THE ACCUMULATION OF ACETALDEHYDE BY SUSPENSIONS OF YEASTS

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[*Manuscript received October* 17, 1952]

Summary

The sherry flor yeast, *Saccharomyces beticus*, which causes accumulation of acetaldehyde when growing as a film on the surface of alcohol-containing media, also causes an accumulation of acetaldehyde when shaken with ethyl alcohol in air. The ability to accumulate aldehyde under these conditions is not confined to this species, however, but was also found in *S. cerevisiae*, *S. carlsbergensis*, and *S. ellipsoideus*.

Added acetaldehyde is consumed by suspensions of the yeast under both aerobic and anaerobic conditions in the absence of alcohol and under anaerobic conditions in the presence of alcohol.

When suspensions are shaken with mixtures of alcohol and aldehyde under aerobic conditions the yeast tends to bring about an equilibrium between the concentrations of these two components. The value of the equilibrium ratio [alcohol]/[aldehyde] is not constant, but varies with the concentration of alcohol and the temperature, and is also affected by the presence of certain other substrates.

I. INTRODUCTION

During the course of a series of investigations carried out by the author (Fornachon 1953) on the metabolism of the sherry flor yeast, *Saccharomyces beticus*, it was found that this yeast causes the accumulation of considerable amounts of acetaldehyde when growing on wine or synthetic culture media containing ethyl alcohol. *Saccharomyces beticus* is a fermentative yeast with a strongly developed film stage of growth. When seeded into grape juice or other suitable media containing fermentable sugar, it brings about a normal alcoholic fermentation, but if the fermented liquid is subsequently allowed to remain under aerobic conditions at a temperature near 20°C. for a few weeks, the yeast then grows as a film or pellicle on the surface of the liquid. During this phase of aerobic film growth, the yeast brings about the oxidation of ethyl alcohol with accumulation of acetaldehyde which is formed as an intermediate. Under certain conditions, the acetaldehyde has been found to reach over 0.05 per cent. in wine and synthetic media containing between 14 and 16 per cent. of alcohol by volume.

It has long been known that yeasts possess the enzyme alcohol dehydrogenase which brings about the reversible dehydrogenation of ethyl alcohol to

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acetaldehyde in the presence of cozymase (co-dehydrogenase I or diphosphopyridine nucleotide = DPN). The reaction is represented by the equation

$$CH_3.CH_2OH + DPN \rightleftharpoons DPNH_2 + CH_3.CHO.$$

Chief interest in this reaction has usually been associated with alcoholic fermentation, where the alcohol dehydrogenase functions as an aldehyde reductase reducing acetaldehyde to ethyl alcohol while the oxidized cozymase resulting from this reaction is again reduced by the dehydrogenation of triose phosphate to phosphoglyceric acid. Although Trillat and Sauton (1910) observed the accumulation of acetaldehyde when aqueous solutions of alcohol were agitated in air with large quantities of yeast cells, the ability of yeasts to bring about such an accumulation seems to have received comparatively little attention. This appears to be due to the fact that the equilibrium in the reaction represented by the equation lies far to the left and to the small concentrations of alcohol commonly used by investigators who have studied the reaction. Thus Green (1940) states that the reaction between the coenzyme and the alcohol system is in favour of the reduction of aldehyde to alcohol and the oxidation of reduced coenzyme. Hence in order to study the aerobic oxidation of alcohol, it is essential to fix the aldehyde with a suitable reagent. A similar view is expressed by McShann (1949).

Gottschalk (1941, 1942) investigating quantitatively the course of acetaldehyde utilization by compressed (baker's) yeast under aerobic and anaerobic conditions in the absence and in the presence of additional substrates obtained the following results:

(*a*) Anaerobically, acetaldehyde undergoes dismutation to ethyl alcohol and acetic acid, a process much enhanced when glucose is simultaneously fermented;

(*b*) Aerobically, aldehyde consumption is much greater than under anaerobic conditions, and here again consumption is increased by the presence of glucose. Under optimal conditions of aeration, acetaldehyde is oxidized by compressed yeast at the same rate as glucose; the oxidation of 1 mole of acetaldehyde to CO_2 and H_2O is accompanied by the reduction of another mole of acetaldehyde to ethyl alcohol according to the equations

$$CH_3.CHO + 2O_2 + H_2O + DPN \rightarrow DPNH_2 + 2CO_2 + 2H_2O$$
$$CH_3.CHO + DPNH_2 \rightarrow DPN + CH_3.CH_2OH.$$

From these data he concluded that the rate of the reaction between reduced cozymase and acetaldehyde (activated by alcohol dehydrogenase) greatly exceeds the rate of the reaction in which the reoxidation of reduced cozymase is effected by the flavoprotein-carrier-oxygen system. He found that yeast cells in contact with a mixture of acetaldehyde, ethyl alcohol, and acetic acid under aerobic conditions attack at first and selectively the acetaldehyde; down to very low concentrations the aldehyde remains the only substrate for the respiratory system of the yeast.

In view of these findings it is somewhat surprising to find that acetaldehyde accumulates to the extent of over 0.05 per cent. under the influence of the sherry

flor yeasts in growing cultures. It was therefore considered appropriate to examine further certain aspects of the accumulation of aldehyde by suspensions of intact yeast cells.

II. Materials and Methods

The yeasts used in these studies were:

Saccharomyces beticus	Strain 81 of the Waite Institute collection,
S. cerevisiae	Strain 190 of the Waite Institute collection,
S. carlsbergensis	Strain 191 of the Waite Institute collection,
S. ellipsoideus (port strain)	Strain 139 of the Waite Institute collection,
S. ellipsoideus (champagne strain)	Strain 138 of the Waite Institute collection.

The yeasts were grown for 48 hr. in 20-oz. flat bottles, each containing 100 ml. of diluted grape juice inoculated with 10 ml. of a 24-hr. culture in similar medium and incubated at 25°C. During incubation the bottles lay flat in the incubator and were frequently shaken by hand.

To prepare suspensions of the yeasts, the 48-hr. cultures were centrifuged and the cells washed three times in sterile tap water and finally resuspended as a 10 per cent. w/v suspension in sterile 0.05M phosphate buffer at pH 3.25. The yield obtained was about 2 g. wet yeast from 100 ml. of culture. The suspensions were stored in the refrigerator till required but were never more than 48 hr. old when used. The phosphate buffer was prepared by adding 5 ml. of 0.05M H_3PO_4 to 100 ml. of 0.05M KH_2PO_4 solution. The value of 3.25 was chosen for the pH of the buffer because it falls within the range of the pH values commonly found in the wines used as flor sherry base.

The diluted grape juice was prepared as follows:

300 ml. of pasteurized grape juice (pH about 3.5 and containing about 24 per cent. reducing sugar) was diluted to 1 l. with distilled water. Then 4.5 g. of $(NH_4)_2SO_4$, 0.75 g. K_2HPO_4, and 0.1 g. $MgSO_4.7H_2O$ were added. The medium was dispensed in tubes and bottles as required, and sterilized in the steamer.

Acetaldehyde used in the experiments was distilled from aldehyde ammonia prepared in the laboratory and was kept in the refrigerator as a 1 per cent. aqueous solution. The solution was redistilled a few hours before use and the acetaldehyde content of the distillate determined.

Experiments were carried out with yeast suspensions added to substrates in 0.05M phosphate buffer in 8-oz. flat bottles. The total volume of suspension and substrate in each bottle was 10 ml. For experiments with an atmosphere of air, the bottles were closed with screw-on caps fitted with rubber linings, and when other gas mixtures were used, the bottles were closed with rubber bungs pierced by capillary tubing carrying stopcocks. During experiments the bottles were placed horizontally on an electric shaking machine and were shaken at 120 oscillations per minute with an amplitude of 4 cm. The whole apparatus was placed in an electric incubator and temperature variation did not exceed ± 0.5°C. during any one experiment.

Analysis of samples of gas drawn from several bottles at the end of experiments showed that the oxygen content of the atmosphere in the bottles decreased only very slightly during an experiment.

At the end of an experiment the reaction was stopped by the addition of 1 ml. per bottle of a saturated solution of $AgSO_4$ as recommended by Janke and Kropacsy (1935). The contents of the bottles were then chilled with crushed ice and centrifuged and the supernatant liquid was decanted and examined.

TABLE 1

EFFECT OF ALCOHOL CONCENTRATION ON ACCUMULATION OF ALDEHYDE IN SUSPENSIONS OF *S. BETICUS*

Yeast 200 mg.; pH 3.25; temperature 18°C.; atmosphere air; time 3 hr.; total volume of liquid 10 ml.

Suspension No.	Alcohol Added		Aldehyde Found (mg./10 ml.)
	Vol. %	Mg./10 ml.	
I	0·016	1·3	<0·10
	0·08	6·4	0·10
	0·8	64	0·65
	4·0	320	2·47
II	1·0	79	0·82
	5·0	400	2·34
	10	790	4·20
	15	1200	5·15
III	15	1200	4·93
	20	1600	4·96
	25	2000	2·21
	30	2400	0·50
IV	12	950	4·86
	14	1200	4·95
	16	1300	5·27
	18	1400	5·40
	20	1600	4·84
	22	1800	3·60
	24	1900	2·53
	26	2100	1·92

Aldehyde was distilled from duplicate 2-ml. portions of the liquid in an all-glass still, care being taken to keep the end of the condenser below the surface of the liquid in the receiving flask, which was surrounded by crushed ice. Aldehyde was determined by an adaptation of the bisulphite method of Jaulmes and Espezel (1935), using one-fifth the original quantities of reagents and titrating with 0.02N iodine from a microburette.

Blank experiments were carried out both without yeast cells and with yeast cells and $AgSO_4$ added at the beginning of the experiments. The results of these blank tests showed that under the experimental conditions, non-enzymic oxidation of ethyl alcohol to acetaldehyde produced 0.1 mg. of aldehyde from 10 ml. of 14 per cent. alcohol in 3 hr. Determinations of aldehyde production and aldehyde consumption by suspensions of yeasts always included control tests in which the substrates were shaken without yeast cells and appropriate corrections were applied to the analytical results.

III. Experimental Results

(*a*) *Effect of Alcohol Concentration on Aldehyde Accumulation*

The amounts of aldehyde that accumulated in suspensions of S. *beticus* when shaken in different concentrations of alcohol under aerobic conditions for 3 hr. are shown in Table 1.

It can be seen that the accumulation of aldehyde is dependent on the concentration of alcohol; it increased markedly with increased concentrations of alcohol and under these conditions greatest accumulation occurred near 18 per cent. of alcohol. When the alcohol was raised above 20 per cent., however, the accumulation of aldehyde was greatly depressed.

(*b*) *Accumulation of Aldehyde by Different Yeasts*

Although these investigations have been chiefly concerned with S. *beticus*, suspensions of several other yeasts have also been prepared and tested. The results of such tests are shown in Table 2, from which it can be seen that although the yeasts vary in their ability to accumulate aldehyde under these conditions, the characteristic is not confined to the sherry flor yeast.

Table 2

ALDEHYDE ACCUMULATION BY SUSPENSIONS OF DIFFERENT YEASTS IN 10 PER CENT. ALCOHOL BY VOLUME

Yeast 200 mg.; pH 3.25; temperature 20°C.; atmosphere air; time 3 hr.; total volume of liquid 10 ml.

Yeast	Aldehyde (mg./bottle)
S. beticus	4·62
S. cerevisiae	2·54
S. carlsbergensis	3·41
S. ellipsoideus (port strain)	3·52
S. ellipsoideus (champagne strain) ..	3·88

(*c*) *Effect of pH on Accumulation of Aldehyde*

Adjusting the pH of the suspensions at various levels over the range from 2.0 to 7.8 had little effect on the accumulation of aldehyde. At pH values of 2.0 and 2.5 the amount of aldehyde was slightly less than at pH 3.0, but no

significant differences were found over the range from pH 3.0 to 7.8. The most likely explanation of this result would seem to be that the pH in the immediate environment of the enzyme systems, that is, of the contents of the yeast cells, was not significantly affected during the experiment by such changes in the pH of the suspending medium. It has been shown by Göttschalk (1943, 1949) that the pH of the yeast cell is strongly buffered at about pH 6, and is not readily altered by changes in the pH of the surrounding medium.

TABLE 3

ALDEHYDE ACCUMULATION BY *S. BETICUS* UNDER DIFFERENT MIXTURES OF AIR AND NITROGEN

Yeast 200 mg.; pH 3.25; temperature 18°C.; alcohol concentration 14 vol. per cent.; time 3 hr.; total volume of liquid 10 ml.

Bottle No.	Treatment	Oxygen in Gas Phase (%) (calculated)	Aldehyde Formed (mg./bottle)
1	Atmospheric air	21	4·55
2			4·37
3	Evacuated to 250 mm. Hg and refilled with N_2 once	7	4·06
4			4·13
5	Evacuated to 125 mm. Hg and refilled with N_2 once	3·5	3·67
6			3·68
7	Evacuated to 125 mm. Hg and refilled with N_2 three times	0·1	1·20
8			1·34
9	Evacuated to 75 mm. Hg and refilled with N_2 five times	None	<0·1
10			<0·1

(*d*) *Effect of Oxygen Tension on Accumulation of Aldehyde*

In order to carry out experiments under different partial pressures of oxygen, the bottles were evacuated to predetermined pressures and refilled with nitrogen purified by passage through three scrubbers of alkaline pyrogallol and allowed to stand over alkaline pyrogallol. It is not, of course, claimed that the oxygen tension in the bottles could be accurately controlled by such means, but it was possible to achieve a range between aerobic and anaerobic conditions. The effects of such treatments on the accumulation of aldehyde by *S. beticus* in 14 vol. per cent. alcohol are shown in Table 3.

In the experiment represented by the results in Table 3, aldehyde accumulation was depressed by each reduction in oxygen tension, but in other experi-

ments the treatment given to bottles 3 and 4 in Table 3 has had no significant effect on the amount of aldehyde found. However, aldehyde accumulation has always been much depressed at the lower oxygen tensions and under anaerobic conditions no accumulation of aldehyde could be detected.

(*e*) *Consumption of Acetaldehyde by the Yeast*

Not only do the cells fail to accumulate aldehyde under anaerobic conditions, but if aldehyde is added at the beginning of the experiment, some of it disappears and the volatile acid increases by an amount equivalent to slightly less than half of the aldehyde consumed. In an experiment using 400 mg. of yeast cells to increase the speed of the reaction and an initial aldehyde concentration of 9.5 mg. per bottle, 5.3 mg. (0.121 millimoles) of aldehyde were consumed, and 3.5 mg. (0.058 millimoles) of acetic acid were formed. These data are in accordance with the results of Gottschalk (1941, 1942) indicating that the chief mechanism by which yeasts metabolize aldehyde under anaerobic conditions in the absence of other substrates is dismutation to acetic acid and ethyl alcohol.

TABLE 4

CONSUMPTION OF ALDEHYDE UNDER DIFFERENT CONDITIONS BY *S. BETICUS*

Yeast 200 mg.; pH 3.25; temperature 20°C.; time 3 hr.; total volume of liquid 10 ml.

Substrate	Aldehyde Metabolized (mg.)	
	Aerobically	Anaerobically
Aldehyde 9·5 mg.	3·8	2·7
Aldehyde 9·5 mg.+20 mg. glucose	5·7	4·8
Aldehyde 9·5 mg.+12 mg. acetate	2·5	1·8
Aldehyde 9·5 mg.+3·5 ml. of 40% alcohol (14 vol. % alcohol)	0·0	0·7

Experimental study of the effects of several factors on the consumption of aldehyde by the yeast have shown that in the absence of alcohol, aldehyde is consumed more rapidly aerobically than anaerobically (see Gottschalk 1941), but in 14 per cent. alcohol the reverse is true. In fact, in the presence of alcohol, aldehyde consumption under aerobic conditions is only apparent if the initial concentration of aldehyde is above a certain minimum value. As will be shown later, this minimum value varies with the alcohol concentration.

In Table 4 are shown the amounts of aldehyde consumed by suspensions of S. *beticus* in the presence and absence of other substrates and under atmospheres of air and of nitrogen. As can be seen, aldehyde consumption was increased by the presence of glucose as was shown previously by Gottschalk (1942), but it was depressed by acetate or alcohol. Anaerobic conditions depressed aldehyde consumption except in the presence of alcohol.

When other substrates were added to alcohol-aldehyde mixtures before treatment with the yeast cells, the results shown in Table 5 were obtained. Since these experiments were carried out with several different suspensions, the results are expressed as increased consumption as compared with controls in which aldehyde and alcohol were the only substrates. Galactose, glycerol, lactate, malate, tartrate, citrate, and succinate were also tested but were without effect on aldehyde consumption under these conditions.

As can be seen from Tables 4 and 5, glucose increased the consumption of aldehyde less in the presence of alcohol than in its absence but acetate, which depressed aldehyde consumption in the absence of alcohol, actually caused increased consumption in the presence of alcohol.

TABLE 5

EFFECT OF OTHER SUBSTRATES ON THE CONSUMPTION OF ALDEHYDE BY *S. BETICUS* IN THE PRESENCE OF 14 PER CENT. ALCOHOL

Yeast 200 mg.; pH 3.25; temperature 20°C.; time 3 hr.; initial aldehyde 9.5 mg.; total volume of liquid 10 ml.

Additional Substrate	Increased Aldehyde Consumption (mg.)	
	Aerobically	Anaerobically
Glucose 20 mg.	1·2	1·6
Glucose 40 mg.		2·5
Glucose 60 mg.		2·6
Acetate 12 mg.	1·2	1·2
Fumarate 20 mg.		0·9

(*f*) *The Alcohol-Aldehyde Equilibrium*

When the yeast cells were shaken with mixtures of alcohol and aldehyde under aerobic conditions, it was noticed that the aldehyde sometimes increased and sometimes decreased in amount, depending on the initial concentration of aldehyde present. This suggested that the aldehyde concentration tended to reach an equilibrium value for any particular set of conditions. Accordingly a series of experiments was carried out with varied amounts of aldehyde. In each experiment the alcohol content of all bottles was the same and different amounts of aldehyde were introduced. The yeast cells were then added, and after the bottles had been shaken for 3 hr. the reaction was stopped and the changes in aldehyde content during the experiment were determined. It was found that aldehyde accumulated in bottles with a low initial aldehyde content, whereas it decreased in those with a high initial content, but somewhere in between was a point of equilibrium at which the aldehyde content remained unchanged during the experiment. The data presented in Table 6 are typical of such experiments and indicate the equilibrium aldehyde concentration in 10 volume per cent. alcohol at 25°C.

If the change in aldehyde content is plotted against the initial aldehyde content, then it is found that the data presented in Table 6 indicate that zero change would occur when the initial aldehyde content was about 7.65 mg. per bottle, a concentration of about 0.0174M.

The concentration of alcohol was not determined at the end of each of the experiments represented by the data in Tables 6 and 7. However, in two experiments suspensions of the yeast were shaken with 10 per cent. and with 15 per cent. by volume of alcohol for 3 hr. and the alcohol contents of the suspensions were then determined by the dichromate method of Joslyn and Amerine

TABLE 6

EFFECT OF INITIAL ALDEHYDE CONCENTRATION ON ALDEHYDE ACCUMULATION AND CONSUMPTION BY *S. BETICUS* UNDER AEROBIC CONDITIONS

Yeast 200 mg.; pH 3.25; temperature 25°C.; atmosphere air; time 3 hr.; alcohol concentration 10 vol. per cent.; total volume of liquid 10 ml.

Aldehyde (mg. per bottle)		
Initial	Final	Change
4·05	6·10	+2·05
5·25	6·42	+1·17
6·46	6·99	+0·53
7·68	7·66	−0·02
8·90	8·45	−0·45
10·10	9·30	−0·80

(1941). It was found that the loss of alcohol during the experiments was too small to be detected by this method, the results of which were reproducible to within ± 0.1 per cent. of alcohol by volume. In four experiments samples of the gas in the bottles were collected after 3 hr. and analysed for oxygen and carbon dioxide. The greatest amount of carbon dioxide found was 5.7 mg. per bottle, corresponding to complete oxidation of about 3 mg. of alcohol. Although the error involved in such measurements is admittedly large, it is considered that these, together with the alcohol determinations mentioned above, justify the assumption that the alcohol did not change significantly during the course of the experiments under consideration, in which the changes in acetaldehyde concentration were quite small (Table 6). Accordingly the amounts of alcohol added at the beginning of experiments, and expressed in terms of molar concentrations, have been used in calculating the values of [alcohol]/[aldehyde] at equilibrium. For the data recorded in Table 6 this value is 99.

Similar experiments have been carried out with different concentrations of alcohol and at different temperatures and the results of these experiments are summarized in Table 7.

It can be seen from Table 7 that the alcohol/aldehyde equilibrium is affected by both temperature and alcohol concentration. The data presented in Table 5, which show that aldehyde consumption in the presence of alcohol and under aerobic conditions is increased by the presence of some other substrates, suggests that these other substrates also affect the equilibrium. In a wine containing 14 per cent. of alcohol by volume and treated with suspensions of S. *beticus*, an alcohol/aldehyde equilibrium was found when the value of [alcohol]/[aldehyde] was 178.

TABLE 7

EFFECT OF ALCOHOL CONCENTRATION AND OF TEMPERATURE ON THE VALUE OF THE EQUILIBRIUM RATIO [ALCOHOL]/[ALDEHYDE] IN SUSPENSIONS OF *S. BETICUS*

Yeast 200 mg.; pH 3.25; atmosphere air; time 3 hr.; total volume of liquid 10 ml.

Temperature (°C.)	Alcohol Concentration			Aldehyde at Equilibrium		[Alcohol]/[Aldehyde]
	Vol. %	Mg./10 ml.	Molarity	Mg./10 ml.	Molarity	
15	5·0	400	0·87	4·7	0·011	79
25	5·0	400	0·87	4·4	0·010	87
15	8·0	640	1·4	7·1	0·016	87
20	8·0	640	1·4	6·7	0·015	93
25	8·0	640	1·4	6·4	0·015	93
15	10·0	790	1·7	8·4	0·019	89
25	10·0	790	1·7	7·7	0·018	94
15	16·0	1270	2·8	12·5	0·028	100
25	16·0	1270	2·8	11·4	0·026	108

IV. DISCUSSION

It is evident that the accumulation of large amounts of acetaldehyde in some of the experiments with suspensions of yeast cells reported here is associated with the high concentrations of alcohol used. Accumulation of the aldehyde formed as an intermediate in the aerobic oxidation of ethyl alcohol by the yeast must depend on the relative rates of the aldehyde-forming and aldehyde-consuming reactions. Acetaldehyde is formed by the reaction between ethyl alcohol and cozymase activated by alcohol dehydrogenase, but the further oxidation of the aldehyde also involves reactions in which cozymase takes part (Gottschalk 1942). It is to be expected therefore that the distribution of cozymase between the alcohol- and aldehyde-oxidizing systems is influenced by the concentrations of these substrates and that a high concentration of alcohol will increase the accumulation of the acetaldehyde.

One would also expect that, other things being equal, those strains of yeasts which dehydrogenate acetaldehyde least rapidly would bring about the greatest accumulation of aldehyde. In this connection it is worthy of note that, whereas Gottschalk (1941) found that baker's yeast consumed 360 per cent. more acetal-

dehyde under aerobic conditions than under anaerobic conditions, the corresponding increase with *S. beticus* was only about 40 per cent. (Table 4).

It is not surprising that the concentration of acetaldehyde should have been found to approach an equilibrium value in the presence of yeast cells and ethyl alcohol under aerobic conditions, but it is clear that the ratio between the concentrations of the aldehyde and the alcohol at equilibrium is not what would be expected from the equation

$$CH_3.CH_2OH + DPN \rightleftharpoons DPNH_2 + CH_3.CHO,$$

for the published values for the equilibrium constant for this reaction are between 10^3 and 10^5, depending on conditions (Green 1940). This means that for half reduction of the DPN the ratio [alcohol]/[aldehyde] would have these values (i.e. 10^3-10^5), and that the values found in these investigations could be obtained only if the DPN was mainly in the oxidized form. It is known, however, that intact yeast cells possess another mechanism besides the alcohol-aldehyde system for the reoxidation of reduced cozymase. Under aerobic conditions, the cell is able to transfer hydrogen from the reduced coenzyme to molecular oxygen by means of the diaphorase-cytochrome-cytochrome oxidase system. Reoxidation of the reduced cozymase by this mechanism would displace the equilibrium in the above equation towards the right and result in greater accumulation of acetaldehyde or a lower [alcohol]/[aldehyde] ratio. Although Gottschalk (1941, 1942) has shown that the reoxidation of reduced cozymase by the diaphorase-cytochrome-oxygen system is very slow by comparison with the reoxidation by the alcohol-aldehyde system in baker's yeast, it appears that under the conditions of our experiments and also in growing film cultures of the flor yeasts, an oxidative mechanism involving free oxygen is sufficiently active to compete with acetaldehyde in the reoxidation of reduced cozymase and so increase the accumulation of acetaldehyde.

It has been found that increased concentrations of alcohol do not bring about a *proportional* increase in aldehyde accumulation so that the [alcohol]/[aldehyde] ratio at equilibrium rises with increased concentrations of alcohol (Table 7). This may be ascribed to greater displacement of the oxygen-activated system from the reduced cozymase by the higher concentrations of acetaldehyde.

V. Acknowledgment

I am much indebted to Dr. A. Gottschalk of the Walter and Eliza Hall Institute of Medical Research, Melbourne, for his helpful criticism in the preparation of the manuscript.

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