

Oxidation of Glucose in presence of Insulin, Glutathione and other Substances and the Probable Mechanism of Biological Oxidations.

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For a number of years, we have investigated the catalytic induced and photochemical oxidations of food materials by air or hydrogen peroxide and in several communications we suggested a probable mechanism of oxidations taking place in the animal body or the plant kingdom or in the soil. This paper is a continuation of the same line of investigation.

It has been shown (*J. Indian Chem. Soc.*, 1934, 11, 661) that the induced oxidation of glucose by air in presence of ferrous hydroxide acting as an inductor, is greatly increased if small amounts of manganous hydroxide is added to the ferrous hydroxide. Thus the oxidation of glucose in presence of ferrous hydroxide ($=0.0468$ g.) alone is 4.33% and after the addition of manganous hydroxide ($=0.00328$ g.) to ferrous hydroxide, the oxidation of glucose is increased to 48.26%. Similarly the induced oxidation of glucose in presence of cerous hydroxide ($=0.1069$ g.) is accelerated by manganous hydroxide. Hence our results show that the joint action of the two inductors is much greater than their additive values.

We have also shown that small amounts of copper stimulate markedly the induced oxidation of glucose and other food materials.

In this communication, the results on the oxidation of glucose in presence of insulin and glutathione acting singly or in mixtures aided by different inorganic catalysts and solid surfaces have been shown. It is well known that both insulin and glutathione are excellent reducing agents taking up oxygen directly. A mechanism of biological oxidations has also been discussed briefly.

E X P E R I M E N T A L.

The experimental work was carried on in the same way as described in a previous paper (Palit and Dhar, *J. Indian Chem. Soc.*, 1934, 11, 661). The following results have been obtained :—

TABLE I.

36.5 litres of air passed in 13 hours. 0.05 G. of glutathione and 10 units of insulin taken. Each of the substances taken as inductor = 1 g. Amount of sodium phosphate added = 0.2880 g. 10 C.c. of glucose = 0.2308 g. of CuO (blank).

Substance used as surface and inductor (1.0 g.).	Percentage amount of oxidation of glucose in presence of			
	substance alone.	substance + 0.2880 g. of Na-phosphate.	substance + 0.05 g. of glutathione.	substance + 0.05 g. of glutathione + 0.2880 g. of Na-phosphate.
Titanium dioxide	19.9	22.4	31.3	49.7
Silica	17.5	25.7	32.9	52.2
Cerous hydroxide	100.0	100.0	97.3	84.5
Ferrous hydroxide	39.8	71.06	34.05	63.86
Manganous hydroxide	65.07	96.4	62.4	83.88

TABLE II.

Conditions same as in Table I.

Substance used as surface and inductor (1.0 g.).	Percentage amount of glucose oxidised in presence of			
	substance + 10 units of insulin.	substance + 10 units of insulin + 0.2880 g. of Na-phosphate.	substance + 10 units of insulin + 0.05 g. of glutathione.	substance + 10 units of insulin + 0.05 g. of glutathione + 0.288 g. of Na-phosphate.
Titanium dioxide	22.0	30.0	27.6	50.4
Silica	18.1	56.1	39.16	53.98
Cerous hydroxide	100.0	100.0	96.66	97.7
Ferrous hydroxide	93.76	86.3	81.8	85.26
Manganous hydroxide	70.5	96.79	64.2	84.7

The results in Table I show that glucose is appreciably oxidised by passing air in presence of solid surface like titanium dioxide, silica, etc., but in presence of the hydroxides of cerium, iron and manganese, which not only act as surfaces but as inductors as well, the oxidation is much greater than with titanium

dioxide or silica. When sodium phosphate is added along with the surface, the oxidation is appreciably increased. When glutathione is added, the oxidation in presence of titanium dioxide and silica is also increased but with the hydroxides of cerium, iron and manganese, there is a slight decrease in the oxidation of glucose. In presence of both phosphate and glutathione, the oxidation of glucose is still further accelerated in the cases of titanium dioxide and silica, but with the hydroxides of cerium, iron and manganese, the oxidation in presence of glutathione and phosphate is appreciably less than that with phosphate alone.

From the results recorded in Table II, it is seen that in presence of insulin, the oxidation of glucose aided by titanium dioxide, silica, cerous hydroxide, ferrous hydroxide and manganese hydroxide respectively is increased. In this respect the behaviour of insulin and glutathione is practically the same but in presence of phosphate, insulin seems also to accelerate the oxidation but glutathione appreciably retards the oxidation in presence of phosphate. A mixture of glutathione and insulin is appreciably better than either insulin or glutathione alone with titanium dioxide or silica as surface but with the inductors cerous hydroxide, ferrous hydroxide or manganous hydroxide, a mixture of insulin and glutathione appears to be less effective.

Oxidation of glucose in presence of (i) insulin, (ii) glutathione and (iii) a mixture of insulin and glutathione containing sodium phosphate and metallic chlorides as inductors.

73.0 Litres of air passed in 30 hrs. 10 C.c. of glucose solution = 0.2308 g. of CuO (blank). 20 C.c. of sodium phosphate solution = 0.288 g. of sodium phosphate.

TABLE III.	Na-phosphate taken in 20 c.c. soln.	Glutathi- one added.	Insulin added.	FeCl ₃ in 20 c.c. soln. in terms of Fe ₂ O ₃ .	CuCl ₂ added.	MnCl ₂ added.	Glucose oxidised.
	0.2880 g.	Nil	10 units	Nil	Nil	Nil	22.2%
	"	"	"	0.01452 g.	"	"	31.7
	"	"	"	"	0.01 g.	"	23.05
	"	"	"	"	Nil	0.01 g.	19.5
	"	"	"	"	0.01	0.01	22.0

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	Na-phosphate taken in 20 c.c. soln.	Glutathione added.	Insulin added.	FeCl ₃ in 20 c.c. soln. in terms of Fe ₂ O ₃ .	CuCl ₂ added.	MnCl ₂ added.	Glucose oxidised.
TABLE IV	0.2880 g.	0.05 g.	Nil	Nil	Nil	Nil	50.3
	"	"	"	0.01452 g.	"	"	64.4
	"	"	"	"	0.01	"	56.8
	"	"	"	"	Nil	0.01	55.6
	"	"	"	"	0.01	0.01	52.5
TABLE V	0.2880 g.	Nil	10 units	Nil	Nil	Nil	21.4
	"	"	"	0.001452 g.	"	"	24.7
	"	"	"	"	0.0001	"	26.5
	"	"	"	"	Nil	0.0001	27.1
	"	"	"	"	0.0001	0.0001	32.2
TABLE VI	0.2880 g.	Nil	10 units	0.0290 g.	Nil	Nil	66.2
	"	"	"	"	0.0001	"	80.4
	"	"	"	"	Nil	0.0001	77.7
	"	"	"	"	0.0001	0.0001	81.1
	"	"	"	"	"	"	"
TABLE VII	0.2880 g.	0.05	Nil	Nil	Nil	Nil	51.5
	"	"	"	0.001452 g.	"	"	57.6
	"	"	"	"	0.0001	"	61.7
	"	"	"	"	Nil	0.0001	62.4
	"	"	"	"	0.0001	0.0001	67.3
TABLE VIII	0.2880 g.	0.05	Nil	0.0290	Nil	Nil	77.6
	"	"	"	"	0.0001	"	82.0
	"	"	"	"	Nil	0.0001	79.8
	"	"	"	"	0.0001	0.0001	85.7
	"	"	"	"	"	"	"
TABLE IX	0.2880 g.	0.05	10 units	Nil	Nil	Nil	58.6
	"	"	"	0.01452	"	"	68.2
	"	"	"	"	0.01	"	48.0
	"	"	"	"	Nil	0.01	52.5
	"	"	"	"	0.01	0.01	48.7
TABLE X	0.2880 g.	0.05	10	Nil	Nil	Nil	56.9
	"	"	"	0.001452	"	"	60.3
	"	"	"	"	0.0001	"	64.8
	"	"	"	"	Nil	0.0001	65.5
	"	"	"	"	0.0001	0.0001	70.2

In a previous publication (Dube and Dhar, *J. Phys. Chem.*, 1932, 36, 444) we have shown that glucose is oxidised in presence of insulin alone and the amount of oxidation increases by the addition of phosphate. The results in Table III show clearly that the amount of oxidation of glucose is appreciably increased when ferric chloride is added to a mixture of phosphate and insulin and that the amount of oxidation of glucose is retarded by the addition of either cupric chloride or manganous chloride or a mixture of cupric and manganous chlorides to the same amount of ferric chloride. Exactly similar results are obtained using glutathione instead of insulin under identical conditions but the amount of oxidation of glucose in presence of glutathione is far greater than that in presence of insulin as will be evident from Table IV.

From the results in Tables V and VI, it is noticed that the amount of oxidation of glucose in a mixture of insulin and phosphate increases with the concentration of ferric chloride. In other words, the greater the amount of ferric chloride, the greater is the amount of oxidation of glucose. Another interesting fact may be observed that with the same amount of ferric chloride, the amount of oxidation of glucose increases by the addition of very minute amount (traces) of either cupric chloride or manganous chloride or a mixture of both. Hence traces of copper or manganese occurring singly or in mixtures of both act as an accelerator in the oxidation of glucose, whereas in presence of larger amount of copper, manganese or a mixture of both, each one acts as a retarder in the oxidation. Exactly similar results are obtained using glutathione instead of insulin as will be evident from Tables VI, VII and IX. In this case also the amount of oxidation of glucose in presence of glutathione is greater than that in presence of insulin.

The results in Tables IX and X show that the amount of oxidation of glucose in presence of a mixture of glutathione and insulin aided by phosphate and metallic salts is slightly greater than that in presence of glutathione alone.

Mechanism of Biological Oxidations.

It seems that the following agencies are important in bringing about the oxidation of food materials not only in animal life but also in plant respiration :—

(i) Reducing agents like glutathione, chlorogenic acid, ascorbic acid, Palladin's "respiratory chromogens," Keilin's cytochrome, Warburg's

"ovoflavin", "lactoflavin", internal secretions, etc., are known to take up oxygen directly from the air and are likely to induce the oxidation of food materials. The experiments recorded in this paper show that glucose is oxidised by air in presence of glutathione or insulin.

(ii) The surface of plant and animal cells, just as surfaces like SiO_2 , TiO_2 , etc., accelerate the oxidation of glucose by air.

(iii) The presence of small quantities of iron and traces of manganese and copper compounds accelerate the oxidation of glucose by air.

(iv) Mild alkalis and phosphates.

(v) Sunlight.

In previous publications (*cf.* Dhar "New Conceptions in Biochemistry", 1932) it has been shown that the oxidation of food materials by air is accelerated by mild alkalis and phosphate and sunlight.

All these agencies seem to have their relative importance in causing the oxidation of the food materials possible in the plant and animal tissues, although they are not oxidised in the air outside. There is reason to believe that in animal oxidation, the internal secretions play an important rôle. It is well known that nature hardly depends on one agency in carrying on its mechanism and in bringing about the oxidation of glucose and other oxidisable materials, seems to take recourse to the foregoing agencies, which have been shown to accelerate the oxidation of food materials by air.

Several years ago one of us stated that the internal secretions act as inductors in the oxidation of food materials (*cf.* Dhar, "*Chemie der Zelle und Gewebe*", 1926, 13, 119). It is gratifying to note that this view is being supported by medical men and Physiologists (*cf.* Wright's "Applied Physiology", Oxford University Press).

SUMMARY.

1. Glucose is appreciably oxidised by passing air in presence of solid surfaces like titanium dioxide, silica, cerium hydroxide, ferrous hydroxide, manganous hydroxide. The oxidation in the case of these hydroxides which also act as inductors, is much greater than with titanium dioxide or silica. In presence of sodium phosphate, the oxidation aided by the above substances is appreciably increased.

2. In presence of glutathione, the oxidation with titanium dioxide or silica is also increased, but with the hydroxides, there is a slight decrease in the oxidation.

3. In presence of both phosphate and glutathione, the oxidation of glucose is still further increased, but with the hydroxides, the oxidation is appreciably decreased and is less than that in presence of phosphate alone.

4. In presence of insulin, the oxidation of glucose with titanium dioxide, silica, and the hydroxides increases. In this respect the behaviour of insulin and glutathione is practically the same. In presence of phosphate, insulin accelerates the oxidations but glutathione retards them. A mixture of insulin and glutathione acts better than either insulin or glutathione with titanium dioxide or silica and appears to be less effective with the hydroxides as inductors.

5. The amount of oxidation of glucose is appreciably increased when ferric chloride is added to a mixture of insulin and phosphate or of glutathione and phosphate, the oxidation in the latter being much greater than in the former.

6. The oxidation in a mixture of insulin and phosphate or of glutathione and phosphate increases with the concentration of ferric chloride. Traces of copper or manganese occurring singly or mixtures of both, act as accelerators, but in presence of larger amounts of the same, each one acts as a retarder in the oxidation of glucose in presence of either insulin and phosphate or glutathione and phosphate.

7. In presence of a mixture of insulin and glutathione aided by phosphate and metallic salts, the oxidation is slightly greater than that of glutathione alone.

8. Reducing agents like glutathione, chlorogenic acid, ascorbic acid, internal secretions, etc., act as inductors taking up oxygen directly from air and thus induce the oxidations of food materials.