for 20 min. at 3000 rev./min. after the second washing. The freezing-point depression was then determined in the usual way. Data are given

Table 1. *Freezing-point depression of frozen and thawed resting baker's yeast, with calculated total intracellular molarity*

Similar observations with diffusates were obtained by dialysis (0°). The values of C_{i0} are expressed as moles/l. of intracellular fluid in equilibrium with external water.

	Freezing-point depression		Calculated total intracellular molarities		Difference of the intracellular molarity from the diffusate and the mixture (%)
Sample	Frozen and thawed mixture	Diffusate corrected for the extra water used	From frozen and thawed mixture (C_{i0})	From diffusate (C_{i0})	
1	0·715°	0·725°	0·620	0·630	+1·6
2	0·675	0·650	0·585	0·565	−3·4
3	0·620	0·635	0·540	0·550	+1·8
4	0·715	0·675	0·620	0·590	−4·9
Averages	0·681	0·671	0·591	0·584	−1·2

Table 2. *Total intracellular molarities of resting and fermented baker's yeast*

The figures in parentheses refer to numbers of experiments. The mean value of the freezing-point depression of resting yeast is given with the S.E.M. Example of calculation of intracellular molarity:

$$\text{For resting yeast } C_{i0} = \frac{0·680}{1·86} \times \frac{0·786}{(0·786 - 0·30)} = 0·59.$$

Yeast	Freezing-point depression	Total water content (l./kg.)	Intracellular molarities (C_{i0})
Resting	0·680 ± 0·040° (12)	0·786 (2)	0·59
Fermented, glucose (5%)	0·935 (2)	0·755 (2)	0·81
Fermented, glucose (5%) + K^+ (0·2 M)	1·005 (2)	0·755 (2)	0·87
Fermented, glucose (5%) + Na^+ (0·2 M)	1·065 (2)	0·752 (2)	0·92
Fermented, glucose (5%) + Mg^{2+} (0·2 M)	1·025 (2)	0·772 (2)	0·88

in Table 2 for yeast fermented in 5% glucose and in 5% glucose containing 200 m-equiv./l. of Na^+, K^+ or Mg^{2+} ions. Na^+ and K^+ ions were added to the suspensions as citrate, and Mg^{2+} ions as acetate. The mean of the total intracellular molarities from the eight experiments with fermented yeast was $0{\cdot}87 \pm 0{\cdot}045$, or 48% higher than for resting yeast. In the two experiments with glucose alone without added electrolytes, the mean was 0·81 M, or 37% higher than for non-fermenting cells.

Total intracellular molarities for vegetable tissues. In addition to yeast, the other vegetable cells examined were contained in slices of potato, carrot and sugar-beet. The non-penetrating sugars chosen for measurement of the extracellular water, and distensibility coefficient, were D-galactose for potato and carrot and L-arabinose for sugar-beet. The fact that over the 60 min. period of immersion the sugar used did not enter the cells appreciably is shown by the data in Table 3. For centrifuged yeast and sugar-beet the value of $(C_0 - C_e)/C_e$ was followed from 10 min. up to 60 and 90 min. respectively. The sugar (0·12 M) was used in the immersion fluid. For the carrot and potato it was observed from 60 min. with the immersion fluid containing 1·0 M- and 0·5 M-D-galactose for the potato and 1·0 M for the carrot. No significant increase in the value of $(C_0 - C_e)/C_e$ occurs from 10 to 60 min. in the centrifuged yeast, nor from about 30 to 90 min. in the sugar-beet slices. Also no significant increase occurs with the carrot and potato slices from 60 to 120 min.

If the sugar were entering the cells one could write:

$$\frac{C_0 - C_e}{C_e} = V_{e0} + V_e + V_x. \qquad (2a)$$

This equation is similar to equation (2) except that an extra V_x value is introduced giving the extra 'space' into which the external sugar may be said to diffuse when it enters the cells. V_x would increase with the time, so that with the tissue slices of sugar-beet, carrot or potato of less than 1 mm. thickness a steady increase of V_x and hence of the value of $(C_0 - C_e)/C_e$ would occur. From Table 3 it is evident that no significant increase occurs over the experimental period. In Table 4 the intracellular molarities (C_{i0} values) for potato, carrot, sugar-beet and yeast are given, being calculated from the other values listed. An example of the calculation for the potato is given in the Table.

Table 3. *Measurement of extracellular water of tissues*

Tissues were incubated with the non-penetrating solutes given below. For further details see text. Each value of $(C_0 - C_e)/C_e$ is the average of three experiments.

Cell species	Substance used	Concn. used (M)	$(C_0 - C_e)/C_e$ after 10 min.	30 min.	60 min.	90 min.	120 min.
Baker's yeast (centrifuged)	L-Arabinose	0·12	0·31	0·30	0·27	—	—
Baker's yeast (centrifuged)	D-Galactose	0·12	0·31	0·29	0·35	—	—
Sugar-beet	L-Arabinose	0·12	0·25	0·32	0·32	0·33	—
Potato	D-Galactose	1·0	—	—	0·51	—	0·51
Potato	D-Galactose	0·5	—	—	0·31	—	0·31
Carrot	D-Galactose	1·0	—	—	0·43	—	0·43

Table 4. *Total and extracellular water of certain vegetable cells; also intracellular molarities*

The numbers in parentheses give the numbers of experiments; in all cases the figures for the single experiments were close to the mean values. Example of calculation of the intracellular molarity: taking the potato values, the molarity is calculated as

$$\frac{0{\cdot}635}{1{\cdot}86} \times \frac{0{\cdot}86}{(0{\cdot}86 - 0{\cdot}14)} = 0{\cdot}41.$$

The symbols used in the Table are defined under Methods.

Tissue or cell species	Freezing-point depression of frozen and thawed mixture	Total water (l./kg.) $(V_{e0} + V_{i0})$	Extracellular water from Fig. 1 (l./kg.) (V_{e0})	Intracellular molarities C_{i0} (M)
Potato	0·635° (3)	0·86 (4)	0·14	0·41
Carrot	1·045 (3)	0·90 (7)	0·09	0·62
Yeast	0·68 (12)	0·79 (3)	0·30	0·59
Sugar-beet	1·54 (5)	0·77 (3)	0·32	1·42

Comparative distensibilities of plant-cell wall. A practical 'coefficient of distensibility' suitable for comparisons between various plant tissues or cells is given under Methods. As pointed out there the relative amount of cell water per kilogram of tissue will vary with varying extracellular water, and it is the litre of cell water that is suitably taken for comparison.

From the slopes of the lines in Fig. 1 the values of V_{ec}/C_e have been determined, and, applying equation (4), values of 'd' for the potato, the carrot, sugar-beet and yeast have been calculated and are given in Table 5.

Note on the V_{e0} *values.* The V_{e0} values for the plant tissues and centrifuged yeast are given by the points where the lines in Fig. 1 cut the ordinate. The data for centrifuged yeast were taken from the results of Conway & Downey (1950). The value of V_{e0} for yeast so found was 0·30 l./kg. This figure is largely made up of the volume of water between the yeast cells (0·22 l./kg.). There is in addition the water in the outer metabolic region of the cells into which various small molecular substances penetrate (Conway & Downey, 1950).

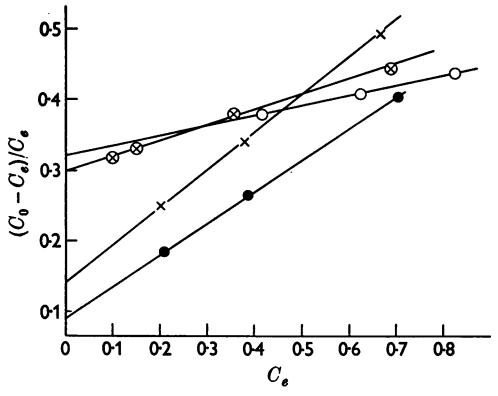

Fig. 1. Values of the external concentration (C_e) of the non-penetrating sugar plotted against the corresponding values of $(C_0-C_e)/C_e = (V_{e0}+V_{ec})$, the symbols being defined under Methods. The data for centrifuged baker's yeast have been taken from the results of Conway & Downey (1950). ×, Potato; ⊗, yeast; ○, sugar-beet; ●, carrot.

The value of 0·22 l./kg. was determined by the use of inulin solutions (the theoretical value for rigid spheres being 26 % of the total volume). This value was very recently confirmed in this Laboratory (J. Veličković, unpublished work). He was extending the investigations of Rasajski & Veličković (1957) and used both inulin and polyvinylpyrrolidone (mol.wt. 38 000) in a number of experiments.

The outer metabolic region, making up the remainder of the 0·30 l./kg., was identified with the cell wall for reasons detailed in the paper of Conway & Downey (1950).

For the V_{e0} value with fermenting yeast no change need be considered, the amount of budding during the 5 hr. of fermentation, under the conditions, making no appreciable change in the volume of water between the centrifuged cells.

Comparison between the total intracellular concentration as determined cryoscopically and analytically in yeast. The total intracellular molarity was found to be 0·59 M in resting, washed yeast. The analytical data in Table 6 show that the sum of the known concentrations of intracellular substances in the yeast cell, of quantitative significance for this purpose, is 378 mM apart from the amino acids. If the amino acid nitrogen were all present in free monoamino acids the amount would be 224 mM of a total of 602 mM. It is very probable at the same time that the figure of 224 mM is somewhat in excess of the true figure through some of the acids being present in di- or tri-peptide form, as well as a proportion existing as the diamino acids. It is also very probable that much of the magnesium and calcium content is not free in the cell water.

It may be concluded, however, that the analytical results with respect to the composition of yeast indicate a total intracellular molarity of a similar order to that found by cryoscopic measurements.

Table 5. *Distensibility coefficients for certain vegetable cells*

The values of V_{i0} were obtained from the values of $(V_{e0}+V_{i0})$ and of V_{e0} in Table 4. The ratios V_{ec}/C_e were obtained from the slopes of the lines in Fig. 1. The values of C_{i0} (intracellular molarities) are given from Table 4 for comparison with the 'd' values.

Tissue or cell species	Intracellular water (V_{i0})	Ratio of water abstracted from cells divided by $C_e=(V_{ec}/C_e)$	Distensibility coefficients (d or V_{ec}/C_eV_{i0})	Intracellular molarities (C_{i0})
Potato	0·72	0·531	0·74	0·41
Carrot	0·81	0·440	0·55	0·62
Yeast				
Resting	0·49	0·214	0·44	0·59
Fermented	—	—	—	0·92
Sugar-beet	0·45	0·147	0·33	1·42

Table 6. *Approximate total intracellular molarity of resting baker's yeast as determined by chemical analyses*

(B) indicates that such data were received from Professor T. Brady, University College, Cork (personal communication).

Substance	m-moles/kg. of resting yeast	No. of observations	Range observed	m-moles/l. of cell water
Potassium	128	34	102–148	261·8
Amino acids (B)	110	3	—	224·0
Orthophosphate	13·2	5	10·8–15·0	26·9
Succinic acid (B)	13·2	3	—	26·9
Magnesium	7·1	8	7·4–8·7	14·5
Hydroxy acids (ether-soluble) (B)	5·2	3	—	10·6
Hexose esters	4·7	5	4·4–5·4	9·6
Calcium	4·7	8	4·0–5·1	9·6
Citric acid (B)	3·5	3	—	7·1
Sodium	2·7	8	2·0–3·3	5·5
Metaphosphate	2·6	5	1·3–3·5	5·3
Keto acid (B)	0·4	3	—	0·8

An intracellular molarity of 0·59 M corresponds to an internal osmotic pressure of 13 atm., but for yeast fermenting in the presence of external Na^+ ions the intracellular molarity was found to be 0·92 M, corresponding approximately to 21 atm. For a sugar-beet sample with high sugar content the intracellular molarity was 1·42 M, corresponding to 32 atm. of osmotic pressure.

Relation between the difference in molarities on each side of the cell wall and the volume of the intracellular water. This relation is derived for yeast from the data given in Table 3 of the paper by Conway & Downey (1950).

If one takes, for example, the value of 0·693 M (mean for D-galactose and L-arabinose experiments) for the external solution when 1 kg. of centrifuged yeast is mixed with 1 l. of the sugar solution, then the value of C_0/C_e is 1·445 so that $(C_0-C_e)/C_e = 0{\cdot}445$. As given under Methods this is equal to $V_{e0}+V_{ec}$. Since V_{e0} is 0·30 (from Fig. 1) V_{ec} is $(0{\cdot}445-0{\cdot}30)$ or 0·145 when there is an external C_e of 0·693 M. As the value for V_{i0} or intracellular water for resting yeast in equilibrium with external water is 0·49 l./kg. the value of V_i in equilibrium with 0·693 M externally is $(0{\cdot}49-0{\cdot}145)$ or 0·345 l. The intracellular molarity is then $0{\cdot}49/(0{\cdot}345\times 0{\cdot}59\,\text{M}) = 0{\cdot}838\,\text{M}$, 0·59 M being the intracellular molarity in equilibrium with water. Proceeding in this way with the other figures in Table 3 of the paper of Conway & Downey (1950), it appears that a linear relation exists between (C_i-C_e) and V_i as shown in Fig. 2. This relation is given by the equation:

$$V_i = (C_i-C_e)\times 0{\cdot}34+0{\cdot}295,$$

or

$$(C_i-C_e)\times 0{\cdot}34 = V_i-0{\cdot}295. \qquad (5)$$

Thus when $C_i = C_e$, $V_i = 0{\cdot}295$, and it is only above this intracellular water volume that the cell wall becomes distended or develops tension, the increase in volume being linearly related to the difference in molarities (or resulting difference in osmotic pressure) of the solutions on each side of the cell wall.

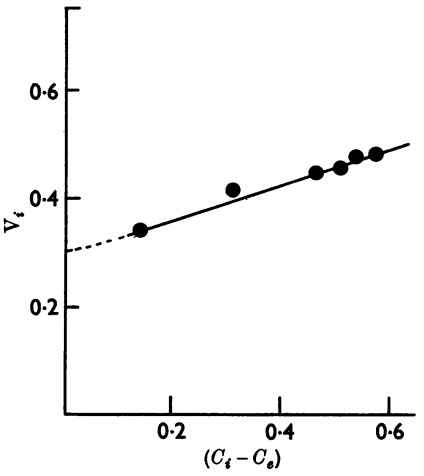

Fig. 2. For baker's yeast, and the non-penetrating sugars D-galactose and L-arabinose the Figure gives the values of (C_i-C_e) or the difference between the intracellular and extracellular molarities, against the values of V_i, the volume of the intracellular fluid (the symbols are defined in Methods). The original data are taken from Table 3 of Conway & Downey (1950).

DISCUSSION

The reality of the high intracellular pressure in plant cells, which even in resting yeast amounts to about 12 atm., is supported by three different kinds of evidence shown here. Thus the cryoscopic data

after bursting the cells by freezing and thawing are of the expected order and agree with the results obtained from the diffusate from this mixture. The total intracellular concentration of solutes in yeast as determined from the sum of the analytical data is in agreement with that indicated by the cryoscopic results; also, there is the fact that from zero to high external concentrations (e.g. to about 0·5M) of a solute to which the cells are impermeable, there is a practically linear relation between the external concentration and the cellular water loss. This is in accordance with a high internal pressure associated with water or dilute solutions outside.

There is in addition to such evidence the experiments of Eddy & Williamson (1957) using the observation of Giaja (1922) that the gut juice of the snail *Helix pomatia* dissolved the cell wall of whole yeast cells. They were able to prepare protoplasts or cells without cell walls which were stable for several hours when suspended in 0·5M-rhamnose. In sugar solutions greater than 0·5M the protoplasts contracted, and with lower concentrations they tended to swell and eventually burst.

Such observations accorded with the evidence given above that the internal concentration of solutes in the resting yeast cells was of the order of 0·5–0·6M. This work of Eddy & Williamson followed the new approach to the structure and functions of the cell surface of certain bacteria as revealed by Weibull (1953), who showed that, in the presence of sucrose, lysozymes dissolved the cell wall, leaving the protoplast essentially intact.

Distensibility of the cell wall. If the classical concept of elasticity and Young's modulus be applied to the cell wall, there arises the difficulty that the thickness of the wall alters with change in volume of the cell; also the thickness of the wall is not easy to determine with exactness. It would seem useful to have a more practical measure of cellular distensibility, such as is given by equation (4).

The results in Table 5 giving the d values of equation (4) for the potato, carrot, yeast and sugar-beet suggest that the total intracellular concentration goes in inverse proportion to the distensibility coefficients, though many more results would be required to test such a hypothesis.

The following comparison may be useful in clarifying the nature of the changes in intracellular volume as the vegetable cells or tissue slices containing them are immersed in solutions of a non-penetrating solute.

One may suppose a porous cylindrical vessel, with a semi-permeable membrane in the wall, and containing a certain concentration of a solute. Surrounding the cylinder is another solution. A piston fits over the surface of the inner fluid closely enough to prevent the passage of fluid between it and the cylinder wall, but otherwise free to move. On entrance of water into the cylinder the solution therein moves the piston up against a spring. When the molarities of the solutions outside and inside the cylindrical vessel are equal, the spring is considered fully extended and exerts no force on the piston. If the external solution is replaced by a weaker solution, water enters the cylinder and the piston presses up against the spring.

Here the compression of the spring is analogous to the distension of the cell walls, and the volume present when the molarities are equal inside and outside is analogous to the 0·295 in equation (5). When the molarity outside is less than inside, the volume of water that enters the cylinder is proportional to the difference between the internal and external molarities at equilibrium.

SUMMARY

1. By means of a cryoscopic method the intracellular molarities of baker's yeast, potato, carrot, and sugar-beet were found to be 0·59 (rising on fermentation to over 0·80), 0·41, 0·62 and 1·42M respectively.

2. The total intracellular molarity of resting yeast from the analysis of its various constituents was of the same order as that determined cryoscopically.

3. A 'coefficient of distensibility' for plant cells has been defined as the volume of intracellular fluid removed from the cells per unit concentration of an external non-penetrating solute and per unit volume of intracellular fluid. It is expressed as V_{ec}/C_eV_{i0}, the symbols being defined in the text. The 'coefficient of distensibility' or 'd' value is relatively easy to determine, as a linear relation has been found between V_{ec} and C_e up to high values of C_e, the external concentration. The values of the 'coefficient' for the potato, carrot, resting baker's yeast and sugar-beet were found to be 0·74, 0·55, 0·44 and 0·33 respectively.

4. The distension of the resting yeast cell, expressed as the volume of the intracellular fluid, gives a linear relation when plotted against the value of $(C_i - C_e)$. Here C_i and C_e are the total intracellular molarities and that of an external non-penetrating solute respectively. Extrapolating the line to zero value of $(C_i - C_e)$ it cuts the ordinate at 0·295 l. of intracellular fluid/kg. of centrifuged yeast.

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Studies in Immunochemistry

20. THE ACTION OF PAPAIN AND FICIN ON BLOOD-GROUP-SPECIFIC SUBSTANCES*

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Studies on the composition and structure of the human blood-group-specific substances, which are macromolecules containing carbohydrate units firmly bound to amino acid residues, have been largely concerned with the carbohydrate components, and the results have contributed much to our understanding of the part played by these structures in the characteristic serological reactions of the specific substances.

The nature and form of the amino acid-containing moiety of the materials has, however, been somewhat neglected and relatively little is known about this component except that it contains a remarkably high content of proline and of the hydroxy amino acids threonine and serine.

In earlier studies on the serologically specific materials isolated from the mucins which occur in human secretions and tissue extracts, proteolytic enzymes were employed to remove protein in the belief that the amino acids present constituted an impurity. Although much extraneous protein could be removed by proteolysis from the native mucins or tissue digests early in the isolation procedure, nevertheless it was found that the final product always contained amino acids. It is now known that amino acids comprise about 15% by weight of all highly purified human blood-group substances, irrespective of their specificity within the ABO and 'Lewis' blood-group systems.

Until recently, it was generally believed that no changes were induced in the materials by digestion with proteolytic enzymes, but a few early observations (Jorpes & Norlin, 1933; Jorpes, 1934; Landsteiner & Harte, 1940) suggested that a modification, or even a destruction of the serological properties of the substances, occurred. However, the changes observed were not further investigated.

In an attempt to obtain the blood-group-specific mucopolysaccharides from human secretions by a method which did not involve the use of phenol (Morgan & King, 1943), native secretions were incubated with ficin at pH 7 (Lawton, McLoughlin & Morgan, 1956). The materials recovered had a much reduced viscosity compared with the mucopolysaccharides isolated from the same native secretions by extraction with phenol, and other properties, such as solubility in 90% phenol and behaviour in the ultracentrifuge, were also changed. There was, at that time, no firm evidence to indicate that digestion with ficin decreased serological activity, but subsequently Pusztai & Morgan (1958) showed that treatment of the materials with ficin or papain lowered their capacity to neutralize the appropriate antibody in haemagglutination-inhibition tests.

* Part 19: Pusztai & Morgan (1961*b*).