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Coupled Oxidation of Alcohol

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I—Introduction

We have seen previously that hydrogen peroxide is formed in several oxidation reactions catalysed by enzymes like xanthine oxidase, uricase, and amino acid oxidase. In all these reactions one molecule of hydrogen peroxide is formed for each molecule of substrate oxidized. It is known that the hydrogen peroxide formed in the primary oxidation reaction can be used in promoting secondary or coupled oxidations of some substances (Thurlow, 1925; Harrison and Thurlow, 1926). The study of secondary oxidations by hydrogen peroxide revealed an unsuspected fact, namely, that it can be used in the oxidation of alcohol to the corresponding aldehydes. In this paper we propose to examine the conditions under which the coupled oxidation of alcohol takes place, the properties of the peroxide formed in the primary reaction, and the mechanism of the secondary oxidation of alcohol promoted by this peroxide.

II—Coupled Oxidation of Alcohol by Uricase System

The study of this reaction was carried out in Barcroft differential manometers in the usual manner but with a series of controls. For instance, in order to detect the oxidation of alcohol by the uricase system, the oxygen uptake of four mixtures was measured separately: (1) uricase + uric acid; (2) uricase + alcohol; (3) uricase + uric acid + alcohol; (4) uric acid + alcohol. The reactions were carried out in buffer solution of p_H 8·9 at 39° C. The quantity of enzyme varied from 25 to 50 mg of dry acetone preparation of liver, that of uric acid varied from 2 to 5 mg, and of alcohol from 5 to 10 mg per flask. The CO_2 was absorbed in the usual way by rolls of filter paper soaked with 10% KOH. The substrate in these experiments was always added in small dangling tubes suspended from the KOH tubes. These tubes were dislodged and their contents mixed with that of the flask after equilibration of the temperature and closing the taps of the manometers. After the experiment the contents

of the flasks were examined for aldehydes. In manometric experiments with uricase the presence of KOH is required for absorption of CO_2, and most of the aldehyde is absorbed by the potash paper where it polymerizes to a dark brown substance. In this way, even a small amount of

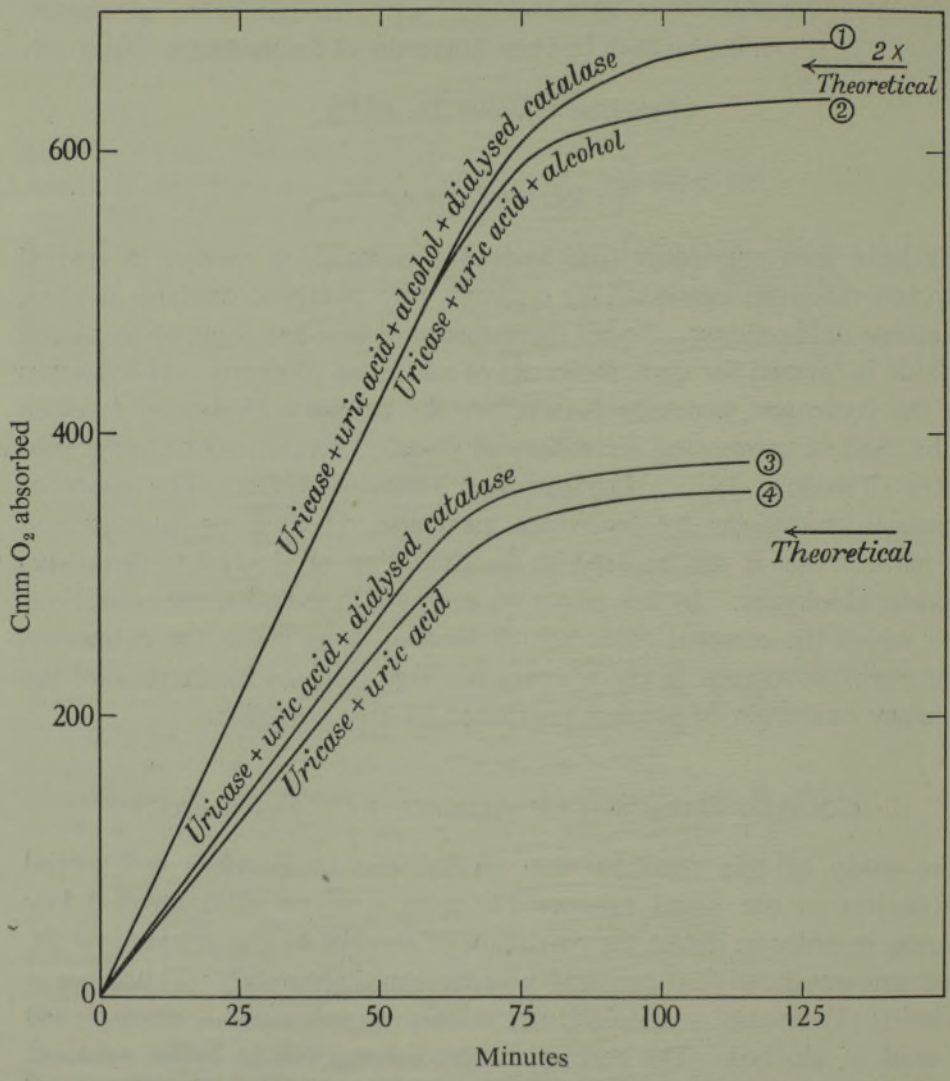

FIG. 1—Coupled oxidation of ethyl alcohol, 10 mg, by uricase + uric acid, 5 mg, at 39° C.

Uricase + catalase + alcohol
Uricase + alcohol
} No oxidation.

aldehyde formed from alcohol gives rise to a yellow fringe on the potash filter paper.

As fig. 1 shows, while uricase + alcohol does not show any appreciable absorption of oxygen, uricase + uric acid takes up just a little more than

the theoretical amount necessary for oxidation of uric acid to allantoin, and uricase + uric acid + alcohol takes up twice this theoretical amount. The experiment was repeated several times with different uricase preparations and always gave the same result. Of other alcohols tried, methyl alcohol undergoes similar oxidation, *n*-propyl alcohol oxidizes

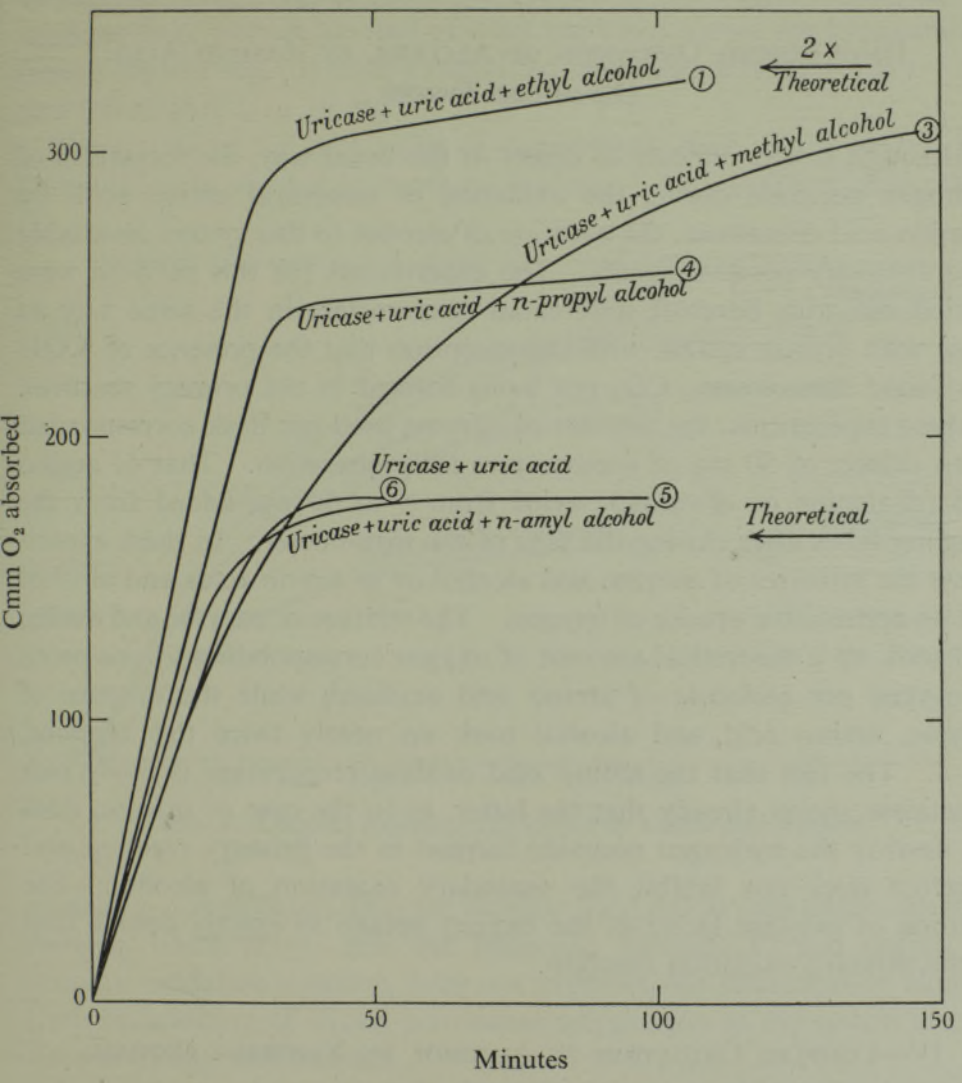

FIG. 2—Coupled oxidation of alcohols, 10 mg, by uricase.

rapidly but does not double the theoretical oxygen uptake, while amyl alcohol does not oxidize at all, fig. 2. The addition of catalase preparation to this system has very little effect on the reaction, because uricase preparation itself contains much catalase. We have found, however, that even when added in great excess, catalase did not remove the hydrogen

peroxide by splitting it in the usual way and did not therefore interfere with the secondary oxidation of alcohol. The fact that catalase did not remove H_2O_2 formed in the primary reaction may be due to its having a much lower affinity for H_2O_2 compared with that of the secondary oxidation system.

III—Coupled Oxidation of Alcohol by *d*-amino Acid Deaminase System

Although it was difficult to detect in the usual way, the formation of hydrogen peroxide during the oxidation of unnatural amino acids by *d*-amino acid deaminase, the addition of alcohol to this system invariably gave distinctly positive results. The experiments for this purpose were carried out with Barcroft differential manometers in the same way as those with uricase system with the exception that the presence of KOH was found unnecessary, CO_2 not being formed in the primary reaction. In these experiments, the amount of enzyme used per flask corresponded to an extract of 50 mg of acetone powder preparation. That of amino acid (*dl*-alanine or *dl*-valine) varied from 5 to 10 mg added from the dangling tubes after closing the taps of the manometer. In these experiments the mixtures of enzyme and alcohol or of amino acids and alcohol had no appreciable uptake of oxygen. The mixture of enzyme and amino acid took up a theoretical amount of oxygen corresponding to one atom of oxygen per molecule of amino acid oxidized, while the mixture of enzyme, amino acid, and alcohol took up nearly twice this amount, fig. 3. The fact that the amino acid oxidase preparation is fairly rich in catalase shows already that the latter, as in the case of uricase, does not destroy the hydrogen peroxide formed in the primary reaction and therefore does not inhibit the secondary oxidation of alcohol. The addition of catalase increases the oxygen uptake to exactly double that of the primary oxidation reaction.

IV—Coupled Oxidation of Alcohol by Xanthine Oxidase System

a—Oxidation of Alcohol in Presence of Catalase Preparation

In order to see whether alcohol undergoes a secondary oxidation in the presence of this system, we have repeated with it a series of experiments similar to those which were carried out with uricase and amino acid oxidase. For this purpose we have measured the velocities and the

total oxygen uptake by xanthine oxidase and its various substrates (hypoxanthine and different aldehydes) with and without the addition of alcohol.

Xanthine oxidase was prepared from milk as caseinogen or as whey preparation and was usually defatted with ether and kept dry. According to the age of the preparation, 25 to 100 mg of dry weight of enzyme was used per flask with an amount of substrate which varied for hypoxanthine from 1 mg to 2 mg, for freshly redistilled acetaldehyde from 0·4 to 1·5 mg, and for alcohol 5 to 10 mg.

The results of the first series of experiments have clearly shown that the addition of alcohol to xanthine oxidase + hypoxanthine or xanthine

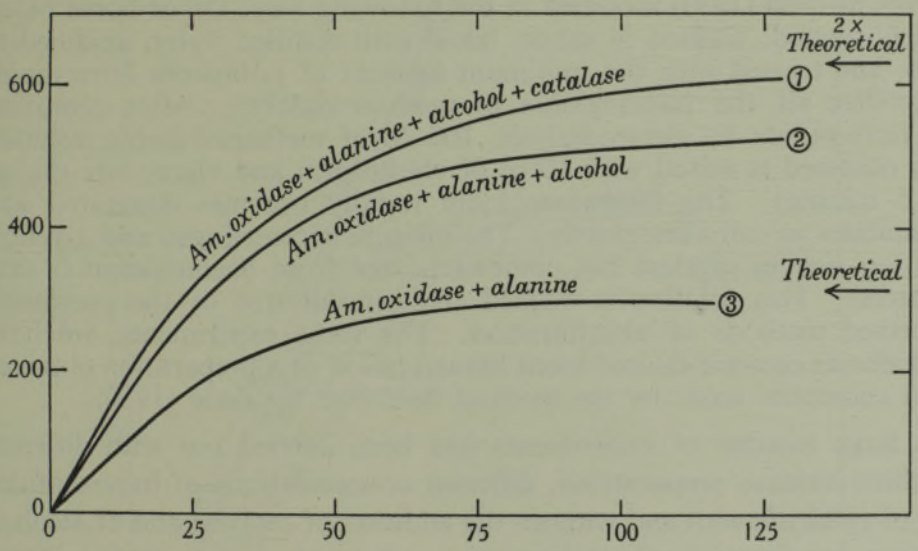

FIG. 3—Coupled oxidation of alcohol by amino acid oxidase.

oxidase + acetaldehyde does not increase the total oxygen uptake by these systems, which shows that the hydrogen peroxide formed during the primary oxidation reaction does not promote the oxidation of alcohol. Even on addition of strong peroxidase preparation to the system alcohol does not undergo oxidation.

It was, however, a matter of some surprise to us to find that on addition of catalase preparation to the system of xanthine oxidase + hypoxanthine + alcohol, the latter undergoes oxidation to aldehyde and doubles the oxygen uptake of the primary reaction. This result was unexpected because catalase is the only enzyme which by its property of rapid decomposition of H_2O_2 was expected to abolish and not to promote the secondary oxidation reaction by H_2O_2 formed in the primary reaction.

The catalase preparation used in these reactions was obtained from horse liver by the method of Zeile and Hellström (1930) slightly modified which gives a strong solution of catalase, containing, however, varying concentrations of alcohol and of chloroform. This strong solution was purified either by electrodialysis against distilled water or by ultrafiltration. The latter was carried out with graded Bechhold's ultra filter membranes supported by a perforated porcelain disc and clamped in a special porcelain funnel fixed into a vacuum flask. The catalase solution was poured on to the membrane impermeable to catalase, the surface of the membrane being constantly swept by a fine brush driven by a small electric motor. The filtration was carried out under a negative pressure, the enzyme being constantly washed by the addition of fresh distilled water. In addition to catalase from liver, we have used also a haemoglobin free catalase preparation obtained from blood by Tsuchihashi's method (1930) modified in the following way: Ox or horse blood is defibrinated, washed in saline, laked with distilled water, acidified to p_H 6, and treated with the minimum amount of potassium ferricyanide to oxidize all the haemoglobin to methaemoglobin. After removing the ferricyanide by electrodialysis, 100 cc of methaemoglobin solution thus obtained is mixed with 10 cc of chloroform and vigorously shaken for 5 minutes. The methaemoglobin rapidly becomes denatured and precipitates as cathaemoglobin. The mixture is centrifuged and a yellow solution rich in catalase but completely free from haemoglobin is thus obtained. This solution is washed and concentrated by the previously described methods of ultrafiltration. For some experiments, we have used also as catalase diluted laked human blood or a preparation obtained from cucumber seeds by the method described by Zeile (1930).

A large number of experiments has been carried out with different xanthine oxidase preparations, different concentrations of hypoxanthine and of aldehyde with and without the addition of catalase and of alcohol. Various preparations of catalase were added either in crude form, containing alcohol, or dialysed and alcohol free. The results of these experiments, which are summarized in Table I and fig. 4, clearly show that in the presence of the primary reaction of oxidation of hypoxanthine or of aldehyde by xanthine oxidase, alcohol does not undergo oxidation. It is only when catalase preparation is added to the system that alcohol is oxidized to aldehyde, with the result that the total oxygen uptake of the system becomes double or even more than double that of the primary oxidation.

b—Cyclic Oxidation

In some of the experiments, in the absence of KOH and especially when the substrate of the primary reaction is an aldehyde, the H_2O_2 formed in this reaction, when used in the secondary oxidation of alcohol to aldehyde, increases the supply of substrate for the primary reaction.

There is, therefore, no reason for this reaction to come to an end while the alcohol is still present in solution and the enzymes involved in the reaction are still intact, figs. 5 and 6. In this reaction, the oxidation proceeds far beyond doubling or even tripling of the primary oxygen

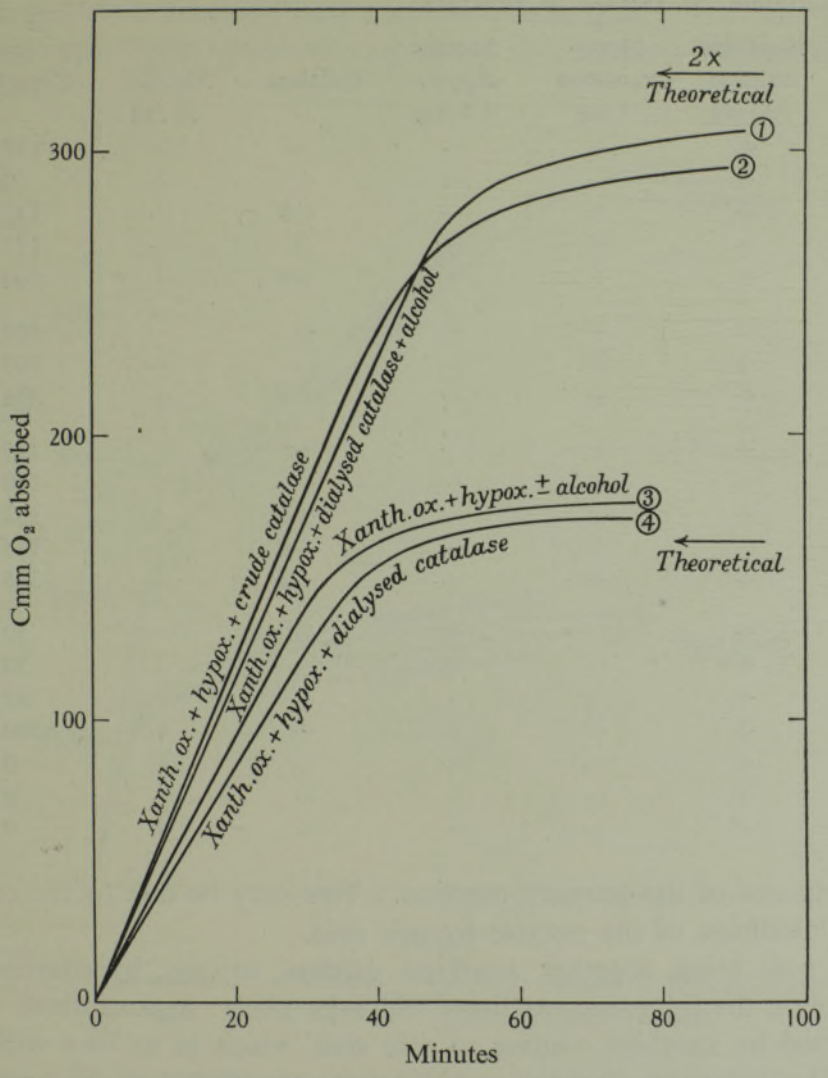

FIG. 4—Coupled oxidation of alcohol by xanthine oxidase (100 mg caseinogen preparation) + hypoxanthine (1 mg) + catalase. KOH papers used.

uptake. Such reaction of oxidation, which we propose to call " cyclic oxidation " may, in fact, occur in liver, which contains all the enzymes and may also contain the substrates necessary for this reaction. If, on the other hand, the primary reaction consists in the oxidation of purines, the secondary, cyclic oxidation of alcohol does not go beyond tripling the

TABLE I

Coupled oxidation of alcohol by xanthine oxidase and hypoxanthine or aldehyde in presence of catalase preparation; theoretical uptake by 1 mg of hypoxanthine = 166 cmm, by 0·4 mg of acetaldehyde = 100 cmm. Catalase: *b*, boiled; *d*, dialysed.

Expt. No.	Xanthine oxidase 100 mg	Hypo-xanthine 1 mg	Acetal-dehyde 0·4 mg	Catalase	Alcohol 10 mg	Cmm O_2
1	+	+	−	−	−	137
	+	−	−	+	+	0
	+	+	−	+*b*	+	142
	+	+	−	−	+	157
	+	+	−	+*d*	+	600
2	+	+	−	−	−	167
	+	+	−	−	+	105
	+	+	−	+*d*	+	326
3	+	+	−	−	−	125
	+	+	−	−	+	140
	+	+	−	+*b*	+	132
	+	+	−	+*d*	−	185
	+	+	−	+*d*	+	325
4	+	−	+	−	−	50
	+	−	+	−	+	50
	+	−	+	+	−	95
	+	−	+	+	+	>350
	+	−	−	+	+	0
	+	−	−	−	+	0
	−	−	+	+	+	0

oxygen uptake of the primary reaction. This may be due to the competitive inhibition of the enzyme by uric acid.

If we now bring together xanthine oxidase, uricase, hypoxanthine, and alcohol, the following reactions will take place: hypoxanthine will be oxidized by xanthine oxidase to uric acid, which in its turn will be oxidized by uricase to allantoin, and in the absence of alcohol, the reaction would come to an end when the total oxygen uptake reached the theoretical amount required for oxidation of hypoxanthine to allantoin. On addition of alcohol, the latter becomes oxidized to aldehyde which forms the substrate for xanthine oxidase and oxidizes further to the corresponding acid, the oxidation reaction thus becoming gradually cyclic. Similar results are obtained by bringing together uricase, uric acid xanthine oxidase, and alcohol, fig. 7.

V—Nature of the Peroxide Formed in the Primary Oxidation Reaction

If the peroxide formed in the primary reaction is ordinary hydrogen peroxide, it should be possible to oxidize alcohol by bringing it together with hydrogen peroxide and catalase preparation. For this purpose several experiments have been carried out with Barcroft differential mano-

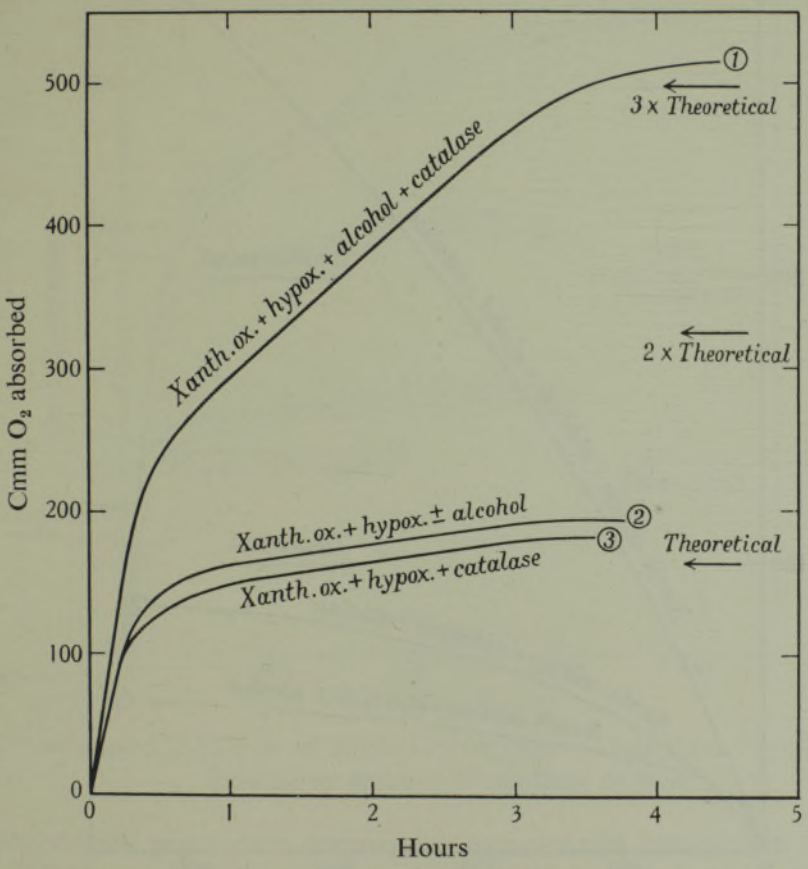

Fig. 5—Coupled oxidation of alcohol by xanthine oxidase + hypoxanthine + catalase. Cyclic reaction in absence of KOH papers.

meters. These experiments have shown, however, that whether alcohol was present or not the hydrogen peroxide added to catalase preparation was decomposed, liberating the theoretical amount of oxygen, and no trace of aldehyde could be detected in the flasks of the manometers. In order to imitate more closely the natural conditions,we have tried in some experiments to add the hydrogen peroxide very slowly. This was obtained by placing into the flasks of the manometers small tubes filled with a dilute solution of H_2O_2 and provided only with very fine capillary outlet

so that H_2O_2 could only slowly diffuse out into the flasks. Even under these conditions, hydrogen peroxide underwent ordinary catalytic decomposition and no aldehyde could be detected within the flasks. It is only when hydrogen peroxide was replaced by some other peroxides such as

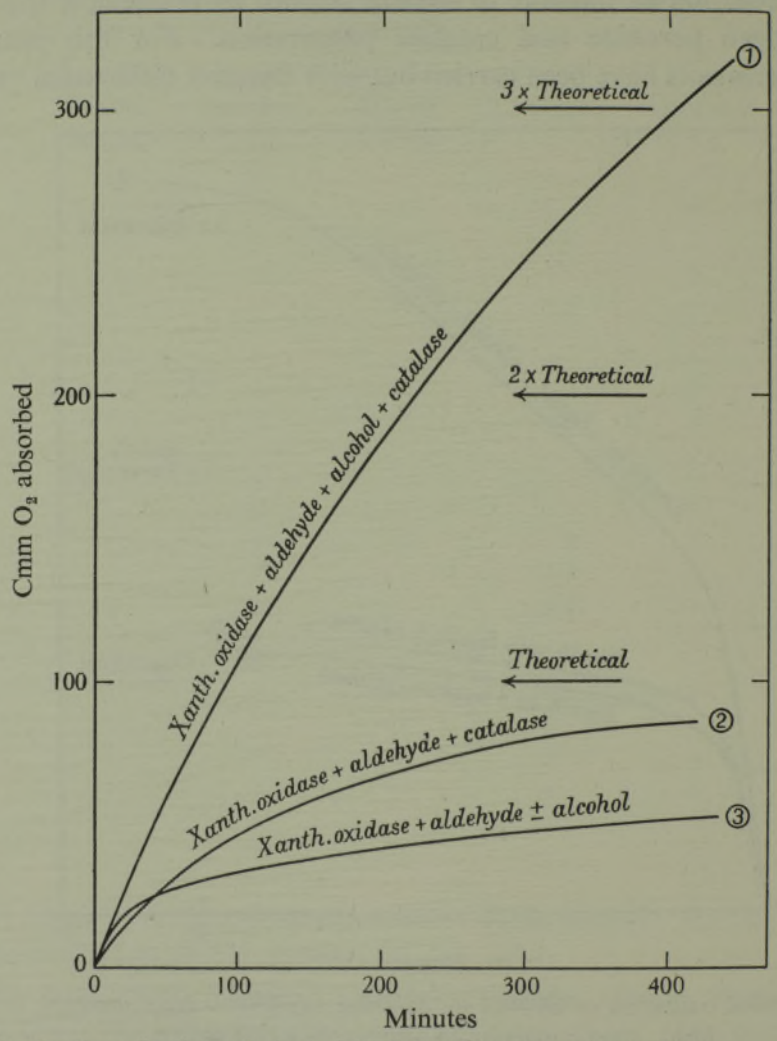

FIG. 6—Cyclic oxidation of alcohol by Schardinger enzyme system.

Aldehyde + alcohol + catalase
Xanthine oxidase + alcohol
Xanthine oxidase + alcohol + dialysed catalase
} No oxidation.

barium peroxide, cerium peroxide, and ethyl hydroperoxide that alcohol underwent oxidation to aldehyde. The easiest way to demonstrate this reaction is to shake in flasks of Barcroft manometers a mixture of alcohol, catalase, and one of these peroxides in the presence of a roll of filter paper soaked in strong KOH. The aldehyde which is formed gradually

becomes absorbed by the potash paper polymerizing into the yellow to brown compound which distinctly colours the paper. The results of these experiments are summarized in Table II.

These experiments clearly show that aldehyde is only formed when catalase preparation, alcohol, and one of these peroxides are present together. They show, moreover, that catalase preparations cannot be

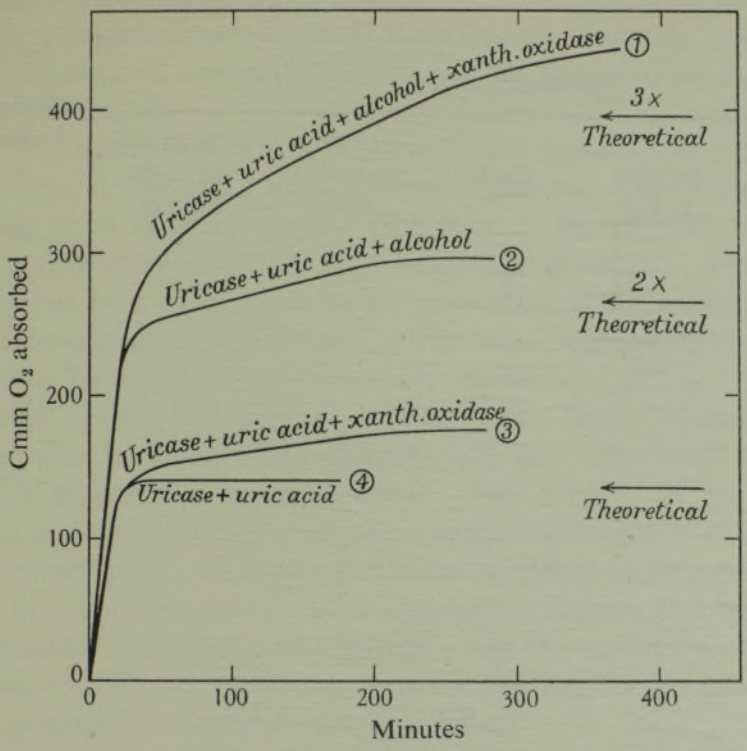

FIG. 7—Coupled oxidation of alcohol by uricase + uric acid. Cyclic oxidation induced by addition of xanthine oxidase.

replaced by a peroxidase preparation, a haematin compound, or a metal like palladium, and that neither of the above-mentioned peroxides can be replaced by H_2O_2 or by benzoyl peroxide. It must be mentioned, however, that ethyl hydroperoxide under the influence of haematin, even in the absence of alcohol, gives rise to acetaldehyde. This reaction resembles the decomposition of diethyl peroxide catalysed by ferrous salts. According to Wieland and Chrometzka (1930), in this reaction two molecules of diethyl peroxide give rise to two molecules of ethyl alcohol and two of acetaldehyde.

On the other hand, one of the properties common to these three peroxides (barium, cerium, and ethyl) is their gradual decomposition to hydrogen peroxide. It is therefore possible that the oxidation of alcohol

to aldehyde in all the above reactions is promoted by what may be called the nascent hydrogen peroxide.

We have seen also that hydrogen peroxide alone, even in the nascent stage, is not sufficient for the oxidation of alcohol and that this reaction requires the co-operation of the second factor which is present in catalase and in some other enzyme preparations.

TABLE II

Substances added					Aldehyde formed
Catalase	Alcohol 10 mg	BaO_2	Cerium peroxide 50 mg	Ethyl hydro-peroxide 6·5 mg	
+	+	−	−	+	+
+	−	−	−	+	−
+	+	+	−	−	+
−	−	−	−	+	−
−	+	−	−	+	−
+	−	−	−	+	−
+	+	−	+	−	+
Peroxidase					
+	+	−	−	+	−
Haematin 0·25 mg					
+	+	+	−	−	−
+	+	−	−	−	−
+	+	−	−	+	+
+	−	−	−	+	+
+	+	−	−	−	−
Palladium 1 mg					
+	+	+	−	−	−
+	+	−	+	−	−
Catalase		Benzoyl peroxide 50 mg			
+	+	+	−	−	−

VI—Mechanism of Secondary Oxidation of Alcohol

In order to determine the constituent of catalase preparation which may be responsible for promoting the secondary oxidation of alcohol,

we have tried to replace the catalase preparation by some other enzyme preparations. We have found, for instance, that the oxidation of alcohol by the xanthine oxidase system takes place also on the addition of uricase or of amino acid oxidase preparations. This explains why in both of these enzyme preparations the secondary oxidation of alcohol takes place without the addition of catalase. Both these enzyme preparations therefore shared with catalase preparation the property of catalysing the secondary oxidation of alcohol. The question that now arises is which of the constituents common to these enzyme preparations is responsible for these reactions.

The first supposition which it is natural to make is the presence in all these preparations of an alcohol dehydrogenase. This enzyme, known since the work of Buchner and especially of Battelli and Stern (1909–1910), is very widely distributed in animals and in plants. A large amount of work has been carried out on the properties of this enzyme within the intact cells and in cell free extracts. The most recent study of this enzyme we owe to Wieland and his co-workers, to Müller (1931 and 1934), Euler and Adler (1934), and Lehmann (1934). The results obtained by these workers are, however, often contradictory and differ with the material used for preparation of the enzyme. The activity of the enzyme in extracts is usually very low and the enzyme was found to be very fragile. In the presence only of substrate it reduces neither oxygen nor methylene blue. It is only on addition of co-zymase (Lehmann) that the methylene blue becomes reduced by this system. For the reduction of oxygen, in addition to the co-enzyme, the system requires also the presence of the yellow pigment or flavine (Euler and Adler).

We could not, however, demonstrate the presence of alcohol dehydrogenase in the enzyme preparations which strongly catalysed the secondary oxidation of alcohol. For instance, xanthine oxidase + alcohol + catalase, even on addition of co-enzyme, did not reduce methylene blue even in 2 hours, while the same system in the presence of hypoxanthine and oxygen rapidly oxidized alcohol to aldehyde. With amino acid oxidase which strongly promoted the secondary oxidation of alcohol, the rate of reduction of methylene blue, even in the presence of co-enzyme, was very slow and if anything inhibited by the addition of catalase. Moreover, catalase preparation kept in solution for over six months was still very active in catalysing the secondary oxidation of alcohol. In spite of this negative result, we cannot definitely dismiss the possibility that some kind of alcohol activating system is present in our enzyme preparations. It is conceivable, for instance, that this enzyme or system has the property of activating the molecules of alcohol only in a special

way so that they can react with hydrogen peroxide formed in the primary reaction but not with other hydrogen acceptors.

The second supposition which is suggested by these experiments is that catalase itself may play some role in promoting the oxidation of alcohol by hydrogen peroxide. The main point which is so far in favour of this supposition is that catalatic property is an obvious property common to the three enzyme preparations (uricase, amino acid oxidase, and catalase) capable of promoting the oxidation of alcohol. This property is absent in xanthine oxidase which without the addition of catalase is incapable of catalysing the secondary oxidation of alcohol. The irreversible destruction of catalase by boiling or treating it with alkali abolishes also completely its power of catalysing the oxidation of alcohol. It was important, however, to determine the effect of some specific catalase poisons on the secondary oxidation of alcohol. It was obvious that such experiments could not be easily carried out in the presence of uricase which is inhibited by KCN and by azide. H_2S, on the other hand, could not be safely used as catalase poison because it can act as a substrate for oxidation induced by metals or haematin compounds present in our preparations. The effect of some of these poisons (KCN, NaN_3, NH_2OH) was tried, however, on the xanthine oxidase system with various catalase preparations added to it, and on the amino acid oxidase preparations which almost always contain catalase. The Tables III and IV and fig. 8 summarize the main results of these experiments.

TABLE III

Xanthine oxidase 100 mg	Hypo-xanthine 1 mg	Catalase	Alcohol 10 mg	Azide m/500	Cmm O_2
+	+	+	−	−	150
+	+	+	−	+	150
+	+	+	+	−	265
+	+	+	+	+	157

All these experiments show that catalase poisons such as cyanide, azide, and hydroxylamine which do not inhibit the primary oxidation reaction promoted by xanthine oxidase and by amino acid oxidase inhibit the secondary oxidation of alcohol.

The third supposition is that the secondary oxidation of alcohol may be due to a specific iron compound like the ferric tannate of de Stoecklin (1909) which is stated to oxidize alcohols in presence of hydrogen peroxide.

Such a compound should be present in preparations of uricase, amino acid oxidase, and catalase; but not in xanthine oxidase. Being, however, always accompanied by catalase, which rapidly decomposes ordinary H_2O_2, this compound will catalyse the oxidation of alcohol only in the presence of a peroxide which by its nature or its low concentration escapes the destructive decomposition by catalase.

TABLE IV

Amino acid oxidase 50 mg	*dl*-alanine 5 mg	Alcohol 10 mg	Poison	Cmm O_2	O_2 uptake due to alcohol
			KCN		
+	+	−	—	272	—
+	+	+	—	475	203
+	+	+	M/250	348	76
+	+	+	M/100	317	45
+	+	−	—	282	—
+	+	−	M/500	272	—
+	+	+	—	475	193
+	+	+	M/500	387	105
+	+	−	—	260	—
+	+	+	—	450	190
+	+	+	M/500	345	85
			NH_2OH		
+	+	−	—	285	—
+	+	+	—	538	253
+	+	+	M/1000	445	160
+	+	+	M/500	405	125
+	+	−	M/250	312	—
+	+	+	M/250	370	68
			Azide		
+	+	−	—	322	—
+	+	+	—	530	+208
+	+	−	M/100	450	—
+	+	+	M/100	410	−40
+	+	−	M/500	382	—
+	+	+	M/500	405	+23

The catalytic activity of such an organic iron compound may also be inhibited by boiling, by treatment with alkali, and by such inhibitors as KCN, NaN_3, and hydroxylamine.

We must admit, however, that all attempts to separate such a compound from catalase itself, by means of dialysis, ultrafiltration, adsorption and

elutions, and fractional precipitation have failed, and the extent of coupled oxidations of alcohol produced by various fractions was so far proportional to their catalatic activity. In other words, if such catalytic iron is present in our enzyme preparations it must be in combination with a protein and must have some properties in common with catalase itself.

We can mention also that we have failed to oxidize alcohol to aldehyde in presence of ferric tannate in experiments carried out under the same

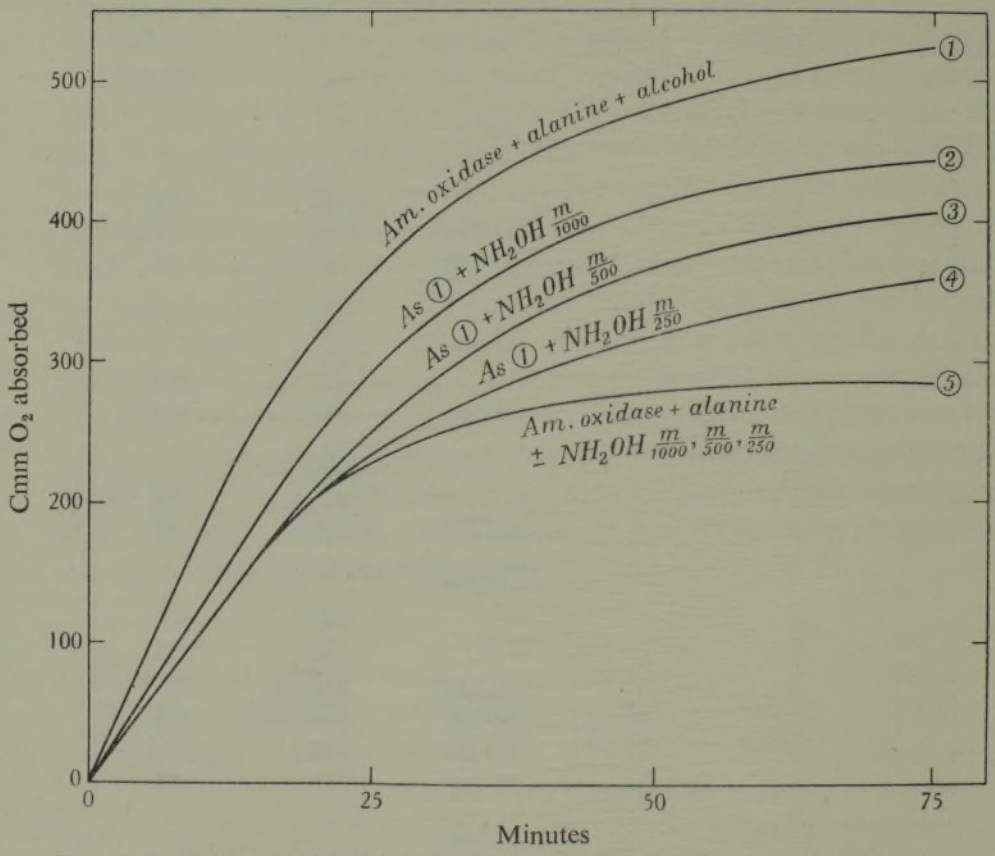

FIG. 8—Inhibition by hydroxylamine of the coupled oxidation of alcohol by amino acid oxidase.

condition as those with catalase. Furthermore, the coupled oxidation of alcohol was not promoted by ferric tannate in presence of the xanthine oxidase system.

We have observed, however, that ferric tannate, like haematin but unlike inorganic iron salts, strongly catalyses the decomposition of ethyl hydroperoxide into aldehyde. It is thus likely that in Stoecklin's experiments, the appearance of aldehyde was due to the action of ferric tannate on an organic peroxide slowly produced in the reaction mixture during 18–20 hours.

We can say, in conclusion, that the coupled oxidation of alcohol requires, in addition to hydrogen peroxide formed in the primary reaction, the co-operation of a second factor, which may be either a specific dehydrogenase activating alcohol, or catalase, or an organic compound of iron present in all catalase preparations so far examined.

The supposition that it may be alcohol dehydrogenase is supported by the specificity of the reaction and by the fact that this dehydrogenase is widely distributed and may be present in all our preparations except that of xanthine oxidase. This supposition is, however, opposed by the marked differences of the properties separating this factor from all known systems activating alcohol, namely: (1) it does not seem to reduce methylene blue; (2) it is highly resistant to acetone, chloroform, ether, and drying; (3) it can remain active for months in solution; and (4) it can be poisoned by KCN, NaN_3, and NH_2OH.

The suggestion that it is catalase itself is strongly supported by the extraordinary parallelism between this reaction and the presence of catalase, by its presence in highly purified preparations of catalase, by great similarity in resistance to some of the organic solvents, and by the effect of specific inhibitors. According to this supposition, the physiological function of catalase would be mainly peroxidatic and only in exceptional cases a catalatic one. This would partly explain the peculiar mutual exclusion of the two enzymes, catalase and peroxidase, namely, that the cells rich in catalase are generally very poor in, or free from, peroxidase and vice versa. This supposition does not, however, help us to understand the specificity of this coupled oxidation of alcohol, especially if the reaction consists mainly in some activation of nascent hydrogen peroxide.

The supposition that it may be an undetermined iron compound is supported by the fact that iron is present in all our enzyme preparations and that some iron compounds are supposed to catalyse the oxidation of alcohol by hydrogen peroxide; but we have not yet succeeded in separating such a compound from catalase itself.

VII—Summary

The addition of alcohol to the mixtures of uricase and uric acid or to amino acid oxidase and amino acids, doubles the oxygen uptake by these primary oxidation systems.

The addition of alcohol to xanthine oxidase and hypoxanthine or to xanthine oxidase and aldehyde has no effect on the oxygen uptake of these systems.

If, however, in addition to alcohol, a little purified catalase preparation is added to the xanthine oxidase system, the oxygen uptake becomes more than double that of the primary oxidation system.

In all these cases alcohol undergoes a secondary or coupled oxidation to aldehyde by H_2O_2 formed in the primary oxidation reaction.

While catalase preparation mixed with ordinary hydrogen peroxide has no effect on alcohol, the same preparation when added to other peroxides such as barium peroxide, cerium peroxide, ethyl hydroperoxide, and peroxide formed in the primary oxidation reaction, catalyses the oxidation of alcohol to aldehyde.

The only property which seems to be common to all these peroxides is the gradual liberation by them of hydrogen peroxide in what may be considered as a nascent state.

The coupled oxidation of alcohol to aldehyde requires, therefore, the co-operation of two factors: (1) a peroxide in nascent state which is being constantly formed in the primary oxidation reactions catalysed by uricase, amino acid oxidase, and xanthine oxidase; and (2) a factor found in uricase, amino acid oxidase, and catalase preparations but not in xanthine oxidase preparation.

As to the nature of this second factor, the study of the secondary oxidation reaction points to three possibilities which have already been discussed in detail, namely, that it may be: (1) an enzyme activating alcohol in a special way so that it can react only with nascent hydrogen peroxide; (2) the catalase itself which may react with this peroxide in a manner different from its reaction with ordinary H_2O_2, and activating it so that it can oxidize alcohol; (3) a special compound of iron and protein present in preparations of uricase, amino acid oxidase, and catalase but not in that of xanthine oxidase.

In the present state of our knowledge, most of the evidence appears to be in favour of the second supposition.

In a system composed of xanthine oxidase + aldehyde + alcohol + catalase preparation, aldehyde undergoes a primary oxidation to the corresponding acid with the formation of H_2O_2 which promotes the secondary oxidation of alcohol to aldehyde, and this in its turn is oxidized to acid. In other words, the secondary oxidation continually supplies the substrate for the primary oxidation reaction and the oxidation proceeds far beyond doubling the oxygen uptake of the primary reaction. This type of oxidation system is described here as cyclic oxidation.

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The Action of Cyanide and Other Respiratory Inhibitors on Xanthine Oxidase

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I—Introduction

It was stated by Dixon and Thurlow (1925) that xanthine oxidase is not inhibited by cyanide. They found that concentrations of cyanide up to M/100 did not produce any inhibition of either the uptake of oxygen or the reduction of methylene blue by hypoxanthine in presence of the enzyme. Some inhibition was, however, observed with higher concentrations of cyanide.

During the course of some other work on xanthine oxidase, Dixon and Lemberg, using the methylene blue technique, observed in 1931 (unpublished) that even small concentrations of cyanide completely inactivated the oxidase, provided that it was incubated with the cyanide for some time at 37° before testing. Similar results were later obtained by