XXIX. THE EFFECT OF SELENIUM ON CELLULAR METABOLISM. THE RATE OF OXYGEN UPTAKE BY LIVING YEAST IN THE PRESENCE OF SODIUM SELENITE.¹

BY VAN RENSSELAER POTTER²
AND CONRAD ARNOLD ELVEHJEM.

*From the Department of Agricultural Chemistry, University of Wisconsin,
Madison, U.S.A.*

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RECENT reports indicate that selenium is chiefly responsible for numerous cases of livestock poisoning in certain parts of the United States [Byers, 1935]. Franke and Potter [1935] have shown that rats develop serious symptoms and almost invariably die on a diet containing as little as 30 parts per million of selenium in the form of sodium selenite. The majority of the animals became extremely anaemic before death.

Moxon and Franke [1935] found that traces of selenium will greatly reduce the fermentation of glucose by yeast although Harden and Norris [1914] found that 1 % Na_2SeO_3 slightly increased the rate of fermentation of glucose by top brewery yeast. Yeast cells are apparently as sensitive to the presence or absence of traces of certain elements as are the higher organisms. In the latter deficiency of iron and copper will result in anaemia due to diminished haemoglobin formation [Hart *et al.*, 1928; Elvehjem and Hart, 1929] whilst, in the case of yeast, Elvehjem [1931] has shown that the rate of oxygen uptake is decreased appreciably by such deficiency.

In the present investigation the authors decided to study the oxygen uptake of living yeast cells in order to determine whether or not the symptoms of selenium poisoning might be fundamentally due to an inhibition of the processes of tissue respiration. It was also felt that selenium might prove to be a valuable tool in the study of the respiratory mechanisms of normal cells.

The respiratory mechanisms in yeast are evidently somewhat simpler than those in most animal tissues since the fact that the oxygen uptake in yeast is practically completely inhibited by cyanide [Dixon and Elliott, 1929] indicates that all the dehydrogenase systems are anaerobic. In the present investigation frequent control cyanide-inhibition experiments were made. Preliminary experiments have shown that selenium does not cause as complete inhibition of the oxygen uptake of yeast as cyanide. This suggests that selenium does not inactivate the cytochrome or indophenol oxidase but rather that it attacks certain dehydrogenating systems.

After a number of experiments to determine the effect of variations in the suspending medium, the authors studied the oxygen uptakes of normal and poisoned yeast cells on a variety of substrates, in order to demonstrate any specific toxicity that might exist for particular enzyme-substrate systems.

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² Wisconsin Alumni Research Foundation Fellow.

Preliminary experiments.

The oxygen uptake was measured by means of a Barcroft differential respirometer as previously described by Stare and Elvehjem [1933]. To each flask were added 1.0 ml. of buffer solution and 1.0 ml. of substrate solution. 1 ml. of water was added to the left-hand flask and 1 ml. of yeast suspension to the right-hand flask. The yeast¹ was suspended in redistilled water and shaken until a homogeneous suspension resulted. The quantity of yeast used was determined by preliminary experiments and was that amount which would produce the greatest oxygen uptake within the range of the apparatus when glucose was used as substrate. The proper concentration (5 mg. yeast per ml.) was accordingly used for all the experiments reported in this paper. When poisons were being tested, 0.1–0.3 ml. of poison was added to both flasks, and the amount of buffer was decreased so that the final volume was always 3 ml.

The buffer used was a mixture of equal parts of 0.15 *M* NaH₂PO₄ and KH₂PO₄ which had been brought to p_H 6.5 by addition of NaOH and KOH respectively. Since 1 ml. of buffer was used, the concentration in the respirometer flasks was 0.05 *M*. Elvehjem [1931] and Dickens [1934] have measured the oxygen uptake of yeast in 0.05 *M* sodium phosphate, which was adjusted to p_H 7.0 in the former case. In the present study no appreciable change in the uptake was noted when the buffer concentration was diminished to 0.025 *M*. The first experiments were carried out in sodium phosphate; when potassium phosphate was substituted it was observed that the rate of uptake increased with time, whereas the rate had either decreased or remained fairly constant when sodium was the cation. Since K⁺ seemed to have a stimulating effect upon the uptake, the buffer which was finally used was a mixture of sodium and potassium phosphates. At this time Ashford and Dixon [1935] published a statement that K⁺ increased aerobic glycolysis in brain slices but had no effect upon the oxidative removal of lactic acid. Lasnitzki and Szörenyi [1935] found that K⁺ increased yeast fermentation, and that Rb⁺ and Cs⁺ also had more marked effects than Na⁺ or Li⁺. Dickens and Greville [1935] have since studied the effects of various cations on tissue respiration in some detail and report that K⁺, Rb⁺ and Cs⁺ produce a greater general stimulation of carbohydrate respiration in brain tissue than Li⁺ or Na⁺. The effect however could not be demonstrated in other tissue. Our work shows that yeast respiration is also susceptible to the so-called "potassium effect", the respiration on glucose being greatly accelerated.

Ehrenfest [1932] and Schroeder *et al.* [1933, 1] showed that iodoacetate inhibition of yeast fermentation was dependent upon the p_H of the suspending medium. They found that the toxicity decreased as the medium became less acid, and attributed their results to a change in the permeability of the yeast cells. Our results show a similar relation between p_H and degree of inhibition.

Seven phosphate buffers were made up of p_H 4.5 to 7.5. The results of a typical experiment are shown in Fig. 1. The uptake in the control flasks was greatest at p_H 6.0 and 6.5 and was somewhat less at 4.5 and 7.5. The Q_{O_2} (μ l. O₂ uptake per g. of dry yeast per hour) of the yeast on the glucose substrate has been calculated to be approximately 100,000, on the basis of the experiments lasting 2 hours. This is over twice as great as has been previously reported [Elvehjem, 1931; Dixon and Elliott, 1929] and may be explained partly by the freshness of the yeast and partly by the fact that the buffer may be nearer the optimum. It

¹ Fresh pressed yeast was obtained from the Department of Agricultural Bacteriology through the courtesy of Dr I. L. Baldwin. Analysis showed the moisture content to be quite uniform at 71–73%.

was found that the p_H of the buffer had a profound effect on the degree of inhibition produced by $M/300$ selenite. From p_H 4.5 to 6.5 the inhibition was maximum, but as soon as neutrality was passed it decreased rapidly. At p_H 7.5 inhibition was very slight, whilst at p_H 7.0 the uptake was between values obtained at p_H 6.5 and 7.5. A large number of experiments uniformly confirmed these results. However, when the selenite concentration was increased to $M/100$, the p_H effect was not observed, probably owing to the large excess of poison. A few experiments were made with sodium arsenite but no diminution in toxicity was observed at p_H 7.5 using arsenite at concentrations of $M/300$, $M/1000$ or $M/3000$.

Fig. 1.

Fig. 1. The effect of p_H on selenium toxicity, Series 38, 5 mg. yeast, 0.05 M NaH_2PO_4 , $M/18$ glucose, $M/300$ Na_2SeO_3 .

Fig. 2.

Fig. 2. Oxygen uptake at various concentrations of selenium, $M/18$ glucose, p_H 6.5, 0.05 M sodium and potassium phosphate buffer, 5 mg. yeast, 2-hour period, Series 52, 69 A1, 69 A2, 70 A, 70 B.

A number of experiments were carried out to determine the proper concentration of sodium selenite. The results are summarised in Fig. 2. It was found that the minimum effective concentration was $M/300$, whilst $M/100$ produced very slight additional inhibition. The selenite was prepared at a concentration of 0.1 M and 0.1 ml. was added to the flasks to make the final concentration in the solution $M/300$. The sodium selenite used¹ had been previously shown to be of high purity [Franke and Potter, 1935] and in addition was shown to have the same order of toxicity as a laboratory preparation.² The arsenic used was in the form of sodium arsenite and was tested at the same level as the selenium, although later experiments have shown that its minimum effective concentration is lower than that of the selenite, being approximately $M/1000$.

The experiments were carried out at two temperatures, 28 and 37°. Although

¹ Eimer and Amend, C. P. Anhydrous.

² Furnished through the kindness of Dr V. W. Meloche, Dept. of Chemistry, University of Wisconsin.

the uptake is more rapid at 37°, the lower temperature is probably nearer the optimum for yeast [Moxon and Franke, 1935] and most of the experiments were carried out at that temperature.

Substrate experiments.

After the preliminary work on experimental conditions, a number of experiments were carried out using various substrates. The oxygen uptake of yeast is largely determined by the substrate. The data in Tables I and III show that the

Table I. *Inhibition of oxygen uptake on glucose substrate.*

Temperature 28°. Buffer: $\text{NaH}_2\text{PO}_4 + \text{KH}_2\text{PO}_4$ neutralised to p_{H} 6.5.

Poison	% inhibition at 2 hours	$\mu\text{l. uptake per 5 mg. yeast}$	
		0-60 min.	60-120 min.
M/300 Se	73	48.2	23.4
Control	—	119.5	141.0
M/300 As	83	26.4	18.1
Control	—	117.5	144.5
M/300 Se	57*	61.0	36.2
Control	—*	120.5	106.5
M/300 Se	56*	48.6	30.4
Control	—*	87.0	94.0
M/300 Se	79	44.2	20.8
Control	—	135.0	176.0
M/300 Se	85†	43.5	29.3
Control	—†	207.0	224.0
Control	—	133.5	163.5
M/25 NaF	2	134.0	156.0
M/75 NaF	—	140.0	177.0
Control	—†	128.0	168.0
M/25 NaF	10†	124.0	143.0
M/75 NaF	1†	128.5	165.5
Control	—	119.8	148.5
M/100 Se	82	31.2	16.3
M/300 As	85	26.4	17.6
Control	—	131.7	164.0

* Red Star Commercial Yeast Cake.

† 37°.

‡ p_{H} 7.5.

Table II. *Inhibition of oxygen uptake on lactate substrate.*

Temperature 28°. Buffer: $\text{NaH}_2\text{PO}_4 + \text{KH}_2\text{PO}_4$ neutralised to p_{H} 6.5.

Poison	% inhibition at 2 hours	$\mu\text{l. uptake per 5 mg. yeast}$	
		0-60 min.	60-120 min.
M/300 Se	7	89.3	74.2
Control	—	94.2	79.3
M/300 As	—*	9.3	5.4
M/300 Se	8*	47.6	22.6
Control	—*	48.9	27.5
M/300 Se	7*	39.4	25.1
Control	—*	39.4	29.6
M/300 Se	5	92.0	79.0
Control	—	92.8	86.2
M/300 Se	7†	140.3	110.7
Control	—†	149.7	119.0
Control	—	87.4	110.6
M/300 Se	12	82.3	91.9
M/300 Se	—	89.4	94.3
M/300 As	—	11.4	3.4
M/300 As	93	9.3	4.6
Control	—	87.1	99.9

* Red Star Commercial Yeast Cake.

† Temperature 37°.

Table III. *Inhibition of oxygen uptake on various substrates.*Temperature 28°. Buffer: $\text{NaH}_2\text{PO}_4 + \text{KH}_2\text{PO}_4$ neutralised to p_{H} 6.5.

Substrate	Poison	% inhibition at 2 hours	$\mu\text{l. uptake per 5 mg. yeast}$	
			0-60 min.	60-120 min.
No substrate	Control	—	17.2	19.4
"	<i>M</i> /300 Se	77	6.1	2.2
"	<i>M</i> /300 As	87	2.8	1.7
"	Control	—	19.1	14.7
Succinate (<i>M</i> /9)	<i>M</i> /300 Se	73	5.9	3.1
"	Control	—	12.7	20.0
Acetate (<i>M</i> /9)	Control	—	33.7	87.8
"	<i>M</i> /300 Se	56	34.1	19.3
"	Control	—	25.7	58.3
"	<i>M</i> /300 Se	39	31.1	20.5
"	Control	—	30.3	80.4
"	<i>M</i> /300 Se	44	36.1	25.7
"	Control	—	23.5	57.1
"	<i>M</i> /300 Se	46	26.1	17.6
"	<i>M</i> /300 As	97	1.7	1.9
"	Control	—	45.8	74.9
Ethyl alcohol (<i>M</i> /9)	Control	—	62.8	96.9
"	<i>M</i> /300 Se	70	34.3	13.7
"	<i>M</i> /300 As	72	42.5	27.9
"	Control	—	109.7	140.3
Fructose (<i>M</i> /18)	Control	—	168.8	234.2
"	<i>M</i> /300 Se	84	43.6	21.7
"	<i>M</i> /300 As	89	15.6	12.3
"	Control	—	112.8	148.9
Mannose (<i>M</i> /18)	Control	—	91.6	130.4
"	<i>M</i> /300 Se	76	32.1	21.1
"	<i>M</i> /300 As	86	26.4	15.3
"	Control	—	130.5	177.8
Pyruvate (<i>M</i> /9)	Control	—	65.7	90.3
"	<i>M</i> /300 Se	4	64.6	85.2
"	<i>M</i> /300 As	97	4.1	2.3
"	Control	—	85.7	98.8
"	<i>M</i> /300 Se	9	51.9	70.9
"	Control	—	55.8	78.8

uptake by the yeast alone is only 10-15 % of the uptake when glucose is present. It is thus possible to determine the ability of the yeast organism to oxidise specific compounds.

Elvehjem [1931] and Dickens [1934] have used *M*/18 glucose as a substrate and this concentration was found to be quite satisfactory in the present investigation. The uptake was not affected by reducing the concentration of the sugar to *M*/36. The concentrations of the other substrates were based on the glucose solution. The substrate solutions were prepared fresh each day, with the concentration three times as great as the final concentration in the respirometer flasks. In these experiments the amount of yeast was so small (5 mg.) that the substrate concentration was not greatly altered during the period of the experiment. The buffer was also strong enough to hold the p_{H} constant.

The substrates were all C.P. chemicals, and in the case of the alcohol and pyruvic acid were repurified. The mannose and fructose were laboratory preparations.¹

¹ The mannose and fructose were kindly supplied from the laboratory of Dr Karl Paul Link.

Most of the substrate experiments were done in triplicate, and the figures reported are the average values. In general, individual manometers agreed within 5%. The uptake was studied for a period of 2 hours, and readings were taken at 20-min. intervals. The results are recorded in Tables I, II and III. Fig. 1 shows the general form of the curves for these data. A few experiments were continued for 6 hours, but the curves were merely extensions of those obtained at 2 hours and yielded no additional information.

It was observed that when glucose, fructose or mannose was used as substrate, the rate of oxygen uptake was reduced about 80% by the addition of selenite (Tables I and III). The results with a sample of commercial yeast showed less inhibition and are included in Table I to show that yeast from various sources may vary in the degree to which it is inhibited by selenium, just as in the case of cyanide [Dixon and Elliott, 1929]. As soon as lactate and pyruvate were used as substrates however it was found that the inhibition of oxygen uptake by selenite became negligible (4–9%). Moxon and Franke [1935] found that the rate of CO_2 evolution from glucose-yeast mixtures was greatly diminished by sodium selenite. Their results show that selenium prevents yeast from metabolising glucose in a normal manner. Our work seemed to indicate that selenium was acting primarily as an inhibitor of the glycolytic system, since it appeared that our results could be explained without postulating an inhibition of oxidative mechanisms, on the assumption that in order for glucose to be oxidised, it must be split into 3-carbon compounds. Barron and Miller [1932] have accumulated excellent evidence to show that this is actually the mechanism in the case of gonococci. It was shown that in the presence of sodium fluoride, which is regarded as a specific poison for the glycolytic system [Dickens and Simer, 1929; Meyerhof and Kiessling, 1933], these organisms were able to oxidise lactate and pyruvate but not glucose. Yeast cells however seem to possess a somewhat greater versatility than gonococci. When sodium fluoride was added to mixtures of yeast and glucose the oxidation proceeded normally (Table I) although the concentration of fluoride exceeded the amount which prevented oxygen uptake by gonococci. Since glucose dehydrogenase is not poisoned by fluoride [Harrison, 1931] there is thus some evidence that this enzyme is present in yeast. The point must remain obscure until the effect of fluoride on living yeast has been more thoroughly investigated. Thus far, most of the yeast glycolysis experiments have been carried out with dried yeast preparations, which may be expected to have lost many of the properties of living yeast. We cannot be certain that glycolysis was inhibited soon enough in our experiments completely to eliminate the formation of oxidisable intermediates. If yeast does in fact possess a glucose dehydrogenase, it is apparent that this part of the oxidative mechanism is poisoned by selenite.

The work with other substrates (Table III) furnishes definite evidence that selenium is toxic to the oxidative as well as the glycolytic systems. Since the uptake with yeast alone is relatively small even without selenium, and since the nature of the compounds being oxidised is unknown, little emphasis can be placed on inhibition in this case. Succinate is poorly oxidised and the uptake may be largely endogenous. However, there is definite evidence that yeast oxidises acetic acid and alcohol and that the oxidation is largely inhibited by selenite.

Arsenite is apparently much more toxic to the oxidative mechanisms than selenite, and produced marked inhibition with all substrates, including lactate and pyruvate. A few experiments were carried out to show that the percentage inhibition was of the same magnitude with lactate as with glucose even when the

arsenite was reduced to concentrations of $M/1000$ and $M/3000$. Thus, arsenic and selenium, although generally described as being pharmacologically similar, actually appear to show fundamental differences in their modes of action.

The inhibition of oxygen uptake by selenite appears to be a time reaction and is quite different in type from the inhibition which is produced by cyanide. The latter blocks the oxygen-carrying activity of cytochrome and exercises its maximum effect at once. Selenite, on the other hand, becomes increasingly toxic with time. It seems unlikely that this delayed action can be explained on the basis of permeability, since increasing the selenite concentration from $M/300$ to $M/100$ increases the inhibition but slightly.

It is a well-known fact that living cells, including yeast, can reduce selenites to selenium. It seems quite possible that the toxic properties of sodium selenite may be related to its ability to react with sulphhydryl groups. Bersin [1935] states that selenite reacts with glutathione, with the intermediate formation of G.S.Se.S.G which then decomposes into oxidised glutathione and free selenium. Voegtlin *et al.* [1931] have also observed the ability of selenium salts to catalyse the oxidation of glutathione. Schroeder *et al.* [1933, 2] have studied the relation between loss of sulphhydryl and inhibition of fermentation by iodoacetic acid. These workers observed that removal of glutathione from suspensions of poisoned yeast was a time reaction and showed that the loss was most marked in the acid range, being virtually negligible after the solution became neutral or alkaline. Their results may thus help to explain the relation between selenium toxicity and p_H . They also found that the decrease in sulphhydryl ran parallel with the degree of inhibition but thought that the decrease in glutathione could not be completely responsible for the results obtained. Geiger [1935] has recently done much toward clarifying the rôle of glutathione in glycolysis. He found that oxidised glutathione even in low concentrations inhibits lactic acid formation from both glucose and glycogen, and suggested that the glycolytic enzyme may be inactivated through oxidation by oxidised glutathione. It thus appears that selenite may inhibit glycolysis indirectly through the production of oxidised glutathione.

On the other hand, the failure of selenite effectively to inhibit the oxidations of pyruvate and lactate may be only apparent. The authors have not been able to prove conclusively that the selenite is not reduced by the substrates, although indications are that no reduction takes place. It is possible that lactate and pyruvate facilitate reduction of selenite by yeast cells and that glucose does not have this property.

At present the glutathione reaction seems to offer the best explanation for the toxic action of selenium, at least so far as glycolysis is concerned. Further work must be done with the intermediate and end-products of glycolysis to discover the real mechanism of the inhibition. Although it is apparent that the oxidative systems are also definitely inhibited, present data are too meagre to warrant any ideas as to mode of action.

SUMMARY.

1. The oxygen uptake of living yeast has been measured using the following substrates: glucose, mannose, fructose, lactic acid, pyruvic acid, acetic acid, alcohol and succinic acid in the absence and presence of sodium selenite.
2. Sodium selenite was found to produce about 80 % inhibition of the oxygen uptake on the sugars and less than 10 % inhibition on lactate and pyruvate substrates. $M/300$ was found to be the minimum effective concentration.

3. Inhibition by selenite was most marked on the acid side and fell off rapidly at p_H 7.0 and 7.5.

4. Parallel experiments with sodium arsenite indicated that this salt is considerably more toxic to the oxidative systems than selenite, the minimum effective concentration being about $M/1000$.

5. A possible mechanism for the inhibition of glycolysis by selenite is discussed.

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