THE INFLUENCE OF IODINE UPON THE GROWTH AND METABOLISM OF YEASTS[1]

J. E. GREAVES, C. E. ZOBELL AND J. DUDLEY GREAVES

Utah Agricultural College Experiment Station, Logan, Utah

Received for publication July 16, 1928

There are certain elements which are required in comparatively large quantities for the normal growth and reproduction of organisms. Other elements are required in smaller quantities whereas still others, while not essential, if supplied modify and often accelerate growth and metabolism. That our knowledge of the required elements is less complete concerning the microscopic forms of life than it is concerning the macroscopic forms is appreciated by all who have tried to grow yeast and some bacteria upon a strictly known mineral salt-sugar solution. Under such conditions growth is often absent or meagre in quantitv, or metabolic activity is perverted. This was forcefully brought to our attention during the past year in a study that was made of new species of nitrogen-fixing microörganisms obtained from a typical arid soil. Some microörganisms were obtained which are energetic nitrogen fixers if grown in soil or soil extract media containing an appropriate source of energy, but, so far, all efforts to obtain fixation upon a known mineral salt-sugar medium have failed. This led to the investigation of the influence of certain substances (which occur in plants, soils, and waters in minute quantities) upon the growth and metabolism of microörganisms. Preliminary experiments have been performed on yeasts, as they lend themselves more readily to growth and metabolic experiments than do the nitrogen-fixing bacteria. The direct microscopic count of the number of cells at varying times was taken as a measure of growth

[1] Approved for publication by Director, July 9, 1928. Contribution from Department of Bacteriology, Utah Experiment Station.

and the carbon dioxide evolved as a measure of metabolism. In this paper are given the results obtained when yeasts are grown in the presence and absence of iodine, in a mineral salt-sugar medium.

METHODS OF EXPERIMENTATION

The early work was with commercial yeasts, and later work with pure cultures. It was necessary to devise means for insuring the continuous purity of the cultures throughout the progress of each specific test. Considerable difficulty was experienced in accomplishing this and at the same time assuring an adequate supply of oxygen to the cultures. The yeasts were cultured in synthetic media in specially arranged Erlenmeyer flasks. At intervals the number of yeasts in the cultures and the quantity of carbon dioxide evolved was determined.

Media

Definite information concerning nutrient requirements of microörganisms has come from studies in which known cultural media are used; i.e., those prepared from pure chemicals, the composition of which is known. After considerable preliminary work with various synthetic media, Mayer's culture fluid was found best suited as the carrier of the iodine. Its composition is as follows:

Sugar	15.0 grams
Potassium phosphate (monobasic)	5.0 grams
Magnesium sulfate	5.0 grams
Calcium phosphate (dibasic)	0.5 gram
Ammonium nitrate	0.75 gram
Distilled water	1000.00 cc.

The inorganic constituents were Baker's highest purity. The water was carefully distilled. This medium was used as the carrier of varying quantities and kinds of iodine which were added from carefully standardized solutions prepared from salts of the highest obtainable purity. In the early work, commercial beet sugar was used as the source of carbon. Later, Baker's highest purity glucose and sucrose which had been carefully washed with

80 per cent hot alcohol was employed. Consequently, the composition of each specific cultural medium is quite accurately known.

Cultural method

The yeasts were cultured in 500-cc. Erlenmeyer flasks fitted with 3-hole rubber stoppers. Through the first hole passed a 3-inch piece of glass tubing having a $\frac{3}{8}$-inch bore and closed at the top with a rubber stopper. Over this was inverted a snugly fitting test tube. Samples were removed through this opening with sterile pipettes, as required for the making of counts. The second hole contained a tube bent at 90°, the end of which entered the flask dipping into the culture fluid. The end was drawn out to a fine point. This served as an inlet for the sterile carbon-dioxide-free-air and insured adequate aeration, which is so essential for the rapid multiplication of yeasts. In the earlier work, air was drawn through cotton and soda lime to free it of microörganisms and carbon dioxide, but, it soon became evident that if tests were to be conducted over long periods microörganisms would be drawn through the cotton. Consequently, in the later tests, air was drawn through bottles containing in the order named, sulfuric acid, potassium hydroxide, tartaric acid and finally sterile distilled water. The third hole in the stopper of the cultural flask contained a bent tube which passed just through the stopper and thence to a second 500-cc. Erlenmeyer flask containing N/5 barium hydroxide. This flask was fitted with a 2-hole stopper, the second opening of which contained a tube leading to an exhaust pump. When the pump was in action sterile air, free from carbon dioxide, was drawn into the culture fluid and the evolved carbon dioxide was drawn into the barium hydroxide, which was carefully protected against outside contamination. The culture flasks were immersed in a water bath kept at 28° to 30°C. by means of an electrical heating device.

In the early experiments the flasks were inoculated from a suspension of yeasts. This was prepared by triturating 1 gram of yeast cake (Fleischman's or Red Star) in 100 cc. of Mayer's culture fluid until the yeasts were evenly distributed. In later work

a loopful of yeast from a known culture was placed into 100 cc. of Mayer's culture fluid and then shaken until the yeasts were uniformly distributed. Measured portions of these suspensions were used for the seeding of the cultural flasks.

The carbon dioxide was determined by collecting in N/5 barium hydroxide and titrating with standard oxalic acid using phenolphthalein as the indicator. The results are reported as milligrams of carbon dioxide produced up to the specified times. At the same time the number of yeasts in the culture fluid was determined by the removal of one cubic centimeter portions using aseptic precautions so as not to contaminate the cultures. Counts were made by diluting to the required extent and then placing a drop on the disk of a hemocytometer. Ten groups of 25 squares were counted; in the absence of agreement among duplicates this was repeated. The reported results are the average of a number of determinations and represent the number of yeasts found in one cubic millimeter of the culture solution at the specified time. One of the greatest obstacles encountered in the work was the obtaining of representative samples, as the yeasts tend to adhere. The constant drawing of air through the culture obviated this difficulty only to a degree.

COMMERCIAL YEASTS WITH SODIUM IODIDE

The first tests were made on commercial yeasts—Fleischman's in some and Red Star in others. One gram portions of the yeast cake were triturated with 500 grams of sterile distilled water. One cc. portions of this suspension were distributed to the various flasks, each containing 100 cc. of Mayer's culture solution with commercial beet sugar as the source of carbon. The initial inoculation in the various sets, the averages of which are reported in tables 1 and 2 varied from 5000 to 10,000 yeast cells. The iodine content of the media varied from 0 to 8000 parts per million. The number of yeasts and the quantity of carbon dioxide produced were determined at intervals. The averages for four such sets of determinations are given in tables 1 and 2.

Earlier tests had shown that when large quantities of the inoculum were used rapid growth occurred in all the cultures. It is

evident from these results that the initial inoculum must have been nearly the same in the various flasks and that growth in Mayer's culture fluid is extremely slow. Growth is accelerated when one part per million of iodine in the form of sodium iodide is added to the culture medium. The maximum stimulation is produced when 10 parts per million is present. In this iodine concentration the yeast cells at the end of thirty seven hours are four times as numerous as they are in the straight Mayer's medium. In the higher concentrations of iodine there is a longer lag

TABLE 1

Number of yeasts per cubic millimeter produced in Mayer's culture fluid containing varying quantities of iodine as sodium iodide with commercial beet sugar as the source of carbon

IODINE AS NaI	THOUSANDS PRODUCED DURING							
	24 hours	26 hours	28 hours	30 hours	32 hours	34 hours	36 hours	37 hours
p.p.m.								
None	5	5	5	6	6	7	7	8
1	5	5	6	8	8	11	12	16
10	5	8	12	12	15	25	25	31
100	5	5	5	6	7	19	16	22
1,000	5	5	7	7	10	26	18	30
2,000	4	4	5	9	14	20	18	25
3,000	4	5	6	7	7	14	15	18
4,000	5	5	5	5	5	14	13	15
5,000	3	4	5	5	5	13	11	15
6,000	3	5	4	8	10	21	35	39
7,000	2	2	3	4	4	8	8	9
8,000	4	4	4	3	4	20	15	22

period, probably due to the yeast becoming acclimated to the iodine-containing medium. There is no evidence of toxicity in any of the concentrations tested, and the results point to a definite stimulation, even with the minute quantity of one part per million.

At the same time that counts were made, the quantities of carbon dioxide which had been evolved were determined. The averages, stated as milligrams of carbon dioxide produced in the various intervals, are given in table 2.

There was a gradual increase from period to period in the carbon dioxide produced. This is not what would be anticipated from the action of a definite number of cells and indicates either increased number of producing cells or increased efficiency. It would seem to be due to multiplication and not increased metabolism. Iodine stimulates even in one part per million and reaches its maximum efficiency in a concentration of 10 parts per million. The low results obtained when 7000 parts per million of iodine were present would be accounted for by errors which may have

TABLE 2

Milligrams of carbon dioxide produced by yeast in 100 cc. of Mayer's solution of culture fluid containing varying quantities of iodine as sodium iodide with commercial beet sugar as the source of carbon

IODINE AS NaI	MGM. CARBON DIOXIDE PRODUCED DURING							
	24 hours	26 hours	28 hours	30 hours	32 hours	34 hours	36 hours	37 hours
p.p.m.								
None	50	61	86	108	138	179	221	255
1	50	67	85	109	148	196	245	281
10	57	77	99	131	176	231	281	316
100	49	64	82	116	162	218	276	330
1,000	51	66	83	114	158	204	253	288
2,000	42	56	74	97	127	165	203	224
3,000	54	70	84	101	121	148	176	199
4,000	47	58	71	85	105	130	154	178
5,000	36	47	61	80	104	127	157	181
6,000	51	67	87	122	170	225	269	306
7,000	13	20	28	38	50	67	84	100
8,000	60	79	98	134	130	212	276	306

crept into the work, were it not for the fact that it appeared to a greater or less extent in all sets and occurred even when the various flasks were interchanged on the apparatus. When the milligrams of carbon dioxide per 1000 yeasts are calculated it is found that in the absence of iodine the production of carbon dioxide for each individual cell gradually increases throughout the reaction, i.e., it follows the law of an autocatalized reaction. In the presence of iodine the production of carbon dioxide is more nearly constant, indicating that the older cells are more efficient in the absence of iodine than in its presence. The results point to the conclusion

that sodium iodide increases growth but does not increase individual metabolic activity.

INFLUENCE OF POTASSIUM IODIDE UPON COMMERCIAL YEASTS

Commercial yeasts were also cultured in the presence of potassium iodide. The same concentrations of iodine were used as in the sodium iodide series with the same results. The iodine stimulated growth when present in the culture medium in small quantities. The quantity of carbon dioxide produced was increased; this was due to an increased number of cells and not to an increased efficiency. In short, the result were so nearly a duplicate of those obtained with the sodium iodide that only these general conclusions need be given.

INFLUENCE OF CALCIUM IODIDE UPON COMMERCIAL YEASTS

When calcium was used as the carrier of iodine the stimulation was also apparent. There was a decrease in the lag period but no evidence of an increased metabolic activity of individual cells. The increase in carbon dioxide came from an increase in the number of cells. The rate of multiplication and also the metabolic activity in all series were markedly influenced by the size of the inoculum, the influence of the iodine being most clearly manifested when small seedings were used.

INFLUENCE OF ELEMENTARY IODINE UPON COMMERCIAL YEASTS

There often appeared in the culture fluid a slightly yellowish tinge, especially in those cultural flasks exposed directly to the light. This was probably elementary iodine; hence the question arises as to whether or not elementary iodine is toxic. Furthermore, in order to make more certain that it was the iodine and not the cation, sodium, potassium, or calcium, which acted as the stimulant, a series was run in which varying quantities of elementary iodine were added to Mayer's culture fluid. A standard alcoholic solution of iodine was prepared and this was used to introduce into the culture media the required quantity of iodine. To those cultures receiving small quantities of iodine or no iodine,

there was added sufficient alcohol so that all received the same amount of alcohol, whether or not they received iodine. Consequently, the only variation in their treatment was in the quantity of elementary iodine which they received. The results are given in table 3.

A slight stimulation is noted in the presence of one part per million of elementary iodine. It is probable that smaller quanti-

TABLE 3

Number of yeasts produced in Mayer's culture fluid containing varying quantities of iodine

IODINE AS I_2	THOUSANDS OF YEASTS IN MEDIUM AT THE END OF						
	24 hours	26 hours	28 hours	30 hours	32 hours	34 hours	36 hours
p.p.m.							
None	6	8	9	11	11	17	11
1	6	7	8	12	12	19	16
10	3	5	6	6	6	6	7
100	2	2	3	5	5	5	7
1,000	0.6	0.6	0.6	0.6	0.6	0.6	0.6
2,000	0.6	0.6	0.6	0.6	0.6	0.6	0.6

TABLE 4

Milligrams of carbon dioxide produced in Mayer's culture fluid containing varying quantities of elementary iodine

IODINE	24 HOURS	26 HOURS	28 HOURS	30 HOURS	32 HOURS	34 HOURS	36 HOURS
p.p.m.							
None	65	89	106	117	128	143	158
1	70	93	106	120	132	148	164
10	51	68	80	94	108	122	132
100	20	33	42	55	68	81	95
1,000	6	15	19	21	24	28	32
2,000	6	15	19	21	23	27	31

ties of elementary iodine would have to be used to obtain the full stimulating effect, as 10 parts per million act as a weak antiseptic; and when 1000 parts per million are present all growth is prevented. It is evident that when one part per million of iodine in the form of the various salts is added to the culture media there would never be sufficient elementary iodine liberated to prove toxic to the yeast. With larger quantities this may not be true,

and it is quite possible that the erratic results which so often appeared have been due to this factor. The quantities of carbon dioxide produced under the same conditions are given in table 4.

One part per million of elementary iodine slightly increases carbon dioxide production, corresponding to the increased number of cells. Higher concentrations are toxic. The iodine retards growth to a greater extent than it does metabolism.

The results with commercial cultures of yeasts warrant the conclusions that various salts of iodine, together with elementary iodine when added to Mayer's cultural fluid, stimulate growth of yeasts. The greatest stimulation, with the concentrations tested,

TABLE 5

Number of yeast cells produced in Mayer's solution with and without iodine and in the presence of various carbohydrates

TREATMENT (I AS KI)	THOUSANDS OF YEASTS PRODUCED IN							
	24 hours	30 hours	34 hours	50 hours	98 hours	146 hours	194 hours	218 hours
Sucrose	3	9	10	25	72	155	158	158
Sucrose + 100 p.p.m. I	5	10	13	56	76	196	175	195
Lactose	1	1	1	3	2	3	3	3
Lactose + 100 p.p.m. I	1	1	1	2	3	3	3	3
Maltose	1	2	2	3	26	53	101	158
Maltose + 100 p.p.m. I	2	3	3	5	23	46	95	98

was when one part per million of iodine is present. Whether even smaller quantities will stimulate cannot be answered from these results.

INFLUENCE OF IODINE IN THE PRESENCE OF VARIOUS CARBOHYDRATES

A series was run in which various carbohydrates were used as the source of carbon, with and without potassium iodide. Unfortunately, one part of iodine was used in 10,000 parts of the media. The results as to numbers are reported in table 5.

Good growth occurred in the presence of sucrose and maltose,

but little occurred in lactose, the yeast being unable to assimilate it. Growth was increased in the sucrose containing iodine, but no effect was produced by iodine in the presence of maltose or lactose in the concentrations of iodine tested. Would it have stimulated had the concentrations been only one part per million?

The quantities of carbon dioxide evolved by yeasts in the presence of iodine and with various carbohydrates as the source of energy are given in table 6.

The increased carbon dioxide resulting from the use of the iodine in the presence of sucrose is in keeping with the results already reported. That is, the carbon dioxide increases with greater

TABLE 6

Milligrams of carbon dioxide produced by yeasts in Mayer's culture fluid with and without iodine with various carbohydrates as the source of energy

TREATMENT (I AS KI)	24 HOURS	30 HOURS	34 HOURS	50 HOURS	98 HOURS	146 HOURS	194 HOURS	218 HOURS
Sucrose	19	50	91	374	609	713	770	796
Sucrose + 100 p.p.m. I	21	55	99	344	586	684	787	807
Lactose	9	22	29	60	108	120	133	152
Lactose + 100 p.p.m. I	8	20	26	66	118	127	139	151
Maltose	11	27	38	114	346	566	633	676
Maltose + 100 p.p.m. I	13	31	41	119	351	580	665	728

number of cells resulting from the stimulation of growth by the iodine. Although the yeasts did not readily multiply in the presence of lactose, yet their metabolic activities were comparatively active, pointing to the conclusion that metabolic activity is increased by iodine in the presence of maltose, and especially lactose.

INFLUENCE OF IODINE ON PURE CULTURES OF YEASTS

The results so far reported were obtained with commercial cultures of yeasts. Therefore, the questions arise: Can they be duplicated with pure cultures: Are they characteristic of yeasts or, are they secondary effects from the action of iodine upon other microörganisms and do these in turn influence the growth and

metabolism of the yeasts? There is also the possibility of substances occurring in the commercial beet sugar or in the minute seeding carried over in the inoculum which may be acted upon by iodine so that the resulting compound will act as a stimulant. For these reasons, cultures of yeasts were obtained from Dr. F. W. Tanner of the University of Illinois. These were grown in cultural media of known composition. Greater precautions were taken to prevent contamination from outside sources, and more

TABLE 7

Yeast cells (Saccharomyces cerevisiae) produced in Mayer's solution containing varying quantities of potassium iodide with commercial beet sugar as the source of carbon

IODINE AS KI	THOUSANDS OF CELLS AFTER					
	50 hours	74 hours	98 hours	122 hours	146 hours	170 hours
p.p.m.						
None	0.3	1.0	6	6	8	9
1	0.3	0.3	3	3	6	13
10	0.3	0.5	2	3	35	35
100	0.3	0.3	1	2	24	28
1,000	0.3	0.4	2	22	33	52
2,000	0.3	2.0	2	21	36	57
3,000	0.3	1.0	5	5	42	46
4,000	0.3	1.0	2	2	16	19
5,000	0.3	0.5	0.6	1	10	34
6,000	0.3	0.3	1	1	24	31
7,000	0.3	1.0	3	3	17	40
8,000	0.3	4.0	10	8	32	

care used to prevent unknown constituents from being carried over in the inoculation. The size of the seeding was also quite accurately known.

INFLUENCE OF IODINE ON TYPES OF SACCHAROMYCES CEREVISIAE

In the first series, *Saccharomyces cerevisiae* were cultured in Mayer's culture fluid containing various quantities of potassium iodide with commercial beet sugar as the source of carbon. The average results for four determinations are given in tables 7 and 8.

Many workers have found, when large numbers of yeasts are

used for seeding the culture media, that growth is better than when small numbers are used, and that if the seeding into synthetic media be extremely small, either no growth or very meagre growth occurs. Consequently, special precautions were taken to insure uniform seeding and to possess actual knowledge concerning the size of the seeding. This was soon accomplished by suspending a loopful of the culture in 100 cc. Mayer's culture fluid. Counts were made on this; it was then diluted so that each cubic centimeter contained approximately 100 cells. One cubic centimeter portion of this suspension was seeded into each cul-

TABLE 8

Milligrams of carbon dioxide produced by yeasts (Saccharomyces cerevisiae) in the presence of varying quantities of potassium iodide with commercial beet sugar as the source of carbon

IODINE AS KI	24 HOURS	50 HOURS	74 HOURS	98 HOURS	122 HOURS	146 HOURS	170 HOURS
p.p.m.							
None	4	17	18	116	323	372	436
1	4	8	20	159	313	363	446
10	6	17	26	71	261	380	428
100	5	22	37	112	258	390	495
1,000	5	17	36	172	364	449	514
2,000	11	22	37	165	389	469	523
3,000	5	27	40	119	279	397	460
4,000	5	18	31	106	156	308	393
5,000	6	18	31	97	144	207	371
6,000	5	16	21	70	187	301	370
7,000	5	18	31	121	258	344	493
8,000	4	19	35	115	188	264	358

tural flask. That this was quite uniform and that all flasks contained living yeasts is evident from the fact that the concentration of yeast at the end of twenty-four hours was over 320 cells per cubic millimeter. It is also evident that the results with pure cultures are not far different from those obtained with commerical cultures. Growth is extremely slow and is stimulated by small quantities of iodine. The spread of the concentration of iodine which stimulates is greater than with the commercial yeasts, and there is no concentration which can be considered toxic. The average results for the metabolism experiments are reported in table 8.

Considerable carbon dioxide was produced in this medium, thus indicating that it contained sufficient bios for growth and metabolism, even though the seeding was small.

The results give evidence of a shortened lag period due to the iodine. The production of carbon dioxide has been increased due to the iodine, and although it does not occur at the same concentration as was found in the commercial cultures yet it occurs when only small quantities of iodine are added. The stimulation becomes more evident as the time of the growth is prolonged. By the end of 194 hours it was very evident, even at a concentration of one part per million of iodine. However, these results were discarded and the experiment terminated as there was evidence in some of the cultural flasks of contamination. It has been our repeated observation that when bacteria, and especially mold growths, appear in cultural flasks, the results are very materially changed. Under such conditions even in the non-iodine containing cultures there was an increased growth and metabolism of the yeasts.

The results with the pure cultures are quite similar to those obtained with the commercial yeasts. When the pure cultures of *Saccharomyces cerevisiae* were grown with sodium iodide in varying concentrations of lactose or maltose the results confirmed those obtained with the commercial yeasts; consequently, the results are not reported here.

The results so far obtained have demonstrated the following: (1) Various salts of iodine increases growth and metabolism of the yeast cells. Apparently multiplication is increased more in proportion than is respiration. (2) Sufficient bios is present in commercial beet sugar to furnish the requisite accessory food factors even when highly purified salts are used as the basis of the nutrient medium. (3) Yeast will readily grow in Mayer's nutrient medium if large quantities of yeast are used to make the inoculation.

PURIFIED CARBOHYDRATES

In an attempt to substitute iodine for bios, yeasts were cultured in the presence of varying quantities of iodine, using Baker's

highest purity glucose as the source of carbon. The glucose was substituted for the sucrose as the latter is not readily fermented by the yeasts used. Mayer's culture fluid was prepared from Baker's highest purity chemicals. The stock culture of yeasts (*Saccharomyces cerevisiae*) was inoculated into 100 cc. of this medium and microscopic counts made of the number of cells in 1 cc. of the medium. It was then diluted to such an extent that each cubic centimeter contained 50 yeasts. One cubic centimeter portion of this solution was distributed to each of the culture media with varying quantities of potassium iodide. At intervals, counts and carbon dioxide determinations were made. The numbers of organisms found in one cubic millimeter are reported in table 9.

TABLE 9

Number of yeasts (Saccharomyces cerevisiae) in Mayer's solution containing varying quantities of potassium iodide and highly purified glucose

IODINE AS KI	74 HOURS	98 HOURS	122 HOURS	146 HOURS	170 HOURS	194 HOURS	218 HOURS	242 HOURS	266 HOURS	290 HOURS	314 HOURS	338 HOURS
p.p.m.												
None	+	+	+	+	+	+	+	+	+	+	+	+
1	+	+	0.6	0.4	19	33	33	35	47	49	10	17
10	+	+	+	+	0.6	3	42	65	13	14	14	14
100	+	0.6	0.6	0.6	0.6	0.6	0.6	0.6	2	5	52	61
1,000	+	+	+	+	+	+	+	+	+	3	52	66
2,000	+	+	+	+	+	+	+	+	+	+	+	8
3,000	+	+	+	+	+	+	+	+	+	+	+	+

These represent the average results of three separate determinations.

The culture media were inoculated with 50 yeasts per cubic-centimeter. This would be only one cell in each 20 c. mm., a number so small that they could not be enumerated by the microscope method with any degree of accuracy; consequently, where only one cell was found in the ruled spaces of the hemocytometer it is listed in the above table with a + sign and may indicate from 1 to 320 in a cubic millimeter of the solution. Where the yeasts were more numerous figures are given which represent the number of thousands of yeasts in 1 c. mm. of the culture fluid.

In the culture medium containing no potassium iodide there

were never over 320 yeasts per cubic millimeter. Consequently, multiplication must have been extremely slow. In the presence of 1 p.p.m. of iodine, the number had reached 19,000,000 at the end of 170 hours; at the end of 290 hours there were 49,000,000 yeasts. After 290 hours there was a decrease. The numbers are even

TABLE 10

Milligrams of carbon dioxide produced by Saccharomyces cerevisiae in the presence of varying quantities of iodine as potassium iodide and with Baker's highest purity glucose as the source of carbon

IODINE AS KI	24 HOURS	50 HOURS	74 HOURS	98 HOURS	122 HOURS	146 HOURS	170 HOURS	194 HOURS	218 HOURS	242 HOURS	266 HOURS	290 HOURS	314 HOURS	338 HOURS
p.p.m.														
None	2	3	4	34	38	41	43	43	43	45	46	56	57	88
1	2	15	16	17	20	61	200	277	314	348	376	442	480	500
10	3	5	5	6	18	22	23	37	90	131	156	190	209	236
100	4	6	7	7	11	15	18	21	28	41	57	118	172	221
1,000	2	3	5	8	12	13	15	32	44	56	60	124	205	248
2,000	2	4	4	6	42	56	60	55	66	69	77	95	126	226
3,000	2	4	6	7	13	13	14	18	18	20	20	24	33	34

TABLE 11

Number of yeasts (Saccharomyces cerevisiae) in Mayer's solution containing varying quantities of potassium iodide and highly purified sucrose

IODINE AS KI	96 HOURS	120 HOURS	144 HOURS	168 HOURS	192 HOURS	216 HOURS	240 HOURS	264 HOURS	288 HOURS	312 HOURS	336 HOURS
p.p.m.											
None	+	+	+	+	+	+	+	1	1	1	6
1	+	+	+	+	+	+	+	4	26	38	37
10	+	+	+	+	+	+	+	+	1	2	2
1,000	+	+	+	+	+	+	+	1	17	22	23
2,000	+	+	+	+	+	+	+	+	+	2	6
3,000	+	+	+	+	+	+	+	+	+	1	2

greater in the presence of 10 p.p.m. of iodine. At 100 and 1000 p.p.m. the lag period is lengthened over what it is in the lower concentrations; apparently the decline does not set in as early. In 2000 p.p.m. of iodine multiplication is very slow and in 3000 p.p.m. it is not perceptible.

A very small, constant quantity of carbon dioxide is evolved in the absence of iodine but in the presence of one part per million of iodine there is an increase in the evolution of carbon dioxide in keeping with the increased number of yeast cells. In the presence of higher concentration there is also an increase of carbon dioxide even up to 2000 parts per million. Above this concentration the iodine apparently is toxic for in the presence of 3000 parts per million of iodine there is no apparent increase in yeast cells and a decrease in the carbon dioxide evolved.

In an attempt to determine if iodine stimulates when carefully purified sucrose is used as a source of carbon, a similar set was

TABLE 12

Milligrams of carbon dioxide produced by Saccharomyces cerevisiae in the presence of varying quantities of iodine as potassium iodide and with purified sucrose as the source of carbon

IODINE AS KI	24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS	144 HOURS	168 HOURS	192 HOURS	216 HOURS	240 HOURS	264 HOURS	288 HOURS	312 HOURS	336 HOURS
p.p.m.														
None	1	3	5	6	7	8	9	9	11	13	13	16	30	83
1	1	3	3	4	6	13	19	25	28	29	53	173	220	236
10	2	2	9	23	30	37	44	50	55	63	86	114	145	175
1,000	6	24	47	75	100	114	139	169	213	237	244	289	387	409
2,000	4	8	17	18	28	46	58	69	77	101	132	147	158	181
3,000	6	8	9	11	11	18	32	48	60	62	70	85	92	101

run through using sucrose as the carbohydrate. Commercial beet sugar was purified by repeated washing with 80 per cent hot ethyl alcohol. This purified sucrose was used in the making of Mayer's nutrient medium. Baker's highest purity chemicals were used throughout and everything with the exception of the carbohydrate, kept as nearly comparable with the preceding series as possible. Each cultural flask was inoculated with a suspension of yeast in sufficient quantities to add 50 yeast cells to each cubic centimeter of the cultural medium. These were then incubated at 28°C. and at intervals determinations were made of the number of yeasts and milligrams of carbon dioxide evolved. The average results for numbers are given in table 11.

The small initial inoculation accounts for the extremely long time which elapsed before growth was perceptible, but the fact that yeasts were always found on microscopic examination proved that all flasks were inoculated. Even one part per million of iodine stimulated growth. These results are almost an exact duplicate of those obtained when this type of yeast was grown with purified glucose as the source of carbon, thus making it certain that the organism is stimulated by iodine or that iodine can substitute for the bios.

At the same time that counts were made the carbon dioxide evolved was determined. The averages for these results are given in table 12.

The results for the metabolism indicate that no appreciable multiplication of the yeast cells occurred in the iodine-free medium, as the quantity of carbon dioxide produced in unit time is a constant. However, in the presence of iodine it increases. This is very noticeable even in the presence of 1 p.p.m. of iodine, but like growth, metabolism reaches its maximum in the presence of 1000 p.p.m. of iodine. It is evident that both multiplication and respiration are influenced by iodine, whether the source of carbon be purified glucose or purified sucrose.

Other series were run in which all conditions were kept as nearly as possible the same as in the above series with the exception of the yeast. In these series, *Saccharomyces cerevisiae* Type Frobergh and *Saccharomyces cerevisiae* were used. The results were similar to those reported.

It required 288 hours in the synthetic media devoid of iodine for the yeasts to become numerous enough to enumerate by the microscopic method. During the following forty-eight hours the yeast made consistent gains in this medium. In similar media containing 1 p.p.m. of iodine as potassium iodide the yeasts were sufficiently numerous to enumerate after 144 hours; by the end of 336 hours there were 102,000 yeasts in each cubic millimeter. In higher concentrations there was also a stimulation, but it was neither as great nor as uniform as in the lower concentrations of iodine. There was unmistakable evidence of stimulation with 1 p.p.m. of iodine which corresponded with the increase in number

of yeast cells and is in keeping with the results obtained in the other reported tests.

In all of the series there was conclusive evidence that iodine increases multiplication, even when added to the nutrient medium in a concentration of one part per million.

Saccharomyces cerevisiae Type Saaz, growing in Mayer's nutrient medium with carefully purified sucrose as the source of carbon, produced only 20 mgm. of carbon dioxide, whereas with some concentrations of iodine there was produced over 25 times this amount. The rate per hour of carbon dioxide production in the absence of iodine was nearly constant throughout the series, indicating little, if any, multiplication, whereas in the presence of iodine it was progressively increased, as is characteristic of an autocatalized chemical reaction.

It was often desired to conduct the experiment for a longer period, but usually the work was terminated through the fact that molds or less often bacteria found their way into the culture solution. In the work so far reported, cotton and large tubes of soda lime were depended upon to keep out microörganisms and carbon dioxide. In order to make it possible to conduct the tests for a longer period and to find whether these yeasts multiply when inoculated into synthetic media in very small numbers, and to determine whether the stimulation by iodine has only a temporary effect or will last over long periods, a series was run with the following modifications: (1) The air was drawn through strong acid and alkali and then through cotton and soda lime. All connections were sealed with wax, thus making it possible to conduct the experiment over long periods without contamination of the cultural solutions. (2) Baker's highest purity sucrose was carefully washed with hot 80 per cent alcohol and this sugar used as the source of carbon. (3) The cultures were seeded with only 150 yeasts to the flask. It is claimed by some workers that a pure synthetic medium seeded very lightly does not promote growth, while others maintain that it does; however, the growth is extremely slow. The average results for three such sets are given in table 13.

Although the results do not show an actual multiplication in the

medium containing no iodine, yet it was evident that there had been a slight multiplication as the cells were more easily found toward the close of the experiment than they were at the beginning. However, growth was very slow, as is illustrated by the fact that even after thirty-one days there were not over 320 cells to the cubic millimeter. When iodine was supplied, multiplication was rapid enough so that toward the close of the experiment in some of the concentrations of iodine they reached thousands in 1 c. mm. However, even in the presence of iodine there is only a slow growth. The quantities of carbon dioxide produced under these conditions are given in table 14.

TABLE 13

Number of yeasts (Saccharomyces cerevisiae) produced in Mayer's nutrient solution with and without iodine and with highly purified sucrose as the source of carbon

IODINE AS KI	9 DAYS	11 DAYS	13 DAYS	15 DAYS	17 DAYS	19 DAYS	21 DAYS	23 DAYS	25 DAYS	27 DAYS	29 DAYS	31 DAYS
p.p.m.												
None	−	+	+	+	+	+	+	+	+	+	+	+
1	−	+	+	+	+	+	+	+	+	+	1	1
10	2	2	4	6	7	7	6	5	7	6	10	11
100	−	+	+	3	5	8	6	8	12	12	13	16
1,000	2	3	4	4	4	7	8	8	9	9	11	11
2,000	−	+	+	+	+	1	1	1	2	2	2	3
3,000	−	+	+	+	+	+	1	1	1	1	1	1
4,000	−	+	+	+	+	+	+	+	+	+	1	1

At the beginning of the experiment the non-iodine-containing series was producing carbon dioxide at the rate of 1 mgm. per twenty-four hours. This regularly increased, so that by the end of the experiment it was being produced at the rate of 7 mgm. per twenty-four hours. If there was no change in efficiency there would have been 7-fold multiplication in thirty-one days. This is extremely slow. By the same reasoning it may be concluded that in the presence of 100 p.p.m. of iodine there had been three times this growth. Counts, however, indicate that there had been an even greater increase than this.

GENERAL CONCLUSIONS

It is evident from these results that the growth and metabolic activities of yeast are extremely slow in Mayer's cultural fluid when it is prepared from carefully purified chemicals. It is also evident that impure chemicals, especially the carbohydrates, may contain sufficient of Wildier's bios growth-promoter, to increase growth appreciably. The same may be the case when large quantities of the yeasts are used for seeding the cultural flasks, presumably due to the carrying of sufficient bios into the new cultures. The results reported in this paper conclusively prove that minute quantities of iodine when added to an appropriate synthetic medium promote the growth of yeasts and raise a number of interesting questions:

1. What is the relationship of Wildier's bios to iodine? If Wildier's, or later Develo's,[2] description of bios be taken as a criterion of properties, it cannot be stated that iodine meets fully the requirements of bios. Some of the properties attributed to bios could not be attributed to iodine. Moreover, the increase in growth attributed to bios is often much greater than we have been able to produce with iodine. These objections, however, do not preclude the possibility of organic or inorganic compounds of iodine being the possible cause of the phenomenon attributed to bios. There is also the possibility of iodine not being the only element required by yeast in minute quantities. It does not appear that the hypothetical bios could have been carried into these cultures by iodine and that it and not the iodine is the stimulator of growth, for: (*a*) Chemicals of high purity were used as the carriers of iodine; (*b*) sodium, potassium, and calcium iodide and also elementary iodine all stimulate and it would have to be assumed that all carried bios; (*c*) iodine stimulates when present in only 1 p.p.m. and possibly in even smaller concentrations. Hence, if it be concluded that the iodine is the carrier of

[2] Inasmuch as the whole bios question has been ably reviewed by Fred W. Tanner in the Physiological Review (vol. 1, no. 4, pp. 397–472), no references to the literature are given here. However, those interested are referred to Dr. Tanner's article.

impurities which are the cause of the increased growth, it would be necessary for them to be effective in almost infinitisimal quantities.

2. The absence of measurable quantities of yeast growth in the absence of iodine and the appreciable growth in its presence raises the question: Is not iodine in minute quantities required for the growth of yeast? These results indicate that it is. Is this iodine which is assimilated by the yeasts built into organic compounds which may be valuable to higher animals? If so, may this not be one of the best methods yet devised for the administration of iodine to man? It would probably be less irritating

TABLE 14

Milligrams of carbon dioxide produced by yeast (Saccharomyces cerevisiae) when grown in Mayer's solution with and without iodine

IODINE AS KI	7 DAYS	8 DAYS	9 DAYS	11 DAYS	13 DAYS	15 DAYS	17 DAYS	19 DAYS	21 DAYS	23 DAYS	25 DAYS	27 DAYS	29 DAYS	31 DAYS
p.p.m.														
None	6	9	15	26	40	56	70	83	121	145	169	200	212	225
1	3	7	14	28	47	71	89	118	138	156	172	192	206	221
10	3	7	12	23	42	64	87	120	164	205	259	306	348	390
100	4	10	13	28	42	70	107	157	228	311	387	464	534	633
1,000	7	15	22	42	99	142	235	313	394	458	494	531	557	590
2,000	4	9	15	28	45	70	93	115	141	166	187	212	235	253
3,000	3	6	11	22	39	65	89	117	142	155	172	186	200	211
4,000	4	9	18	31	50	74	109	140	175	200	235	280	313	356

than the inorganic salts of iodine and the distribution, and, consequently, dosage could be nicely controlled. Later work must answer these questions.

SUMMARY

Growth and metabolism of yeasts are extremely slow in a mineral salt-sugar solution such as Mayer's cultural fluid. Heavy seeding, the presence of impurities such as may occur in commercial beet sugar, and bacterial (and especially mold) growth accelerate yeast multiplication.

Small quantities of iodine, 1 p.p.m. either as elementary iodine

or as the salts of sodium, potassium, or calcium accelerate yeast growth.

The relationship of iodine to Wildier's bios and the possibility of the use of iodine-cultured yeasts as a means of furnishing iodine to man is considered. The results indicate that iodine is essential to yeast growth and metabolism.