CCLVI. THE RELATION OF THIOL COMPOUNDS TO GLUCOSE FERMENTATION.

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IN the course of experiments on the toxicity of iodoacetic acid to biological processes, it was found that addition to the acid of sodium thiosulphate or thiol compounds brought about a considerable diminution in the intensity of the toxic effect. This observation led the writers to consider the possibility that thiol compounds may normally play an important part in the processes affected by iodoacetic acid. Experiments were accordingly devised to test this possibility, especially with regard to yeast fermentation. The results which are recorded below showed that both cysteine and glutathione markedly affect the relationship of aerobic fermentation of glucose by baker's yeast to the respiration in presence of glucose. It would seem that glutathione plays some controlling part in determining the relationship between aerobic fermentation and respiration of the cell in presence of glucose.

A. EXPERIMENTS WITH IODOACETIC ACID.

Experiments with animals showed that the toxicity of iodoacetic acid was greatly diminished by admixture with sodium thiosulphate. For instance, an intramuscular injection of 3 cc. of a 2 % solution of iodoacetic acid (neutralised with NaOH) was invariably fatal to guinea-pigs, death occurring within 1 hour after the injection. When, however, 0.3 g. sodium thiosulphate was dissolved in this dose of iodoacetic acid death of the animal did not occur. A separate injection of thiosulphate did not relieve the animal from the toxic action of iodoacetic acid and it was apparent that the diminution of toxicity of this acid by the presence of thiosulphate was due to interaction between the two substances.

This was confirmed by iodimetric titration of sodium thiosulphate solution after the addition of iodoacetic acid. Two solutions were prepared (a) 50 cc. N/100 sodium thiosulphate, (b) 50 cc. N/100 sodium thiosulphate to which was added sodium iodoacetate to give a concentration of N/250. The solutions were placed at 37° for 3 hours after which titrations on 5 cc. with N/100 iodine solution were made. The titrations were (a) 5.0 cc., (b) 3.15 cc. The theoretical

figure for (b) was 3.0 cc. had there been complete interaction between one molecule of sodium thiosulphate and one molecule of iodoacetic acid.

Experiments were then carried out to determine whether the halogen atom of iodoacetic acid was sufficiently labile to react with the thiol groups of cysteine and glutathione.

In performing these experiments, advantage was taken of the fact that exposure of brain tissue to dilute iodoacetic acid resulted in an inability of the tissue to oxidise glucose and other sugars [Krebs, 1931; Quastel and Wheatley, 1932].

Fresh brain tissue was added to solutions containing (a) iodoacetic acid, (b) iodoacetic acid + cysteine, (c) iodoacetic acid + glutathione, (d) iodoacetic acid + sodium thiosulphate. It was found that the inhibitory action of iodoacetic acid on the oxidation of glucose by brain was much reduced by its previous admixture with thiol compounds or sodium thiosulphate.

The experimental technique, which involved the use of the Barcroft differential manometer, was that fully described in an earlier communication [Quastel and Wheatley, 1932]. Briefly it consisted in placing in the right-hand vessel of the manometer 1 cc. *M*/5 phosphate buffer solution p_H 7.4, 2 cc. saline (containing the iodoacetic acid or mixture of iodoacetic acid and sulphur compound) and 0.5 g. fresh chopped guinea-pig brain tissue. 3 cc. saline were placed in the left-hand vessel. The apparatus was shaken at the temperature of the bath (37°) for 2½ hours when the rate of autoxidation of the brain had fallen very considerably. Glucose was then added to the tissue to give a final concentration of 0.025 % and the new rate of oxygen uptake was observed. This was compared with the rate given by brain tissue which had not been treated with iodoacetic acid. The p_H of all solutions added to the brain tissue was 7.4—acids being neutralised with sodium hydroxide. The results are shown in Table I.

Table I.

Oxygen uptakes in mm.³ by 0.5 g. guinea-pig brain tissue (in presence of 0.025 % glucose) after treatment with iodoacetic acid and mixtures of iodoacetic acid and sulphur compounds. Autoxidation had been allowed to proceed for 2½ hours before addition of the glucose.

Mode of treatment	Time (in mins.)			
	30	60	90	120
Control (no iodoacetic acid)	144	312	474	618
<i>M</i> /4000 iodoacetic acid	52	95	124	150
„ + <i>M</i> /160 cysteine	108	235	356	482
„ + <i>M</i> /100 glutathione	128	270	408	548
„ + 0.7 % sodium thiosulphate	92	187	285	382
Cysteine <i>M</i> /160	124	266	404	538
Glutathione <i>M</i> /100	142	302	460	612
Sodium thiosulphate (0.7 %)	147	313	471	610

It will be observed that cysteine, glutathione and sodium thiosulphate diminish the toxic action of iodoacetic acid, a considerably greater rate of oxygen uptake being secured. Control experiments showed that none of those

sulphur compounds, when added alone, appreciably affected the oxygen uptake of brain tissue in the presence of glucose. Cysteine had the most marked action.

Experiments were then carried out to determine whether the addition of cysteine *etc.* to brain tissue already exposed to iodoacetic acid for $2\frac{1}{2}$ hours, would restore the normal rate of O_2 uptake in the presence of glucose. The results were negative showing that the alleviating action of the sulphur compounds when added together with the iodoacetic acid was due to direct interaction with the halogen acid.

Some evidence for the interaction of thioglycollic acid with iodoacetic acid has been obtained by Bersin [1932].

These experiments demonstrate the lability of the halogen atom of iodoacetic acid. The comparative ease with which it combines with thiol groups would lead one to consider the possibility that the physiological activity of iodoacetic acid may be connected with an interaction with naturally occurring thiol groups in the living cell. If this were so, it would be anticipated that thiol compounds may play a part in some of the biochemical reactions specifically affected by iodoacetic acid.

In view of the fact that fermentation of glucose by yeast is inhibited by small concentrations of iodoacetic acid [*cf.* Lundsgaard, 1932] experiments were undertaken to determine whether thiol compounds play any rôle in the process of glucose fermentation by yeast.

B. EXPERIMENTS WITH YEAST.

Technique.

In the experiments to be described the Barcroft differential manometer was used. Observations were made of the O_2 uptake and CO_2 output by yeast in the presence of phosphate buffer solution of p_H 6.0 containing 1.6 % glucose. Two manometers were used for the estimations. In the first manometer 0.3 cc. 20 % KOH was placed in the inner tube of the reaction vessel, the O_2 uptake alone of the yeast being obtained. In the second manometer potash was omitted from the reaction vessel, the readings obtained representing differences between CO_2 output and O_2 uptake. The CO_2 output was determined from the readings of the two manometers. It is important in this work that the p_H of the fluids in the vessels of the manometers should remain the same throughout the experimental period. Using *M*/15 phosphate buffer of p_H 6.0 it was found that no appreciable difference in p_H occurred.

The effect of a thiol compound on the metabolism of yeast was obtained either by adding the substance to the yeast at the commencement of an experiment or by adding it to the yeast after fermentation had proceeded for some time. Actually it was found that the effects of the thiol compound were the same by either method.

Experiments were carried out at 37° , air being present in the Barcroft

manometer vessels. Readings were taken after preliminary shaking for 15–30 minutes to ensure temperature equilibrium and steady rates of O_2 uptake and CO_2 output.

Most of the work so far has been carried out with fresh baker's yeasts—two Belgian varieties and one Dutch (Delft) being used.

The exact procedure was as follows.

In the right-hand vessels A, B, C, D of four Barcroft differential manometers were placed:

(A) 1 cc. $M/5$ phosphate buffer solution p_H 6.0, 1 cc. saline, 0.5 cc. 10 % glucose solution (in saline), 0.5 cc. 2 % yeast suspension (in saline); no potash was present;

(B) as in (A), but potash was placed in the inner tube;

(C) as in (A), with the exception that 1 cc. saline was replaced by 0.5 cc. saline and 0.5 cc. of 0.5 % solution of thiol compound (in saline); no potash was present;

(D) as in (C), but potash was placed in the inner tube.

The left-hand vessels each contained 3 cc. saline. Readings were taken every 15 minutes over a period of 3 hours. At the close of the experiment p_H estimations were made to determine whether material changes of p_H had occurred. As a rule no difference in p_H greater than 0.2 was observed.

Results with cysteine.

The rate of CO_2 evolution is slightly greater than the rate of O_2 uptake with all specimens of baker's yeast used, under the experimental conditions described.

The addition of cysteine to the yeast entirely changes the rates of CO_2 output and O_2 uptake. There is an enhanced rate of production of CO_2 and a greatly diminished rate of O_2 uptake.

The results in Table II are typical of cysteine (a Belgian yeast being used). 2.5 mg. cysteine hydrochloride (neutralised with NaOH) were present.

Table II.

		Time (in mins.)			
		30	60	90	150
No cysteine present.	O_2 uptake, mm. ³	290	513	753	1204
	CO_2 output, mm. ³	334	582	847	1355
Cysteine present.	O_2 uptake, mm. ³	79	148	209	308
	CO_2 output, mm. ³	414	762	1078	1664

Similar results were obtained with Delft baker's yeast. It is evident that cysteine exercises a considerable inhibitory action on the O_2 uptake of yeast in the presence of glucose. This result is quite different from that obtained with brain tissue where cysteine has relatively little inhibitory action on the oxygen uptake in the presence of glucose (Table I).

The effect of cysteine on the O_2 uptake and CO_2 output resembles that of cyanide, with the exception that the inhibitory effect of cyanide on yeast respiration is much more pronounced.

The increase in the rate of CO_2 formation by the cysteine only occurs under aerobic conditions. Under anaerobic conditions or in the presence of cyanide cysteine does not increase the rate of CO_2 formation—it has on the contrary a small inhibitory action. Thus, in one experiment, in the presence of 0.015 % HCN the evolution of CO_2 , under the conditions given above (and using a Belgian yeast), was 503 mm.³ in 60 mins. and 930 mm.³ in 120 mins. In the presence of 0.015 % HCN and 0.15 % cysteine the evolution of gas was 459 mm.³ in 60 mins., and 821 mm.³ in 120 mins., indicating some toxicity of cysteine to the fermenting complex.

Results with glutathione.

The action of glutathione is similar to that of cysteine in bringing about a considerable change in the relative rates of CO_2 output and O_2 uptake. Whereas, however, cysteine greatly diminishes the O_2 uptake of yeast in the presence of glucose, glutathione has a relatively small effect.

The following results (Table III) are typical for glutathione (a Delft yeast being used).

Table III.

		Time (in mins.)		
		30	60	90
No glutathione added.	O_2 uptake, mm. ³	314	577	833
	CO_2 output, mm. ³	414	761	1120
2.5 mg. glutathione added.	O_2 uptake, mm. ³	293	512	702
	CO_2 output, mm. ³	534	1149	1784

Glutathione brings about a gradual decrease in the rate of O_2 uptake and a decided increase in the rate of CO_2 output. Moreover the rate of CO_2 output appears to increase with time in presence of added glutathione.

Preliminary experiments have indicated that the greater the quantity of glutathione added, the greater is the rate of evolution of CO_2 , the limiting rate being equal to that obtained when glucose is fermented under anaerobic conditions (*i.e.* in nitrogen and in the absence of added SH compounds¹). Thus the ratios of the rates of formation of CO_2 with baker's yeast in the presence of 1.6 % glucose in nitrogen, in air and in the presence of $M/400$ glutathione and air were 100 : 60 : 94.

Effect of cysteine on fermentation of fructose.

Cysteine behaves in the same way towards the fermentation of fructose as towards that of glucose.

The following results (Table IV) were obtained using 0.5 cc. of a 5 % suspension of a baker's yeast, the conditions being those described for glucose. 2.5 mg. cysteine hydrochloride (neutral) were added.

¹ The effect of glutathione varies quantitatively with the type of yeast. The tripeptide was found to enhance the rate of CO_2 formation with a Delft baker's yeast at concentrations which were ineffective with a Belgian yeast.

Table IV.

		Time (in mins.)			
		30	60	90	120
No cysteine present.	O ₂ uptake, mm. ³	547	1055	1532	1942
	CO ₂ output, mm. ³	597	1189	1837	2499
Cysteine present.	O ₂ uptake, mm. ³	77	134	189	211
	CO ₂ output, mm. ³	864	1726	2584	3341

Effect of cysteine on respiration of yeast in presence of glycerol.

The action of cysteine on the respiration of yeast in the presence of glycerol (0.5 cc. of 10 % solution) was investigated. The following results (Table V) were obtained, using 0.5 cc. of a 5 % suspension of baker's yeast.

Table V.

		Time (in mins.)		
		30	60	90
Cysteine absent.	O ₂ uptake, mm. ³	150	279	391
	CO ₂ output, mm. ³	100	191	273
4 mg. cysteine added.	O ₂ uptake, mm. ³	133	244	336
	CO ₂ output, mm. ³	113	214	298

It will be observed that cysteine had a relatively small effect either on the CO₂ output or on the O₂ uptake of yeast in the presence of glycerol.

It is evident from these results with glycerol and from the lack of a marked inhibition of O₂ uptake by brain in the presence of glucose that the inhibitory effect of cysteine on hexose oxidation by yeast is intimately associated with a specific process occurring in the breakdown of the hexose molecule by yeast.

Mode of behaviour of cysteine and glutathione.

It is apparent from the results cited that thiol compounds as represented by cysteine and glutathione affect markedly the relationship between the oxidation and the fermentation of glucose by yeast. The liberation of CO₂ approaches the rate obtained when yeast ferments glucose under anaerobic conditions. Whereas, however, the O₂ uptake by yeast in presence of glucose is greatly inhibited by cysteine, glutathione has but a small effect. The phenomena observed with these thiol compounds are similar to those described by Warburg [1926] in his studies of the relative effects of cyanide and carbylamine on the Pasteur reaction. Carbylamine intensified aerobic fermentation without any marked action on the respiration, whilst cyanide greatly decreased the respiration.

As Warburg has pointed out, many substances, *e.g.* narcotics, will affect the Pasteur reaction, the normal relationship between aerobic fermentation and respiration being disturbed. Glutathione, however, is a substance which occurs naturally in the living cell and hence results with it must have some significance in the dynamics of normal cell processes.

Warburg [1926] suggests that the behaviour of carbylamine in affecting aerobic fermentation but not respiration is due to two properties of the sub-

stance: an ability to enter into complex combinations with heavy metals and an inability to react with the heavy metal of the respiration ferment. The latter is more sensitive towards free cyanide than the catalyst of the Pasteur reaction.

Perhaps the effects of cysteine and glutathione may be interpreted in a similar manner. Cysteine is able to enter into combination both with the catalysts of respiration and of the Pasteur phenomenon, whilst glutathione can only affect the latter. This explanation, however, is not very satisfactory, for the marked inhibitory effect of cysteine on the rate of respiration in the presence of glucose is not found to occur with the respiratory rate in the presence of glycerol. If cysteine enters into combination with a respiratory catalyst it would be anticipated that all oxidations of yeast would be affected. The conclusion would be that either there is a particular catalyst which comes into play during glucose breakdown and which is affected by cysteine and not by glutathione, or that there is a particular process occurring in the aerobic breakdown of glucose by yeast whose course is affected to a much greater extent by the reducing action of cysteine than by that of glutathione. Further investigation is necessary to settle this problem.

Bumm and Appel [1932] have demonstrated that glutathione increases aerobic glycolysis by tumour cells to the limits found anaerobically, and it seems likely that this effect is closely related to the phenomenon already described.

The inference would be that glutathione, present in the living cell, plays some controlling part in the relationship between respiration and fermentation (or glycolysis) in the presence of glucose.

Preliminary experiments with brewer's yeast have shown a behaviour with glutathione similar to that experienced with baker's yeast, but the effect is not so well marked. This is to be correlated with the fact that the respiration of the brewer's yeast was found to be extremely small compared with the fermentation. Moreover, iodine titrations showed a larger content of —SH groups in the brewer's yeast than in the baker's yeast. The addition of an extract of brewer's yeast to baker's yeast was found to increase the rate of aerobic fermentation of glucose, this being probably due to glutathione present in the extract.

The fact that cysteine or glutathione has the property of securing a rate of fermentation by yeast which approximates to that obtained under anaerobic conditions recalls the observation [Quastel and Stephenson, 1926] that thiol compounds enable a strict anaerobe such as *B. sporogenes* to proliferate under aerobic conditions. The suggestion made then was that the presence of thiol compounds established a limiting reduction potential which was necessary for proliferation of this organism. In an analogous manner it may be suggested that the Pasteur phenomenon—the relationship between aerobic fermentation and respiration—is associated in the cell with the existence of a particular reduction potential, whose magnitude will depend in part on the presence of

thiol compounds. Whether this be the case or not there seems little question that thiol compounds normally play some part in determining the relationship between fermentation and respiration of yeast in the presence of glucose.

Further work on this question is now in progress.

SUMMARY.

1. Iodoacetic acid reacts with sodium thiosulphate, cysteine and glutathione. These substances diminish the toxicity of iodoacetic acid towards biological processes.

2. Cysteine and glutathione markedly affect the relationship between fermentation and respiration of baker's yeast in the presence of glucose.

3. Cysteine inhibits the oxygen uptake of baker's yeast in the presence of glucose; glutathione has but little effect. Both thiol compounds increase the rate of CO₂ formation, glutathione having the greater action.

4. The fermentation of fructose is affected in a similar manner to that of glucose by cysteine.

5. The O₂ uptake and CO₂ output of yeast in presence of glycerol are only slightly affected by cysteine.

6. The controlling action of glutathione on aerobic fermentation is probably of significance in the normal metabolism of the cell.

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