The Permeability of the Yeast-Cell.

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The question of the permeability of plant membranes and of the protoplasm lining plant cells has received just attention from time to time, the method usually employed being based upon plasmolysis, a phenomenon first described by Nägeli in 1855 (1), and subsequently investigated by Pfeffer (2), De Vries (3), and Overton (4). The results of their experiments tend to show that purely physical diffusion laws cannot always interpret osmotic phenomena as exhibited by living plant cells, but that in some cases there is evidence of specific permeability.

Nathanson (6) finds that the permeability of protoplasm for any substance is not constant, but varies according to the concentration within and without the cells, and he holds that these variations cannot be accounted for in a purely physical manner.

In 1899 Overton (5), in a series of very comprehensive investigations, observed the similarity existing between solutions in oils and in the ectoplasmic layer of the cytoplasm; he showed especially that many substances could be made to enter the plasma by dissolving them in oils, and suggested the hypothesis that the absorption of such substances by living plants might be due to the presence of lecithin and cholesterol in the plasmatic layer. This hypothesis, however, does not account for the semi-permeability exhibited by various plant membranes towards inorganic salts. Again Adrian Brown (7) has shown that the seed coat of *Hordeum vulgare* exhibits a remarkable degree of impenetrability to strong acids and to metallic salts, while it admits of ready diffusion of such substances as alcohol, aldehyde, acetone, iodine, and certain salts of mercury and cadmium. Armstrong (8) has attempted to explain these results on the theory of "hormones," but it appears to the author that strong support to Overton's hypothesis is afforded by these experiments of Adrian Brown. It has been shown by Overton that iodine and mercuric chloride, as well as the above-mentioned organic substances, are readily soluble in cholesterol. Since these include most of the substances which were found by Brown to be capable of entering the barley grain, it seemed advisable to ascertain whether the remaining substances found by him to enter the seed, namely, cadmium iodide and trichloracetic acid, also possessed this property of solubility

in oils. Of these, cadmium iodide is not soluble in oil or cholesterol, but is known to form a double compound with lecithin; trichloracetic acid was found to dissolve with great readiness in both xylene and cholesterol. It would, however, be unfair to assume from these facts that the presence or absence of cholesterol and lecithin in the cells is the determining factor for the diffusion of these substances. It seems to the author more likely that there may be some characteristic of the molecule, or possibly a determinate size of particle, which gives to certain substances the property of solubility both in oils and in living protoplasm.

Diffusion must play a very important part in the technique of cytological investigation, since proper fixation depends largely upon the rapid and uniform diffusion of such agents as mercuric chloride, iodine, osmic and chromic acids, etc. Variations in diffusion capacity may possibly account in large measure for the differences observable under the influence of the several fixing agents. It is conceivable, for instance, that in nuclear investigations the extreme sharpness of definition with which the astral rays and spindle-fibres have been brought out by some workers may be due to inferior fixation; in other words, to a contraction along lines of dynamic activity, wherein the protoplasm has become so altered as to possess a coefficient of penetrability for the special fixing agent employed which is different from that of the surrounding medium.

The primary object of this research has been to investigate the osmotic behaviour of the yeast-cell towards those substances which have been found to influence alcoholic fermentation. Harden and Young (9) have shown that when phosphates, arsenites or arsenates are added to yeast-juice and sugar a considerable increase of the rate of fermentation is produced. When, however, these substances are applied to living yeast it has been found that in almost all cases no such result occurs. Slator (10) has shown that when neutral potassium phosphate is added to living yeast the only effect produced on the initial rate is that of a small inhibition. Iwanoff (11) on the contrary, and more recently Euler and Lundeqvist (17), have demonstrated a small increase in the total fermentation produced by addition of phosphates to living yeast and glucose, but these effects are not comparable with that produced on yeast-juice.

Another phenomenon of a similar character is observed in the case of hexosephosphates, which are freely hydrolysed and fermented by yeast-juice, but are scarcely affected by living yeast. As the formation and decomposition of hexosephosphates plays an essential part in alcoholic fermentation by yeast-juice, it is a matter of great importance to ascertain whether these salts can penetrate the cell.

The Plasmolysis of Yeast by Various Substances.

Preliminary experiments were first made on the degree of plasmolysis of yeast-cells produced by immersion of the cells in solutions of different substances. For this purpose equal weights of pressed brewer's yeast were intimately mixed with equal volumes of the several solutions and allowed to stand for varying intervals of time. Well-mixed samples were then drawn up into capillary tubes of 10 cm. length, which were afterwards sealed at one end and spun simultaneously in a centrifuge. The columns of residue and of the clear liquid were then measured in millimetres, and from these the ratio of the length of the column of residue to that of the whole column (residue + liquid) was calculated.

In all these experiments wort and 7 per cent. alcohol were employed as standards, the effect produced being practically the same.

Table I.

No.	Solution.	Percentage length of column of residue after—		
		2 hrs.	20 hrs.	70 hrs.
22*a*	Wort	63·7	63·5	63·5
b	Water	70·0	62·2	63·4
c	Alcohol, 7 per cent.	60·5	63·3	63·4
d	Sodium chloride, 0·3 molar.....	59·9	55·2	54·0
e	„ 0·1 „	66·2	61·5	61·0
f	Sodium phosphate, 0·3 molar	63·3	57·1	54·9
g	„ 0·1 „	70·6	63·3	62·5

It was found that when yeast was treated with water the cells at first increased in volume, but later returned to their original state. An initial dilatation also occurred with decimolar solutions of sodium chloride and sodium phosphate, but eventually, in both these cases, a slight amount of plasmolysis was noted. With 0·3 molar concentrations of these substances no increase in volume was observed, and a considerably greater final degree of plasmolysis was produced than was the case with the weaker solutions.

These numbers show, further, that equilibrium is practically established in 20 hours at air temperature, but not in 2 hours.

Adrian Brown (*loc. cit.*) finds that solutions of certain non-electrolytes seem to possess the power of entering the barley grain, whilst others, such as sugar and urea, do not; also that trichloracetic acid, an acid which becomes strongly ionised in dilute solution, enters quite freely. The fact that most of the entering substances are non-electrolytes, he observes,

cannot be taken as an explanation of the diffusion phenomena. A possible solution of the problem has already been advanced (p. 289).

It seemed desirable to ascertain whether these substances would act in a similar manner towards the yeast-cell, it being at first thought that permanent plasmolysis of the cell might be taken as an indication that no diffusion of the dissolved substance into the cell had occurred, an idea which further experiments proved to be untenable (p. 294). The following table contains the results of experiments with acetone, urea, mercuric chloride, cadmium iodide, sulphuric and trichloracetic acids. Since the volumes measured in the narrow tubes were very small, these experiments were made on a larger scale; 50 grm. of pressed yeast were stirred up with 50 c.c. of solutions of the various substances which Adrian Brown found to be of interest. They were allowed to stand for varying lengths of time in the cold room at a temperature ranging from $-2°$ to $+2°$, and were then all spun simultaneously in the centrifuge and the columns of residue and liquid carefully measured. The corresponding volumes were ascertained by gauging the capacity of the vessel.

Table II.

No.	Solute.	Percentage volume of spun residue after—					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	20 hrs.	25 hrs.
176*a*	Sulphuric acid, molar	43·0	41·0	36·5	37·0	36·5	36·5
b	Sodium chloride, molar ...	43·0	43·0	45·0	43·0	40·0	40·0
c	Trichloracetic acid, molar ..	42·0	42·0	37·5	37·5	37·5	37·5
d	Alcohol, 7 p.c. (control) ...	62·0	62·0	58·0	58·0	62·0	60·0
e	Acetone, molar	64·0	62·0	60·0	60·0	64·0	62·0
f	Urea, molar	53·0	57·0	58·0	58·0	61·0	61·0
g	Cadmium iodide, molar ...	43·0	43·0	42·0	42·0	41·0	41·0
h	Mercuric chloride (satd.)...	54·5	50·5	50·5	43·0	38·5	35·5

These results exhibit striking differences when compared with Adrian Brown's experiments. When this observer immersed dried grains of barley in different solutions, water entered as freely and rapidly from solutions of alcohol, acetone, and trichloracetic acid as from pure water, a fact which was interpreted as showing that these substances readily penetrated through the diffusion membrane. In *d* and *e* above alcohol of 7 per cent. and acetone produced no permanent plasmolysis and would seem to diffuse quite readily. Urea also produced no permanent plasmolysis, in striking contrast to Brown's result, where the entrance of water was strongly inhibited. The behaviour of trichloracetic acid also stands in contrast to its behaviour towards the barley grain.

In the experiments with cadmium iodide and mercuric chloride considerable plasmolysis occurred, but this fact cannot be taken to indicate that no diffusion of these substances had taken place, since a marked change was observed in the appearance of the yeast. The cells became much paler in colour and more opaque, while the liquid assumed a dark brownish-grey colour. From the solid appearance of the cells, it would seem that these salts had penetrated through the membrane and coagulated and contracted the cytoplasm; this appears the more evident in the case of the relatively weaker solution of mercuric chloride, where plasmolysis went on slowly up to the end of three hours, after which a strong and rapid contraction took place. These facts are explicable on the assumption that the proteins of the ectoplasm are slowly coagulated during the first three hours and by contraction leave open access for the solution to the inner layers.

In another series of experiments 10 grm. of pressed yeast were weighed out into each of several Nessler glasses and treated with 20 c.c. of the solutions tabulated below. The tubes were allowed to stand in ice water during about 20 hours. They were then centrifuged in batches of four, each batch being spun for exactly 21 minutes. The tubes were then weighed, the liquids poured off into measuring vessels and the weights of the residues ascertained by re-weighing the tubes.

No plasmolysis was produced by solutions of acetone, urea and the lower concentrations of alcohol up to 10 per cent. With the higher concentrations

Table III.

No.	Solute.	Total weight.	Weight of residue.	Percentage of residue.	Volume of liquid poured off.
178, 1	Water	29·8	14·3	48·0	15·5
2	Alcohol, 7 per cent.	29·5	14·0	47·5	15·6
3	„ 10 „	29·3	13·9	47·1	15·5
4	„ 20 „	29·1	13·0	44·7	16·5
5	„ 25 „	29·0	12·6	43·4	17·2
6	„ 30 „	28·7	11·2	39·0	18·3
7	Acetone, molar	29·9	14·7	49·2	15·0
8	Urea, molar	29·9	14·3	47·8	15·5
9	Glycerine, molar	29·8	12·9	43·3	16·5
10	„ $\frac{1}{10}$ molar	29·7	14·6	49·1	15·5
11	Sulphuric acid, $\frac{1}{2}$ molar	30·4	8·7	28·6	21·7
12	Acetic acid, molar	29·8	8·6	28·9	21·0
13	Sodium chloride, molar	30·5	10·5	34·4	19·0
14	„ $\frac{1}{10}$ molar	29·9	14·0	46·8	15·8
15	Sodium acetate, molar	30·4	11·7	38·5	18·0
16	Sodium sulphate, $\frac{1}{2}$ molar	30·9	12·1	39·1	18·0
17	Magnesium sulphate, molar	31·5	11·8	37·5	18·5
18	„ $\frac{1}{10}$ molar	30·9	14·6	47·3	15·0
19	Sodium phosphate, $\frac{3}{10}$ molar	30·4	12·8	42·1	17·4
20	Sodium arsenate, $\frac{2}{10}$ molar	30·4	12·7	41·8	17·5

of the latter plasmolysis was well marked, the effect increasing with increasing concentration. Since no appreciable effect is produced by concentrations up to 10 per cent. it seems possible that diffusion of alcohol is freely permitted. The plasmolysis produced by more concentrated alcohol may be a result of changes in the molecular constitution of the protoplasm. Comparison with the case of mercuric chloride tends to strengthen this view.

The liability to plasmolysis by 20 per cent. alcohol exhibited by different samples of yeast seems to vary with the physical condition of the yeast.

In the experiments given in Table III 10 grm. of pressed yeast were stirred up with 20 c.c. solution, allowed to stand over night in the cold room, and centrifuged next morning.

Table IV.

Solution.	Yeast A.		Yeast B.		Yeast C.		Yeast D.		Yeast E.	
	Volume of liquid poured off.	Weight of residue.	Volume of liquid poured off.	Weight of residue.	Volume of liquid poured off.	Weight of residue.	Volume of liquid poured off.	Weight of residue.	Volume of liquid poured off.	Weight of residue.
Water	14·0	—	15·0	14·8	15·5	14·0	13·7	16·1	15·5	14·3
Alcohol, 5 per cent....	—	—	14·5	14·7	16·0	14·1				
„ 7 „ ...	14·0	—	15·0	14·6	14·5	13·9	14·1	15·7	15·6	14·0
„ 10 „ ...	—	—	14·5	14·5	15·0	14·0	14·0	15·7	15·5	13·9
„ 15 „ ...	15·0	—	14·0	14·5	15·0	14·1				
„ 20 „ ...	17·0	—	15·5	14·0	15·5	14·4	15·0	13·8	16·5	13·0
„ 25 „ ...	—	—	—	—	—	—	16·2	13·1	17·2	12·6
„ 30 „ ...	—	—	—	—	—	—	21·0	9·4	18·3	11·2

Yeast C was an old sample which had been kept in the cold room for about 24 hours after being received from the brewery, and it is worthy of note that no plasmolysis of this yeast by concentrations of alcohol up to 20 per cent. could be detected by this method.

The fact that permanent plasmolysis of yeast is produced by higher concentrations of alcohol, by mercuric and cadmium salts which precipitate the proteins within the cell, and by acids which prevent the activity of the cell, shows that for this cell the existence of permanent plasmolysis is no criterion of the non-diffusibility of the solutions producing it.

In order to arrive at definite results on this subject, it was clearly seen that quantitative estimations of the substance under investigation in the yeast-

cells and in the liquid surrounding the cells would be necessary. Attempts were therefore made to obtain the yeast-cells minus the liquid which normally fills the interstices in an ordinary cake of yeast.

This was eventually accomplished by enclosing the moist yeast cake of the brewery, or yeast obtained as residue after centrifuging, in chain cloth and subjecting it to the pressure of a small hand press. A white friable cake of yeast was obtained which appeared to be composed of dry cells. This was proved to be the actual fact by the following series of experiments:—(1) Total solid estimations in the same pressed cake gave uniform results, showing the cake to be homogeneous. (2) Two pressings of the same yeast-paste gave dry pressed cakes with the same total solid content. (3) Samples of brewery yeast were pressed out and subsequently suspended in the expressed wort, centrifuged and again pressed out, the total solids in the two press cakes were exactly equal. (4) Direct estimations of a salt solution left in the interstices of press cake showed that the greatest volume of liquid thus held by 100 grm. of dry pressed yeast was 0·5 c.c.

Method of Experiment.

The yeast was prepared by pressing out the cake of yeast as received from the press of the brewery, washing being avoided in order to prevent disturbance of the equilibrium of the cell contents. A considerable amount of wort was thus removed. A known weight of this dry yeast was then suspended in a certain volume of the liquid under experiment and allowed to stand for about 20 hours in the cold, after which it was found that osmotic equilibrium between the cells and the solution was attained. The mixture was then centrifuged until the liquid portion was cleared from suspended yeast-cells. The clear fluid was then poured off and the pasty yeast residue was pressed out as described above.

In order to ascertain the weights of yeast-cells and liquid after the experiment, and the distribution of the solute under examination, the following determinations were necessary:—Total solid estimations of the initial and final liquid, and of the initial and final pressed yeast, together with estimations of the dissolved substance in the initial and final liquid, and, in all cases where this was possible, in the initial and final yeast.

The assumption has been made that the total solid matter present in the mixture remains constant during the experiment, an assumption only justified when no loss of carbon dioxide owing to auto-fermentation of the yeast takes place.

Effect of Auto-fermentation.

A small loss of carbon dioxide, accompanied by production of alcohol, does always take place, and the results are subject to error arising from this cause. It was found by direct experiment that, under the conditions employed, a maximum loss of about 0·9 grm. of solid was caused by auto-fermentation.

A loss of 1 grm. in total solids during an experiment has been found to produce a positive error of 5 per cent. on the calculated weight of liquid outside the yeast-cells, so that the results to be given later must be considered to be liable to an error of this order. To eliminate this factor as far as possible, the mixture was allowed to stand in the cold room at a temperature ranging from -2 to $+1°$.

Calculation of the Formula for Obtaining the Weight of Liquid outside the Yeast-cells.

Let l = weight of initial liquid, y = weight of initial yeast, then the total weight $W = l + y$; and if g = percentage of solids in l, and e = percentage of solids in y, then the total solid matter present $V = \frac{lg}{100} + \frac{ye}{100}$.

Both these values W and V are assumed to remain constant during the experiment.

Further, let L = weight of final liquid, Y = weight of final yeast, then $Y = W - L$; and if G = percentage of solids in L, and E = percentage of solids in Y, then the total solid matter $V = \frac{LG}{100} + \frac{YE}{100}$. Substituting for Y in terms of W and L,

$$\frac{LG}{100} + \frac{(W-L)E}{100} = V,$$

whence $L = \frac{EW - 100V}{E-G}$, and Y is obtained by difference from W.

Having thus calculated the weights of liquid and yeast at the end of the experiment, the distribution of the substance under investigation, before and after treatment, is found from the analyses of the initial and final liquid and the initial and final yeast; at the same time, any interchange of other solid matter and of water is made manifest.

Alcohol.

Table V shows the results of experiments with alcohol of various concentrations. This substance was chosen as it might be expected to diffuse freely through the envelope of the cell. Assuming the whole of the water

within the cells to be available for mixture with alcohol, the concentrations of alcohol inside and outside the cells, after osmotic equilibrium had been established, would be equal.

Now the grammes of substance (in this case alcohol) per 100 grm. of water within the yeast (P), divided by the grammes of substance per 100 grm. of water outside the cells (P_1), gives a measure (K) of the amount of diffusion which has taken place. In the case under discussion, therefore, K would be expected to be equal to unity.

In these experiments the increase of alcohol due to auto-fermentation could not be neglected. The total amount of alcohol present at the end of the experiment was therefore determined by analysis of the liquid poured off and of the residue after centrifuging. The weight of alcohol due to auto-fermentation was thus found by difference from the original amount, and was embodied in the calculation for the weight of liquid outside the cells. The formation of an amount of alcohol (F) during an experiment occasions a loss of an approximately equal amount of carbon dioxide to be

Table V.—Diffusion of Alcohol of varying Concentrations.

No.	Conditions.		Yeast.		Liquid.		P.	P_1.	K.
			Initial.	Final.	Initial.	Final.			
75	Alcohol, 2·5 molar, stood 3 hrs. at room temperature	Total ...	100·00	95·70	98·00	101·90			
		Alcohol	4·29	5·80	11·47	10·78	9·91	12·08	0·82
76	„	Total ...	100·00	95·50	98·00	102·30			
		Alcohol	4·50	5·60	11·47	10·42	9·13	10·95	0·83
77	Alcohol, 2·5 molar, stood 20 hrs. in cold room	Total ...	100·00	98·17	98·00	99·57			
		Alcohol	3·14	5·50	11·47	9·50	8·97	10·65	0·84
80	„	Total ...	100·00	91·08	98·00	106·16			
		Alcohol	3·48	5·36	11·47	10·62	9·62	11·30	0·85
100	Alcohol, 1·3 molar, stood 20 hrs. in cold room	Total ...	100·00	97·56	98·80	100·47			
		Alcohol	3·39	3·42	6·00	6·63	5·31	7·13	0·74
101	„	Total ...	100·00	94·01	98·80	104·31			
		Alcohol	3·47	3·55	6·00	6·84	5·95	7·07	0·84
81	Water, stood 20 hrs. in cold room	Total ...	50·00	47·07	100·00	102·59			
		Alcohol	2·39	0·64	—	2·11	1·96	2·11	0·92
85	„	Total ...	100·00	94·16	100·00	105·48			
		Alcohol	4·44	1·37	—	3·42	2·16	3·38	0·64
90	„	Total ...	100·00	99·83	100·00	99·90			
		Alcohol	4·65	1·86	—	3·19	2·66	3·34	0·79

subtracted from the total weight (W), and a loss of approximately twice the amount of solid matter (2F) to be subtracted from V, since the alcohol is formed according to the equation

$$C_6H_{12}O_6 = 2CO_2 + 2C_2H_5OH.$$

If F = weight of alcohol by auto-fermentation, W − F = the total weight at the end of the experiment, and V − 2F = the weight of solid matter finally present, and the formula given on p. 296 becomes

$$L = \frac{E(W-F)-100(V-2F)}{E-G} = \frac{EW-100V-F(E-200)}{E-G},$$

and Y is obtained by difference from W − F.

The table shows very clearly that alcohol penetrates freely through the cytoplasm of yeast, but the interesting fact is observed that when equilibrium is established the ratio of alcohol to water is, in every case, less within the cell (P) than it is outside (P_1), and that these ratios stand to one another in a fairly constant proportion (K).

This points to the possibility that some of the water of the protoplasm is bound up in such a manner as to render it unavailable as a solvent for alcohol. This view is supported by the high value for K found in Experiment 81, wherein old yeast was employed which contained a very large vacuolar space and a correspondingly decreased layer of cytoplasm.

The method is specially interesting, as it affords a very clear insight into the interchange of material occurring between the cells and the surrounding liquid. For instance, in Experiment 81 (yeast in water), 0·36 grm. of alcohol have been formed by auto-fermentation within the yeast, bringing the total alcohol up to 2·75 grm. Of this 2·11 grm. have passed out into the surrounding water, leaving 0·64 grm. in the final yeast; 0·70 grm. of solid matter have passed out from the yeast, and 0·73 grm. of solids have been fermented. At the same time there has been an entrance of 0·21 grm. of water into the cells, which is also accounted for as having left the liquid.

Since in these experiments the value of K appeared to be independent of the concentration of the alcohol, it seemed advisable to investigate this further, and also to try the effect of variations in other directions. Since the factor K depends solely upon the analyses of the components of the final system, in each of the experiments about to be described only two estimations of total solids and two of alcohol were necessary.

The results of these experiments are contained in the following Table VI. In all cases, except where otherwise stated, the duration of the diffusion was 20 hours at the temperature of the cold room :—

Table VI.—Shewing Diffusion of Alcohol.

No.	Alcohol.	Time of standing.	Yeast.			Liquid.			P.	P_1.	K.
			Solids.	Alcohol.	Water.	Solids.	Alcohol.	Water.			
	per cent.	hrs.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.			
Effect of varying Concentration.											
119	20	20	38·63	8·15	53·22	1·52	15·02	83·46	15·31	17·99	0·85
120	10	20	27·95	5·97	66·08	0·83	9·33	89·84	9·04	10·38	0·87
121	5	20	30·49	3·48	66·03	0·91	5·75	93·34	5·27	6·16	0·85
122	0	20	28·71	1·72	69·57	0·92	2·94	96·14	2·47	2·98	0·83
Effect of varying Time of Standing.											
131	20	17	33·00	7·67	59·33	0·69	13·67	85·64	12·93	15·96	0·81
132	20	41	34·49	8·14	57·37	0·74	13·90	85·36	14·19	16·28	0·87
133	20	65	34·99	7·74	57·27	1·03	13·80	85·17	13·52	16·20	0·83
134	20	89	33·46	9·88	56·66	1·32	13·87	84·81	13·85	16·35	0·85
In Auto-fermented Yeast.											
81	0	20	29·26	1·37	69·37	0·69	2·06	97·25	1·96	2·11	0·92
139	10	20	30·82	4·87	64·31	1·29	8·27	90·44	7·57	9·14	0·83
144	10	20	30·76	6·06	63·18	4·07	9·40	86·53	9·59	10·86	0·88
Effect of varying Quantity of Liquid.											
	Yeast.	Alcohol, 10 per cent.									
141	50 grm.	50 cc.	34·50	4·11	61·39	0·95	7·73	91·32	6·69	8·46	0·79
142	50 „	100 „	34·61	4·35	61·04	0·47	7·86	91·67	7·13	8·57	0·83
143	50 „	200 „	34·88	4·10	61·02	0·27	8·05	91·68	6·72	8·78	0·76

In this table, the factor K is found to be remarkably uniform, and to be uninfluenced by variations in the conditions. It is further noteworthy that, under the action of concentrations ranging from 0 to 10 per cent. (Experiments 119–122), no marked variation in the solid content of the yeast is produced. This stands in further confirmation of the results obtained during the earlier experiments on plasmolysis given in Table III, p. 293, in which it is shown that the plasmolysing effect of alcohol is inappreciable until a concentration of 20 per cent. is employed. Again, as was previously observed, the extent of plasmolysis by the higher concentration is not uniform in different samples of yeast; for instance, in the yeast of Experiment 119, an increase of 10 per cent. in the total solids is observed, while in the yeast of similar experiments the increase was only 1·8 and 4·7 per cent. It was noticed also that, with this concentration, a change had taken place within

the cell, making it impossible to obtain the usual white friable press cake, but rather a brown-coloured and putty-like mass.

This change may possibly take the form of a contraction of the cytoplasm of the cell, and, since the depth of the layer of cytoplasm varies under different conditions of the yeast, the amount of plasmolysis sustained by a given sample of yeast when immersed in 20 per cent. alcohol may possibly be determined by the relative proportions of the cell occupied by the cytoplasm and by the vacuole.

In Experiments 131–134 a gradual diffusion of solid matter from the yeast into the surrounding liquid is observed to have taken place; this is probably due to the production of diffusible solids by autolysis of the cell contents.

In Experiments 81 and 139 yeast was employed which had been kept in the cold room for three days. The yeast of 144 was a sample which had been dried in the air at room temperature. The original percentage of total solids in this dry yeast was 68·4 per cent. On immersion in 10 per cent. alcohol the yeast absorbed liquid very quickly, and eventually, after 20 hours, 50 grm. had increased to about 110 grm. Notwithstanding this large influx of liquid, the diffusion factor is seen to be not widely different from the normal.

Sodium Chloride.

This substance was taken as being a typical salt dissociated into its ions more or less completely in dilute solution. Four experiments were made, the results of which are given below. The salt was estimated in the liquid by the method of Carius, the amount in the yeast being calculated by difference. Preliminary experiments had shown that when 50 grm. of pressed yeast

Table VII.—Diffusion of M/10 Sodium Chloride.

No.	Conditions.		Liquid.		Yeast.		P.	P_1.	K.
			Initial.	Final.	Initial.	Final.			
60	Allowed to stand 20 hrs. in cold store	Total ...	100·50	101·00	50·00	49·50			
		NaCl ...	0·58	0·54	nil	0·04	0·11	0·54	0·21
61	Allowed to stand 20 hrs. in cold store	Total ...	100·50	100·30	50·00	50·20			
		NaCl ...	0·58	0·55	nil	0·03	0·08	0·55	0·15
64	Allowed to stand 3 hrs. at room temperature	Total ...	100·50	104·30	50·00	46·20			
		NaCl ...	0·58	0·58	nil	nil	—	0·56	—
65	Allowed to stand 3 hrs. at room temperature	Total ...	100·50	101·60	50·00	48·90			
		NaCl ...	0·58	0·58	nil	nil	—	0·58	—

were suspended in distilled water and the mixture centrifuged, only a faint trace of milkiness was produced by the addition of acid silver nitrate to the liquid poured off.

These results show that the diffusion of sodium chloride is slow. A definite quantity of substance enters the cell when yeast is suspended in M/10 sodium chloride solution and allowed to stand over night, although no diffusion is noticed after a suspension of three hours only.

Ammonium Sulphate.

The next experiments were made with ammonium sulphate, as being a substance which is of service to the yeast, and which is a sufficient source of nitrogen in artificial culture. In these experiments two concentrations of the solution have been employed, of approximately one-tenth and three-tenths molar respectively.

Table VIII.

No.	Conditions.		Liquid.		Yeast.		P.	P_1.	K.
			Initial.	Final.	Initial.	Final.			
67	Stood 20 hrs. in cold store. Solution, 0·1 molar	Total	100·40	97·84	50·00	52·56			
		$(NH_4)_2SO_4$	1·31	1·21	nil	0·10	0·28	1·26	0·22
68	Stood 20 hrs. in cold store. Solution, 0·1 molar	Total	100·50	99·21	50·00	51·29			
		$(NH_4)_2SO_4$	1·31	1·19	nil	0·12	0·34	1·22	0·28
69	Stood 3 hrs. in cold store. Solution 0·1 molar	Total	100·60	97·30	100·00	103·30			
		$(NH_4)_2SO_4$	1·29	1·23	nil	0·06	0·09	1·28	0·07
70	Stood 3 hrs. at room temperature. Solution, 0·1 molar	Total	100·60	99·48	50·00	51·12			
		$(NH_4)_2SO_4$	1·31	1·21	nil	0·10	0·28	1·24	0·23
71	Stood 3 hrs. at room temperature. Solution, 0·1 molar	Total	100·60	102·50	50·00	48·10			
		$(NH_4)_2SO_4$	1·31	1·28	nil	0·03	0·09	1·27	0·07
145	Stood 20 hrs. in cold store. Solution, 0·1 molar. Air-dried yeast	Total	90·10	58·78	30·00	61·32			
		$(NH_4)_2SO_4$	1·14	0·98	nil	0·16	0·37	1·76	0·22
149	Stood 20 hrs. in cold store. Solution, 0·3 molar	Total	50·50	58·87	50·00	41·63			
		$(NH_4)_2SO_4$	1·84	1·71	nil	0·13	0·47	3·00	0·15
150	Stood 20 hrs. in cold store. Solution, 0·3 molar. Air-dried yeast	Total	100·00	90·86	50·00	59·14			
		$(NH_4)_2SO_4$	3·65	3·40	nil	0·25	0·61	3·91	0·16
151	Stood 20 hrs. in cold store. Solution, 0·3 molar. Air-dried yeast	Total	100·00	75·44	50·00	74·56			
		$(NH_4)_2SO_4$	3·65	3·13	nil	0·52	1·03	4·41	0·23

The ammonium sulphate was estimated in the initial and final liquids only, the amount in the final yeast being found by difference.

The results with ammonium sulphate are found to be very similar to those obtained with sodium chloride.

Experiments 145 and 151 are specially interesting as the initial yeast in these cases contained a large percentage of solid matter. In the former, where one-tenth molar ammonium sulphate was employed, 33·17 grm. of water have entered the yeast while only 0·16 grm. of the salt have been carried in, and in the latter from three-tenths molar solution 25·47 grm. of water and 0·52 grm. of salt have entered. The ratio of the concentration inside the cells to the concentration outside is the same in both cases.

The rate at which a sample of air-dried yeast will absorb water and recover turgescence is very remarkable. In No. 145 when 30 grm. of the yeast were mixed with 60 c.c. of solution such a stiff paste was obtained, within two minutes, that it could only be stirred with difficulty. It is further worthy of note that yeast which has been dried in air returns to its normal condition of turgescence when immersed in water, as shown by the percentage of total solids. In all samples of fresh pressed yeast the total solid content has been found to vary from 28 to 35 per cent. Total solid estimations of three air-dried samples gave 68·4, 50·5, and 37·5 per cent.; when immersed in water these yeasts became turgid with a normal solid content of 30·5, 31, and 32·4 per cent. respectively.

The envelope of such dried and shrivelled cells, though readily permeable by water, does not admit of the entrance of a 1/10 molar solution of ammonium sulphate, but selects from it a large quantity of water and only a relatively small quantity of the salt. The liquid surrounding the cells therefore becomes considerably concentrated. In Experiment 145, for instance, the concentrations of salt in the initial and final liquids were 1·26 and 1·66 respectively.

Copper Sulphate.

A peculiar resistance to the entrance of copper salts is exhibited by the protoplasm of *Penicillium glaucum* (12), growth of which has been found possible on a medium containing as much as 21 per cent. copper sulphate, although very much smaller quantities down to 3 per cent. have occasionally proved destructive. In view of this result it seemed advisable to investigate the effect of solutions containing copper upon the protoplasm of the yeast-cell. 100 grm. of yeast were suspended in 100 grm. 1/10 molar $CuSO_4$, allowed to stand for the usual time in the cold room, and the distribution of the copper determined.

Table IX.

Initial liquid.		Initial yeast.		Final liquid.		Final yeast.	
Weight.	$CuSO_4$.	Weight.	$CuSO_4$.	Weight.	$CuSO_4$.	Weight.	$CuSO_4$.
grm. 100	grm. 0·78	grm. 100	grm. —	grm. 128·4	grm. 0·36	grm. 71·6	grm. 0·43

A remarkable degree of plasmolysis was observed and the shrunken cells were of a pale green colour and solid appearance, the cytoplasm had evidently entered into combination with the $CuSO_4$ and had been precipitated thereby, so that the factor K in this case is much greater than unity, namely, 3·36. When this yeast was added to sugar solution no fermentation was produced.

Sodium Phosphate.

These experiments have been made with solutions of the di-sodium salt of two concentrations, roughly 1/10 and 3/10 molar, and with water.

Table X.

No.	Conditions.		Yeast.		Liquid.	
			Initial.	Final.	Initial.	Final.
103	Liquid containing 1·42 grm./100 c.c. Na_2HPO_4, stood 20 hrs. in cold store	Total	50·00	50·34	50·50	50·16
		P_2O_5	0·75	0·76	0·35	0·38
104	,, ,,	Total	50·00	49·91	50·50	50·59
		P_2O_5	0·81	0·87	0·35	0·36
107	,, ,,	Total	100·00	99·90	101·30	101·40
		P_2O_5	1·63	1·64	0·71	0·71
109	,, ,,	Total	100·00	100·74	101·50	100·76
		P_2O_5	1·39	1·55	0·71	0·63
111	Liquid containing 4·16 grm./100 c.c. Na_2HPO_4	Total	100·00	95·50	104·00	108·50
		P_2O_5	1·63	1·97	2·08	1·80
112	,, ,,	Total	100·00	96·60	104·00	107·40
		P_2O_5	1·59	2·01	2·08	1·80
105	Water	Total	100·00	102·02	100·00	97·98
		P_2O_5	1·43	1·49	—	trace
108	Water	Total	50·00	53·16	50·00	46·84
		P_2O_5	0·81	0·80	—	0·01
114	Water	Total	100·00	107·80	100·00	92·20
		P_2O_5	1·53	1·56	—	trace

In the estimations, the organic matter was destroyed by Neumann's method and the phosphoric acid was precipitated with magnesium citrate mixture.

With the weaker concentration, which was found to be isotonic with yeast, no exchange of phosphoric acid took place, but from a solution containing 4 per cent. of sodium phosphate, approximately 0·3 molar, entrance of phosphoric acid into the cells was well marked.

Sodium Hexosephosphate.

For the purpose of this research hexosephosphate was of all substances of greatest interest, since Harden and Young (13) have found that hexosephosphoric acid is continually being built up and broken down again in the fermentation of sugar by yeast-juice. When they added this substance to living yeast, however, no evidence of its fermentation could be obtained. It was of special importance, therefore, to determine whether any of the substance had been able to diffuse into the yeast-cells.

The solution of hexosephosphoric acid was prepared by the method described by Young (14) and was neutralised to litmus with caustic soda. Four concentrations of the salt have been employed and the results are given in the following table. In each case the time of standing was 20 hours at a temperature between −2 and 0°.

Table XI.—Diffusion of Sodium Hexosephosphate.

No.	Conditions.		Yeast.		Liquid.	
			Initial.	Final.	Initial.	Final.
169	Concentration, 0·035 molar = 0·14 normal. Standing 20 hrs. in cold room	Total	100·00	89·80	100·00	110·20
		P_2O_5	1·42	1·36	0·50	0·51
174	Concentration, 0·06 molar = 0·24 normal	Total	100·00	102·50	100·00	97·50
		P_2O_5	1·42	1·55	0·84	0·80
172	Concentration, 0·126 molar = 0·504 normal	Total	100·00	92·90	100·00	107·10
		P_2O_5	1·26	1·52	1·80	1·56
177	Concentration, 0·23 molar = 0·93 normal	Total	100·00	92·66	100·00	107·34
		P_2O_5	1·66	1·99	3·33	2·98

The results are strikingly similar to those obtained with sodium phosphate. Where the concentration was small, as, for instance, in Experiment 169, no definite entrance of phosphorus took place; with higher concentrations, however, the increase of P_2O_5 in the yeast became well marked. Experiment 177 showed that 0·33 grm. of P_2O_5, equal to 2·5 c.c. of molar solution

and to 1/10 of the total amount in the liquid, have been transferred from the liquid to the yeast. The influence of this solution upon the fermentation of yeast was studied according to the method described in a preliminary communication by Harden and Paine (15), and, although the initial rate of auto-fermentation was increased, the total volume of gas yielded was not greater than that given by a water control, and, moreover, the rate of auto-fermentation produced was exactly comparable to the rate under the influence of sodium phosphate of the same normality. It would seem from this that, although this substance is capable of entering the yeast-cell, it is not able to penetrate through to the sphere of activity of the hydrolysing enzyme.

Sodium Arsenate.

Sodium arsenate was specially interesting, since Harden and Young (16) have found that solutions of arsenates have an enhancing influence on the rate of fermentation of sugar by yeast-juice.

The following table gives results of three experiments. The estimations were made by digesting the yeast and liquid with nitric and sulphuric acids, and, after dispelling the nitric acid, reducing the arsenic acid with hydriodic acid. The liberated iodine was removed by titration with thio-sulphate and the arsenious acid precipitated with sulphuretted hydrogen, collected on a tared filter, washed successively with water, alcohol and carbon bisulphide, dried at 100° and weighed. The results are expressed in terms of anhydrous sodium arsenate.

Table XII.—Diffusion of Sodium Arsenate.

No.	Conditions.		Yeast.		Liquid.		P.	P_1.	K.
			Initial.	Final.	Initial.	Final.			
164	Concentration, 2·02 per cent. = 0·11 molar. Stood 20 hrs. in cold room	Total ...	100·00	102·40	100·00	97·60			
		Arsenate	nil	0·15	2·02	1·86	0·22	1·95	0·11
165	„ „	Total ...	100·00	97·90	100·00	102·10			
		Arsenate	nil	0·26	2·02	2·10	0·39	2·12	0·18
166	Concentration, 3·35 per cent. = 0·18 molar	Total ...	100·00	88·20	100·00	111·80			
		Arsenate	nil	0·28	3·35	3·27	0·49	3·03	0·16

These results are essentially similar to those obtained with sodium chloride and ammonium sulphate, the factor K shows a fair degree of uniformity and indicates definite but very imperfect diffusion of the substance.

Summary and Conclusions.

The early experiments on plasmolysis of yeast seemed to indicate that the envelope of the yeast-cell was impermeable by inorganic salts generally while it allowed of the ready diffusion of such substances as alcohol, acetone, and urea, which have been known to pass with ease through many forms of living protoplasm.

Quantitative estimations have shown the power of diffusion of alcohol to be very different from that of inorganic salts. On immersion of yeast in dilute alcohol, varying from 5 per cent. to 20 per cent., the ratio of the concentration within the cells to that of the liquid outside becomes practically constant, and independent of the absolute concentration. Alcohol is believed to diffuse quite readily into the cell, but at the same time this ratio is not unity, but a constant which deviates only slightly from 0·85. Probably the whole of the water in the cell, which is removed by drying at 98° C., is not available for diffusion of alcohol. The amount of water thus bound up, possibly as a constituent of the protoplasmic complex, appears to vary somewhat at different stages in the life-history of the cell, but the method was not considered sufficiently delicate to render further study of this interesting phenomenon advisable in this way.

All salts which have been tried have been taken up by yeast from moderately concentrated solutions, and in the cases of sodium chloride and ammonium sulphate even from dilute solutions. But, whereas with alcohol the amount entering the yeast during three hours was practically equal to the amount which entered on prolonged immersion, with these salts the process was a slow one. After three hours no sodium chloride had entered from a decimolar solution, and considerably less ammonium sulphate was found in the yeast than was the case after longer standing. From decimolar solution of sodium phosphate no entrance of phosphorus was appreciable even after 20 hours' standing, but from more concentrated solution, 0·3 molar, a well marked entrance was observed. Since phosphates are essential for the life of the yeast and are gradually assimilated and accumulated from very dilute solutions, the envelope must admit the necessary amount of these substances required by the cell for its metabolism. The amount thus absorbed during the time of these experiments would naturally be very small and indeterminable.

With regard to the entrance of salts, which the experiments have shown to occur, the following considerations are of interest. Since the yeast must of necessity be analysed as a whole, the question as to how far into the cells the various substances have penetrated must, at present, remain in doubt. While most salts do show some entrance into the cells, the factor which is taken as an expression of permeability is, except in the case of copper

sulphate, comparatively small (0·1—0·25 as against 0·85 in the case of alcohol). It seems very probable that the apparent entrance of salts is a result of adsorption in the surface layers of the cell rather than absorption, or it may be that the salt particles are kept back by a differential septum according to the hypothesis of H. E. Armstrong (8), and that they remain in the interstices of such membrane.

The experiments with hexosephosphate are particularly interesting in this connection, since this substance is present in yeast and is readily hydrolysed and fermented by yeast-juice. The fact that when this substance is added to yeast there is no evidence whatever of its being fermented would seem to indicate that it had not been able to penetrate through to the seat of fermentative activity. It thus seems highly probable that the apparent entrance of this salt, which is well marked, is merely a surface phenomenon.

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