THE INFLUENCE OF SODIUM CHLORIDE ON THE GROWTH AND METABOLISM OF YEAST

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The effects of the commoner salts on the physiology of the higher animals and plants have been studied by numerous investigators. In order to obtain quantitative data workers in the animal field, e.g., Loeb (1906), Lillie (1901), and Moore (1901), have studied normal rhythmical movements such as heart beat, the contractions of Polyorchis or the contractions of the lymph hearts of frogs. This type of research provided the experimental basis for several important conclusions: (*a*) in low concentrations the common salts exert a favorable and stimulating effect, (*b*) a toxic effect is exerted in higher concentrations, and (*c*) the influence of a salt is modified by the presence of other salts in the solution. In the case of higher plants investigators have concerned themselves particularly with the influence of salts on the dry weight of the plant.

The unicellular organisms provide a means of analyzing some of these effects in greater detail by chemical methods. Already there is a considerable literature dealing with this branch of the field. Several workers have shown recently that low concentrations of many different salts stimulate the growth of bacteria. Hotchkiss (1922) in a study of *B. coli* reported that 15 of a total of 23 chlorides stimulated growth as measured by the turbidity method. This was true of those salts which are usually considered to be particularly toxic. Holm and Sherman (1921) found that 0.2 M concentration of NaCl hastens the appearance of turbidity in media inoculated with *B. coli*. No measurements were made of total growth. Brooks (1919) made the important observation that an increased output of CO_2 by *B. subtilis* took place in the

presence of 0.15 M NaCl, 0.2 M KCl or 0.05 M $CaCl_2$. She also observed the antagonistic effects when mixtures were used. Gustafson (1919) made similar observations in connection with *Aspergillus niger*. Lipman (1909) in an earlier research had found that nitrification and ammonification by bacteria were stimulated in a similar manner. Mitra (1917) investigated the effect of chlorides, singly and in mixtures, on the vegetative reproduction of a wine yeast growing in a synthetic medium. Larger crops of yeast were obtained when low concentrations of different salts were added.

We have attempted a more detailed investigation of the influence of several chlorides on the following characteristics of a yeast fermentation: (*a*) vegetative growth, (*b*) nitrogen content of crop, (*c*) rate of CO_2 production and total output, and (*d*) the quantitative relationship between several of these characteristics.

METHODS

The organism used was a type of *S. cerevisiae* used in a local brewery. Pure cultures were maintained by the usual bacteriological methods.

Medium

In choosing a medium for work of this nature it is impossible to avoid the complications due to "antagonism" between the normal salt content of the medium and the salt under investigation. It is obvious that in all the microbiological work which we have reviewed this factor is present to an undetermined extent, and this is particularly so when synthetic media were used. Mitra's (1917) medium contained 0.1 per cent ammonium phosphate, and his experimental findings include the effect of 0.058 per cent NaCl. Recognizing this difficulty we have used wort made according to a carefully standardized method. This medium contained approximately 0.25 per cent of salts, but it gives a control crop of yeast which is more than 10 times the maximum crop obtained by Mitra as a result of stimulation. In other words any changes observed in our experiments are changes from a normal and not from an abnormal type of fermentation. Furthermore the

effects which we regard as significant are produced by large amounts of added salt, and not by quantities significantly less than the initial salt content of our medium.

Our medium was prepared as follows: Extract 600 grams of fresh malt with 3000 cc. of distilled H_2O at 60°C. for three hours. Strain through cheese-cloth, and sterilize for one and one-half hours at 15 pounds steam pressure. Remove the precipitate by filtration, and sterilize for twenty minutes. Determine the sugar concentration by the Shaffer-Hartmann method. Our standard medium was made up to contain 10 per cent sugar. The various amounts of NaCl were added to the wort in the experimental tubes previous to the final sterilization, except in certain experiments when the salt was held in a special container above the medium in the tubes.

Cultures

The experimental cultures were contained in bent tubes similar to that described by Fraser (1921). The bath surrounding the moving rack was maintained at 26.5°C. The upright arm of the culture tube passed through an arc of 20° 50 times per minute. Each tube contained 70 cc. of medium, and the volume of the horizontal arm was approximately 80 cc. (see fig. 1). The CO_2 given off was absorbed without loss in large test-tubes containing NaOH. Specially designed absorption tubes were used. The amount of CO_2 taken up by the soda was determined by a double titration of an aliquot using a standard HCl, phenolphthalein and methyl orange. The efficiency of these methods will be more clearly demonstrated by the results recorded for several controls.

The objection might be raised that the CO_2 absorbed by the NaOH is not quantitatively representative of the amount produced at any given time owing to, (*a*) the solubility of CO_2 in the medium, and (*b*) the different solubilities of CO_2 in media containing various concentrations of NaCl. We consider, however, that the findings of Findlay and co-workers (1910; 1912; 1913) on the solubility of CO_2 in H_2O and solutions of KCl support our conclusion that CO_2 is not held by the medium under the

experimental conditions outlined above. We desire to emphasize the necessity for adequate and uniform motion in the culture tubes throughout the entire period of the experiment. Our experience has been that only results of limited value are obtained when the shaking is performed by hand or by a variable-speed motor.

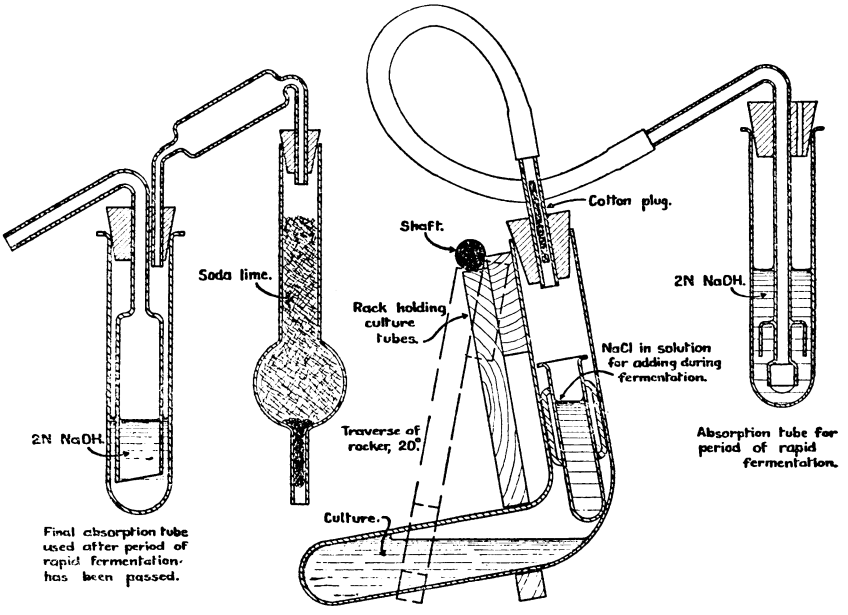

FIG. 1. ROCKER TUBE FOR CULTURE. ABSORPTION TUBES FOR CARBON DIOXIDE

Inoculum

The culture used to inoculate the experimental tubes was grown in 2 per cent wort for twenty-four hours on the moving rack. Each tube received 1 cc., 2 cc. or 5 cc. of culture accurately measured. In order to obtain agreement among controls inequalities in the number of cells added must be avoided, and this cannot be achieved if the yeast in the parent culture is flocculent.

Yeast crops

Alundum crucibles (Norton, porosity R. A. 360) were used to remove the crops from the fermented wort. Controls showed an agreement to within 2.0 per cent by weight after two hours in an oven at 100°C. To maintain this agreement it is necessary to standardize the washing of the crops. Hot cleaning solution will prepare the crucibles for further experiments, but their porosity becomes too great after 4 or 5 experiments.

TABLE 1

SERIES	SUGAR IN WORT	FERMENTATION PERIOD	NaCl	YEAST
	per cent	*hours*	*per cent*	*grams*
1	10	61	0	0.4423
			0	0.4440
			0.02	0.4224
			0.05	0.4330
			0.10	0.4177
			0.50	0.4025
2	7	112	0	0.3839
			0	0.3942
			0.5	0.3674
			0.5	0.3467
			1.5	0.2970
			1.5	0.2887
3	8	112	0	0.4204
			0	0.4179
			2.0	0.3156
			4.0	0.1611
			7.0	0.0863
			10.0	0.0819

THE INFLUENCE OF SODIUM CHLORIDE ON VEGETATIVE GROWTH

Culture tubes containing 70 cc. of sterile wort free from suspended matter and containing various quantities of NaCl were inoculated with 2 cc. of a twenty-four-hour culture. The tubes were then placed on the rocker, and were allowed to ferment to completion. The crops of yeast obtained were weighed in the dry state, and the results from a few experiments are summarized

in table 1. This range of salt concentration has been covered several times, and the above results are characteristic. They show very definitely that in wort, a medium which is particularly suitable for growth and fermentation, there is a gradual diminution in the size of the yeast crop as the concentration of NaCl is increased. We have no convincing evidence of a concentration which acts as a stimulant.

TABLE 2

CONTROL	AGE OF CULTURE		CO_2 PER HOUR	CO_2 IN FORTY-EIGHT HOURS
	hours	*minutes*	*grams*	*grams*
1	13		0.1758	2.068
	14		0.2070	
	15	25	0.2070	
	16		0.2034	
	17		0.1842	
2	13	37	0.1704	2.070
	14	36	0.2028	
	15		0.2100	
	16		0.1968	
	17		0.1650	
3	12	48	0.1278	2.010
	13	49	0.1806	
	14	48	0.2070	
	15	48	0.2040	
	16	49	0.1986	

THE INFLUENCE OF SODIUM CHLORIDE ON GAS PRODUCTION

Experiment I

Before proceeding to investigate the influence of NaCl on the rate of CO_2 evolution and on the total CO_2 production it was necessary to satisfy ourselves that a satisfactory degree of uniformity could be obtained in control fermentations and in our methods of measurement. Accordingly at regular intervals during the progress of this research two or more controls have been followed under standard conditions. Unless the same degree of accuracy was obtained in the controls the results were discarded.

Tubes containing 70 cc. of 10 per cent wort were inoculated with 5 cc. of a twenty-four-hour culture in 2 per cent wort. The CO_2

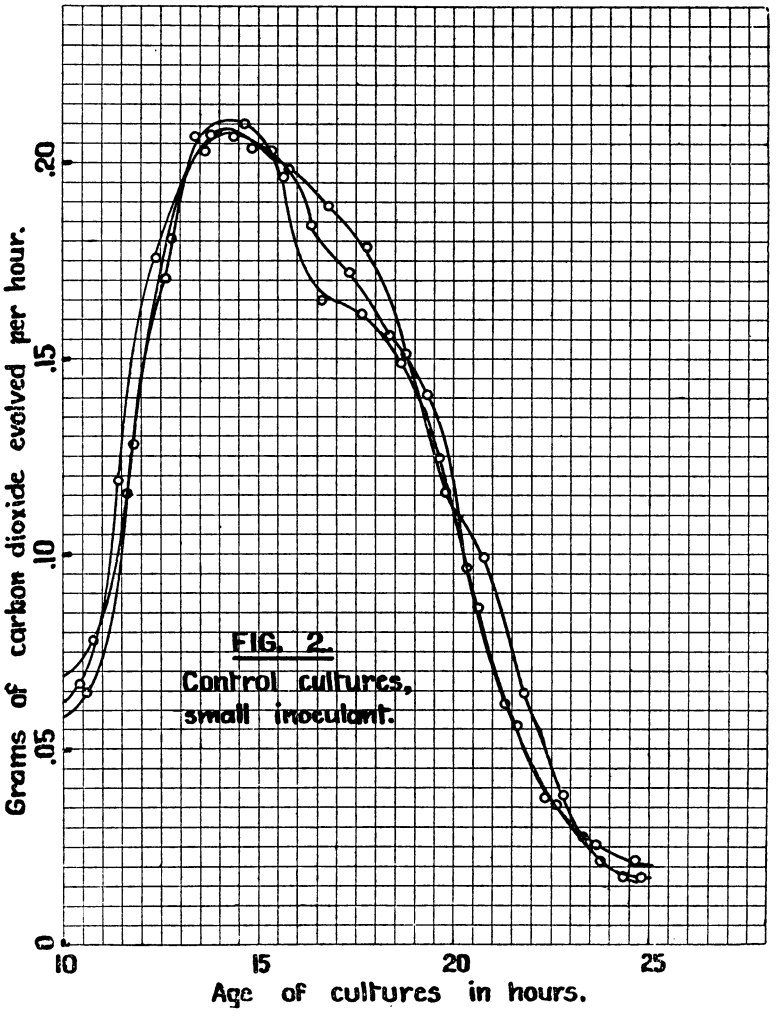

FIG. 2. CONTROL CULTURES, SMALL INOCULANT

given off was determined every hour for each tube, and the total figures for a forty-eight-hour period were also obtained.

The curves in figure 2 show the extent of the agreement between the results as a whole, and in table 2 the rates at the peaks of the curves are given. Total yields of CO_2 are included in the table.

TABLE 3

AGE OF CULTURE		CO_2 PRODUCTION PER HOUR					
		Control	1 per cent NaCl	2 per cent NaCl	3 per cent NaCl	4 per cent NaCl	5 per cent NaCl
hours	*minutes*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
13		0.0706					
13	40		0.1090				
13	50			0.0727			
14	10	**0·1300**					
14	20		**0·1880**				
14	45			0.1210			
15	30	0.1210					
15	40		0.1770				
15	50			0.1350			
16	39	0.0835					
16	45		0.1270				
16	53			**0·1380**			
17	50			0.1260			
20					0.0712		
21					0.0788		
22					0.0790		
23					**0·0815**		
24					0.0762		
32	10					0.0496	
33	10					0.0521	
34	10					**0·0560**	
35	10					0.0445	
41	20						0.0499
42	20						**0·0554**
43	20						0.0531
44	20						0.0461
CO_2 produced in 107 hours.....		1.473	1.560	1.701	1.362	1.238	0.896

Experiment II

Six tubes containing 70 cc. of wort, 10 per cent sugar, were prepared and sterilized. The first tube was a control, and the

remainder contained from 1 to 5 per cent NaCl. Each tube was inoculated with 1.0 cc. of a twenty-four-hour culture. The rates of CO_2 evolution and the total yields were determined. In table 3 we have grouped together the rate figures for each fermentation in the region of maximum gas evolution. The following conclusions may be made from these observations. This strain of yeast will grow and ferment in wort containing 10 per cent sugar and up to 5 per cent NaCl. The maximum rate of gas production is raised by the presence of 1 to 2 per cent of NaCl, and the total production is also greater than in the control. Above this salt concentration the maximum rate becomes steadily smaller, and in the concentrations used the time required to reach the maximum rate gradually increases. This flattening out of the rate curves is partially due to a steady increase in the initial lag phase of the fermentation period.

The stimulation of CO_2 production by low salt concentrations has been confirmed by numerous experiments of this type. The concentrations of NaCl used have been varied up to 2 per cent. Individual experiments vary in the concentration within this range which gives the highest maximum rate, but they agree in showing that between 1 and 2 per cent of NaCl bring about an increase in the maximum rate and total CO_2 production.

Experiment III

In this experiment the tubes were inoculated with 5 cc. of culture, and the same type of quantitative observations were made. Six tubes were studied: 2 controls, 2 containing 0.5 per cent NaCl, and 2 containing 1.0 per cent NaCl. The important figures are given in table 4.

In the presence of 0.5 per cent NaCl the lag-phase is slightly prolonged, the maximum rate of CO_2 evolution is raised, and total CO_2 production is greater than in the controls. When the concentration of salt is raised to 1.0 per cent we find no satisfactory evidence of a stimulation either of the rate or total CO_2 figures. The lag-phase is prolonged, but otherwise the results fall within the range of variation which is possible with controls.

The results for the 1.0 per cent fermentations are therefore essentially different from those obtained in experiment II, and attention is called to the only change in the conditions, namely, an increase from 2 to 5 cc. in the inoculum used.

TABLE 4

CULTURE	AGE		CO_2 PER HOUR	CULTURE	AGE		CO_2 PER HOUR
	hours	*minutes*	*grams*		*hours*	*minutes*	*grams*
Control	18	30	0.213	Control	18	40	0.234
	19	30	0.294		19	40	0.292
	20	30	0.298		20	40	0.294
	21	30	0.297		21	40	0.259
	22	30	0.268		22	40	0.191
	23	30	0.181		23	40	0.092
0.5 per cent NaCl	18	50	0.233	0.5 per cent NaCl	19		0.207
	19	50	0.293		20		0.260
	20	50	0.314		21		0.276
	21	50	0.313		22		0.297
	22	50	0.310		23		0.310
	23	50	0.253		24		0.285
1.0 per cent NaCl	19	10	0.220	1.0 per cent NaCl	19	20	0.285
	20	10	0.269		20	20	0.288
	21	10	0.288		21	20	0.258
	22	10	0.296		22	20	0.167
	23	10	0.271		23	20	0.067
	24	10	0.180				

Total CO_2 in 94 hours

Controls, 2.863 grams, 2.756 grams
0.5 per cent NaCl, 2.913 grams, 2.870 grams
1.0 per cent NaCl, 2.824 grams, 2.637 grams

Experiment IV

A still larger number of cells were used to inoculate the experimental cultures. The entire crops from normal control tubes were separated from the media by careful filtration through double filter-cones. The crops were washed with a little distilled water, and dried at the pump. The paper cone and one crop were added to each tube. In previous experiments we had found that

proper agreement was possible among several controls inoculated in this way. The lag-phase is considerably reduced, the peak of gas evolution is reached in about four hours, and the fermentation is completed in twelve hours. Brown (1892; 1894; 1903) used

TABLE 5

CULTURE	AGE		CO_2 PER HOUR	CULTURE	AGE		CO_2 PER HOUR
	hours	*minutes*	*grams*		*hours*	*minutes*	*grams*
Control IV A	2		0.256	Control IV A	2	10	0.300
	3		0.440		3	10	0.441
	4		0.561		4	10	0.550
	4	20	0.552		4	30	0.528
	4	40	0.531		4	50	0.531
	5		0.501		5	10	0.507
	5	30	0.498		5	40	0.468
Control IV B	2		0.200	0.5 per cent	2	10	0.196
	3		0.445	NaCl, IV B	3	10	0.467
	3	30	0.590		3	40	0.584
	4		0.608		4	10	0.630
	4	30	0.616		4	40	0.610
	5		0.600		5	10	0.610
	5	30	0.540		5	40	0.540
Control IV C	2		0.224	0.75 per cent	2	10	0.232
	3		0.531	NaCl, IV C	3	10	0.518
	3	30	0.602		3	40	0.618
	4		0.608		4	10	0.618
	4	30	0.664		4	40	0.620
	5		0.608		5	10	0.640
	5	30	0.506		5	40	0.542

Total CO_2

IV A. $48\frac{3}{4}$ hours, Control—2.712 grams, Control —2.707 grams
IV B. 25 hours, Control—2.695 grams, 0.5 per cent NaCl—2.709 grams
IV C. $23\frac{1}{2}$ hours, Control—2.932 grams, 0.75 per cent NaCl—3.019 grams

this method in his studies of normal yeast fermentation, and states that the crop from a given volume of a suitable medium does not increase when it is added to a similar volume of fresh material. We hoped by this technique to be able to study the influence of NaCl on fermentation in the absence of vegetative

reproduction, but we found that under these conditions the crop doubles in weight during the second fermentation. The divergence from Brown's findings is probably due to the fact that our cultures were agitated continuously.

Total crops of yeast were added to tubes of the same batch of medium containing, (*a*) no NaCl, (*b*) 0.5 per cent NaCl, and (*c*) 0.75 per cent NaCl. The rate of gas evolution and the total produced were determined in each case. Experimental results from such a group of cultures are given in table 5. Experiment IV shows quite clearly that when a large amount of yeast is used to inoculate wort containing from 0.5 to 0.75 per cent of NaCl the maximum rate of CO_2 production and the total amount produced are similar to the results obtained in the absence of salt. Similar experiments have been performed in which the salt concentration was raised to 1.0 per cent. In this case both the maximum rate and the total CO_2 figures are depressed. These results as a whole are in striking contrast with our earlier ones obtained from experiments in which a small inoculum was used. We shall discuss the theoretical aspect of these differences late in our report.

Experiment V

An attempt was then made to measure the effects of additions of NaCl which were made during the course of normal fermentations. The salt was carefully weighed, and dissolved in a known volume of distilled water. This solution was placed in a small test-tube which rested in the vertical arm of the fermentation tube (fig. 1). By tilting the apparatus slightly the NaCl could be added at the desired point in the fermentation without additional changes in the conditions of the experiment. By the same method a corresponding volume of H_2O was added to the control fermentation.

The crops of yeast from two normal fermentations were separated, and added to fresh portions of the same medium. The rate of CO_2 evolution was followed until the peaks of the curves were approaching. An addition of 0.5 per cent of NaCl was made to one fermentation. We continued the observations of

rates, and measured the total volumes of CO_2 produced. These figures are given in table 6. These results show that when 0.5 per cent NaCl is added to a normal, vigorous fermentation there is no immediate effect on the rate of CO_2 production. The total amount produced is also unchanged. In a similar experiment the salt was added after the maximum rate had passed, and we observed no stimulation.

Experiment VI

The last experiment was repeated with the exception that 1.25 per cent NaCl was added to one of two cultures before the

TABLE 6

CULTURE	AGE		CO_2 PER HOUR	CULTURE	AGE		CO_2 PER HOUR
	hours	*minutes*	*grams*		*hours*	*minutes*	*grams*
Control	1		0.0460	Control	1		0.0406
	2		0.2369		2		0.2307
	3		0.3415		3		0.3251
H_2O added	3	05		0.5 per cent	3	05	
	3	30	0.3874	NaCl added	3	30	0.3886
	4		0.4204		4		0.4204
	4	30	0.4162		4	30	0.4120
	5		0.3874		5		0.3812
	5	30	0.3700		5	30	0.3648
	6	30	0.2830		6	30	0.3077

Total CO_2 produced

Control, $96\frac{1}{2}$ hours, 2.4476 grams
0.5 per cent NaCl, $96\frac{1}{2}$ hours, 2.4409 grams

maximum rate of CO_2 evolution had been reached. The results from the experiment are represented by curves in figure 3. There is an immediate effect on the rate curve when this amount of NaCl is added to the culture. Previously the two cultures had been almost identical, but the salted fermentation at once developed a rate curve distinctly below that of the control. Later on the control curve cut through the "salted" one, and the fermentation was completed in a shorter period. The total yields of CO_2 were: control 2.3693 grams and 1.25 per cent NaCl 2.3687 grams.

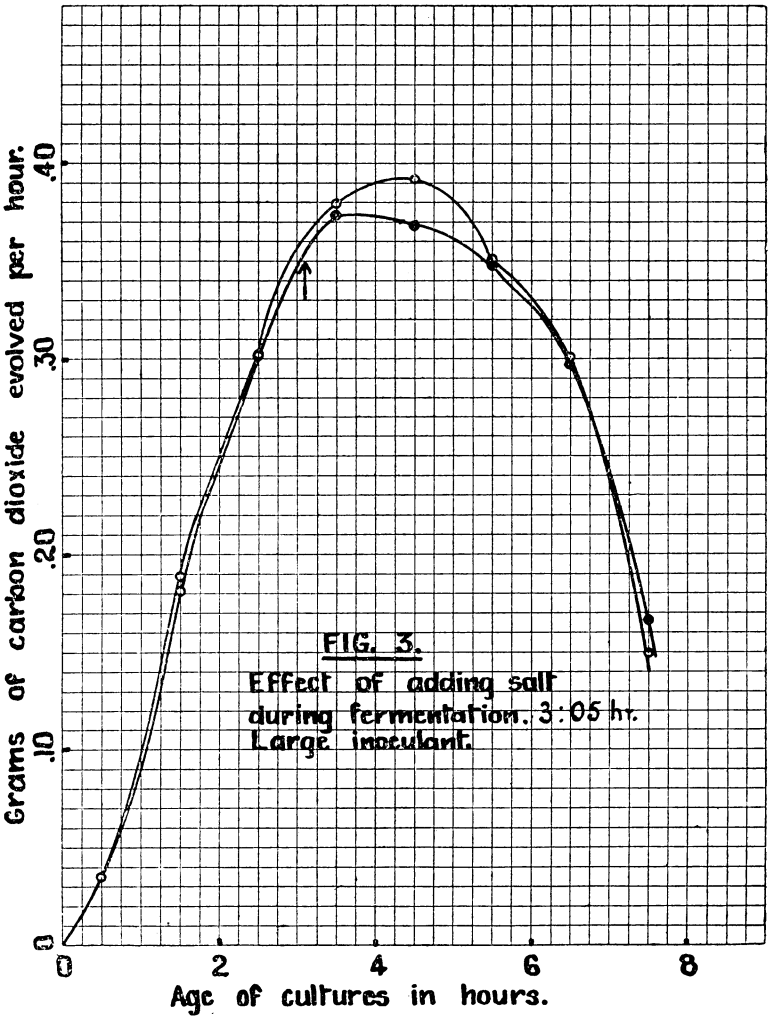

FIG. 3. EFFECT OF ADDING SALT DURING FERMENTATION. 3.05 HOURS. LARGE INOCULANT

THE NITROGEN CONTENT OF YEAST

In order to obtain information regarding the effect of sodium chloride on the chemical composition of the yeast cells the

percentage of nitrogen was determined in crops obtained over a wide range of salt concentrations.

Experiment VII

Six tubes containing 70 cc. of medium were inoculated with 2 cc. of a culture in 10 per cent wort. The crops at the end of thirty-eight hours were removed from the control cultures and those containing 2 per cent of NaCl. After washing and centrifuging 3 times the material was placed in separate, clean tubes, and dried in the oven for six hours at 100°C. Portions of the brittle mass were removed from each tube, and weighed. The crops from cultures containing 4 and 6 per cent of salt were similarly treated

TABLE 7

CULTURE	SAMPLE OF YEAST	NITROGEN IN SAMPLE	NITROGEN IN CROP
	grams	*grams*	*per cent*
Control	0.2474	0.0185	7.47
	0.2256	0.0174	7.72
2.0 per cent NaCl	0.1892	0.0148	7.80
2.0 per cent NaCl	0.1111	0.0087	7.88
4.0 per cent NaCl	0.1947	0.0161	8.27
6.0 per cent NaCl	0.0750	0.0066	8.77

at the end of eighty-five hours. In all cases the N content was determined by the Kjeldahl method. Our methods of procedure had been previously tested by determining the N content of different portions of the same control crop of yeast. Experimental results are given in table 7. Our results indicate a gradual rise in the N content of the crops within this range of salt concentrations. Similar experiments were performed in which the concentration was carried to higher figures, and in these also the N content of the crops was determined. They all agree in showing that when more than 6 per cent of NaCl is present the N figure falls, and when 10 per cent is reached the N figure is similar to that for the controls. We have not the same confidence, however, in these results because under these conditions it is impossible to obtain samples from the dry crops which

are comparable in weight with those from control cultures and those containing only low concentrations of salt. As we have shown earlier in our report the amount of vegetative reproduction is extremely small.

Experiment VIII

The crops from control tubes were isolated, and these were used to inoculate tubes of the same medium containing different concentrations of NaCl. We have found that the control crop in the second fermentation increases in weight approximately 100 per cent. Even in high salt concentrations there is no difficulty in isolating a satisfactory sample of the crop for N determinations. These results are given in table 8. Again we find a gradual rise

TABLE 8

CULTURE	SAMPLE OF YEAST	NITROGEN IN YEAST	NITROGEN IN CROP
	grams	*grams*	*per cent*
Control	0.3839	0.0281	7.34
	0.3646	0.0259	7.10
2.0 per cent NaCl	0.3295	0.0241	7.31
4.0 per cent NaCl	0.1836	0.0136	7.40
6.0 per cent NaCl	0.1135	0.0090	7.94
8.0 per cent NaCl	0.0890	0.0068	7.64
10.0 per cent NaCl	0.1250	0.0086	7.44

and fall in the N content of the yeast crops with the peak of the curve at 6.0 per cent NaCl. In the presence of 10 per cent NaCl little or no growth takes place, and the N content of the yeast crop is similar to that of the control crops. Also the extent of the rise in N is less in this type of experiment than in experiment VII, in which a small inoculum was used. This difference will be considered more fully in our discussion.

THE INFLUENCE OF NaCl ON FERMENTATION RATIOS

It seemed probable that NaCl was influencing the qualitative aspects of the fermentation of wort in addition to its effects on the extent of sugar utilization and gas production. As a prelim-

inary to a more detailed chemical study of the products we have been able to detect a definite change from the normal by a simple calculation of the ratio between sugar utilization and CO_2 output. In addition it is of some importance to measure, if possible, the relative degrees of efficiency of yeast fermenting in different salt concentrations by comparing the weight of sugar utilized with the mass of cells operating. This ratio was used in Pasteur's early investigations to indicate a greater efficiency when yeast fermented under anaerobic conditions. It has since been pointed out that unless the time factor is also included no true comparison of degrees of efficiency can be made. We have overcome this valid objection by stopping certain experiments before the end of any fermentation, i.e., in all the cultures there was residual sugar when the final figures were obtained, and consequently the time factor was constant.

Experiment IX

A series of tubes containing 70 cc. of wort and various concentrations of NaCl were inoculated in the usual way. At the end of the experiment the following quantitative measurements were made: yeast crop, total CO_2 evolved and sugar utilized. From these figures these ratios were determined, $\frac{\text{yeast}}{CO_2}$, $\frac{\text{yeast}}{\text{sugar}}$ and $\frac{CO_2}{\text{sugar}}$. In table 9 the ratios from a number of experiments of this type are assembled. The results from experiments IX A, B show that there is a gradual increase in the fermenting efficiency of yeast, measured by sugar utilization and gas production, as the NaCl concentration is increased up to 4 to 5 per cent. Beyond this amount the efficiency falls. We are justified in accepting the qualitative significance of these results by those obtained in experiment IX C. In all the tubes fermentation was still in progress when the experiment was terminated. In other words the fermentation period was constant in all. During this unit of time the yeast in the 2 and 4 per cent tubes was more efficient than in the controls.

The ratio $\frac{CO_2}{\text{sugar}}$ changes show a rise from the control figure in

low salt concentrations when the total CO_2 output is stimulated, e.g., 1.0 and 2.0 per cent NaCl in experiment IX B. When this does not occur the ratio figure falls with increasing concentrations of salt. Before discussing the possible significance of this change we shall record an attempt to analyze more fully the $\frac{CO_2}{\text{sugar}}$ ratio for a normal fermentation and one containing NaCl.

TABLE 9

EXPERIMENT	NaCl	TIME OF FERMENTATION		SUGAR IN MEDIUM	$\frac{\text{SUGAR}}{\text{YEAST}}$	$\frac{CO_2}{\text{YEAST}}$	$\frac{CO_2}{\text{SUGAR}}$
	per cent	*hours*	*minutes*	*per cent*			
A	0	112	00	8.17	12.2	7.00	
	0				12.3	6.98	
	2				16.2	8.93	
	4				31.0	11.53	
	7				18.6	7.44	
	10				12.3	3.60	
B	0	107	00	8.0	16.6	5.2	0.310
	1				19.1	6.2	0.322
	2				23.6	8.3	0.350
	3				27.0	7.8	0.288
	4				31.1	8.4	0.270
	5				32.6	6.7	0.201
C	0	37	30	9.06	11.6	4.43	0.382
	0				12.4	4.82	0.389
	2				16.8	6.36	0.380
	4				18.2	5.43	0.298

Experiment X

Six tubes were inoculated with 1 cc. of culture. Three tubes were controls, and three contained 5.0 per cent NaCl. Tubes from the two groups were taken at intervals, and the CO_2 output and sugar utilization were determined.

In this experiment the culture tubes were equipped with aeration tubes to allow residual CO_2 to be replaced by air. The CO_2 was slowly washed into the absorption tubes just before the total CO_2 was determined for individual cultures. Small quantities

of medium were rapidly cleared for the sugar determinations. The experimental results and ratios are given in table 10. This type of experiment has been repeated several times, and it is quite definitely shown that the ratio $\frac{CO_2}{\text{sugar}}$ is not a constant throughout a normal yeast fermentation, but rises from about 0.350 to 0.450. It is necessary, therefore, to think not merely of a reaction or a group of coupled reactions rising and falling in intensity and giving uniform products, but rather of qualitative changes in addition to changes in activity. If we accept current views regarding the mechanism of yeast fermentation the ratio figures suggest an accumulation in the early stages of the fermen-

TABLE 10

CULTURE	TIME OF FERMENTATION		SUGAR UTILIZED	CO_2	$\frac{CO_2}{\text{SUGAR}}$
	hours	*minutes*	*grams*	*grams*	
Control	16	15	3.67	1.488	0.406
	21		6.27	2.587	0.413
	129	30	7.02	3.088	0.440
5.0 per cent NaCl	36	30	2.00	0.635	0.318
	61		3.05	1.023	0.335
	137		4.72	1.711	0.362

tation of one or more intermediates which precede the loss of CO_2 by pyruvic acid. It is possible to interpret the figures for cultures containing NaCl in one of two ways. The simplest interpretation is to regard them as relating essentially to an incomplete fermentation, and not qualitatively dissimilar from a control. The results from experiment X when plotted show two parallel curves which are similar in form, but the "salt" curve is below the control for similar degrees of sugar utilization. There is a suggestion, therefore, that NaCl accentuates an accumulation of labile products which is also characteristic of the early stages of a normal fermentation. Only a careful chemical examination of the products will enable us to say what is the precise significance of these changes in the ratios.

CONCLUSIONS

1. Wort containing NaCl up to a concentration of 10 per cent can be fermented by *S. cerevisiae*. The weight of the yeast crop obtained is reduced as the salt concentration is increased. We have no satisfactory evidence of growth stimulation by NaCl in this medium.

2. The lag-phase of the fermentation period is progressively lengthened by increasing concentrations of NaCl.

3. The influence of NaCl on the maximum rate of CO_2 production is dependent upon the size of the inoculum used and the salt concentration. Our experimental observations are summarized in table 11. We have observed similar effects on the total

TABLE 11

INOCULUM (DRY WEIGHT)	EFFECT OF NaCl ON MAXIMUM CO_2 RATE		
	0.5 per cent	1.0 to 1.5 per cent	1.5 to 10.0 per cent
gram			
0.004	Stimulation	Stimulation	Depression
0.020	Stimulation	Depression	Depression
0.400	No change	Depression	Depression

quantity of CO_2 produced. When NaCl is added at some point during an active fermentation low concentrations, i.e., 0.5 to 1.5 per cent, have no immediate stimulatory effect on CO_2 production. Higher salt concentrations under similar conditions depress the rate immediately.

We suggest that these differences are due to the following causes. When low concentrations of NaCl are brought into contact with actively fermenting cells the production rate of CO_2 is not stimulated, and as the salt concentration is increased an increased depression is observed. Yeast cells which are formed by vegetative growth in a medium containing the same low concentration of NaCl produce CO_2 more rapidly, i.e., following upon an increased lag-phase, and in greater quantities than in control cultures. The stimulating effect is not produced by an exposure of cells to the salt solution, but is intimately associated with cell

division. Therefore the observed effect of NaCl, e.g., 1.0 per cent, is determined by the relative numbers of old and new cells in the medium, and it may represent a balance between stimulation and depression. An indication of the possible difference in these numbers of cells is obtained by observing that 0.004 gram of yeast divides 7 times to become 0.400 gram, which is our average yeast crop, whereas an inoculum of 0.400 gram divides once to become 0.800 gram, the crop obtained when Brown's (1892; 1894; 1903) technique is followed.

4. The nitrogen content of yeast crops obtained from media containing NaCl, and inoculated with 0.002 to 0.040 gram of yeast increases until a concentration of approximately 6 per cent NaCl is used. From this point up to 10 per cent the N figure falls to that found for controls. When the inoculum used is a total crop of yeast, 0.400 gram, which ferments the medium with great rapidity and doubles in weight, the N figure changes qualitatively in a similar manner over the same range of salt concentration. Quantitatively the changes are much smaller. Yeast crops of this type exposed to the salted media for times equal to the length of normal fermentations do not show any greater changes in N content, indicating that the time factor is not important in this comparison. We consider that this quantitative difference between the two types of experiment is also due to the different effect of NaCl on existing cells and those formed by division in the experimental media. Recently it has been shown by Sohngen and Coolhaas (1924) that yeast cultures capable of fermenting galactose acquire this property by changes in the enzyme-complex of new cells formed in the medium containing the pentose. Acclimatization, therefore, is dependent upon a capacity to grow under the conditions of the experiment.

5. The ratio sugar fermented: yeast crop increases with the concentration of NaCl up to approximately 5.0 per cent. In higher concentrations the crops have a gradually diminishing efficiency. This relationship holds when the time factor for controls and salted cultures is constant, and its significance is increased in the light of a gradual prolongation of the lag-phase by these concentrations of salt. The changes is efficiency parallel those in N content.

6. As the concentration of NaCl increases (up to 2.0 per cent) the ratio CO_2:sugar utilized shows a gradual rise. In higher concentrations the ratio gradually falls to considerably below the control figure. We have found that during a normal wort fermentation this ratio rises, suggesting an accumulation of intermediates in the early stages. At present we cannot say whether the low ratios for high concentrations of NaCl represent incomplete normal fermentations, or whether they are qualitatively abnormal in their end-products.

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