THE INFLUENCE OF ACRIDINE COMPOUNDS ON BAKERS' YEAST

by

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INTRODUCTION

Numerous papers have been devoted to the influence of acridine compounds on micro-organisms. Only a fraction of them try to explain the activity of such compounds. ALBERT *et al.*[1] studied a series of aminoacridines and correlated their activities with the positions of the amino groups. RUBBO *et al.*[2] suggest that the monoaminoacridines may be divided into three distinctive groups and that the bactericidal activity closely parallels the relative basicity of the various isomers. HINSHELWOOD *et al.*[3] made interesting researches on the resistance of micro-organisms to acridines. Only a few papers, however, try to localise the action of the acridines and to interpret the mechanism of their activity. BERNHEIM[4] stipulates that acridines inhibit respiration. HINSHELWOOD *et al.*[3] admit that elongation of the cell is normal, but that division is stopped. MCILLWAN[5] shows that the inhibition of the growth of *B. coli* and STAPH aureus is reversed by nucleic acid and adenylic acid.

In this paper we try to localise the inhibition due to acridine compounds and to understand the mechanism of this inhibition.

EXPERIMENTAL PART

In all the experiments described in this paper, commercial bakers' yeast was employed. This yeast was suspended in $\frac{M}{15}$ phosphate buffer at P_H 5.9. The temperature was 28° C. As acridine compound we used trypaflavin; this is 3.6 diamino.10.methylacridinium chloride (see however (6) and (7)). The substrate was glucose (end concentration 0.5 %).

The experiments were run in the usual WARBURG vessels, which always contained a total volume of 2 ml liquid. The acridine compound was always added last.

I. THE INHIBITION OF THE RESPIRATION OF BAKERS' YEAST

This inhibition is a progressive one; this means that it becomes greater as the time proceeds. Under the conditions of our experimentals (1 h at 28° C), it is very great in concentrations of 10^{-3}M, not so pronounced in concentrations of $5 \cdot 10^{-4}$M and $2.5 \cdot 10^{-4}$M, and non-existent at 10^{-4}M. The results of such an experiment are shown in Fig. 1.

The WARBURG vessels were filled as follows:

A. 1 ml yeast suspension 1 % (= Y)
0.1 ml glucose 10 % (= G)
0.9 ml water (= W)

B. 1 ml Y
0.1 ml G.
0.5 ml W
0.4 ml trypaflavin (= T) $\frac{M}{200}$

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C. 1 ml Y, 0.1 ml G, 0.5 ml W, 0.4 ml T $\frac{M}{400}$ D. 1 ml Y, 0.1 ml G, 0.5 ml W, 0.4 ml T $\frac{M}{800}$ E. 1 ml Y, 0.1 ml G, 0.5 ml W, 0.4 ml T $\frac{M}{2,000}$

2. REVERSION OF THE INHIBITION BY NUCLEIC ACID AND NUCLEOTIDES

WAGNER JAUREGG[8] has demonstrated the existence of stoichiometric compounds between adenylic acid and adenosine triphosphoric acid on the one side, and acridine compounds on the other. MCILLWAIN[5] prepared such compounds starting with yeast nucleic acid and proflavin. One of us (J. D. L.) isolated such a compound between rivanol

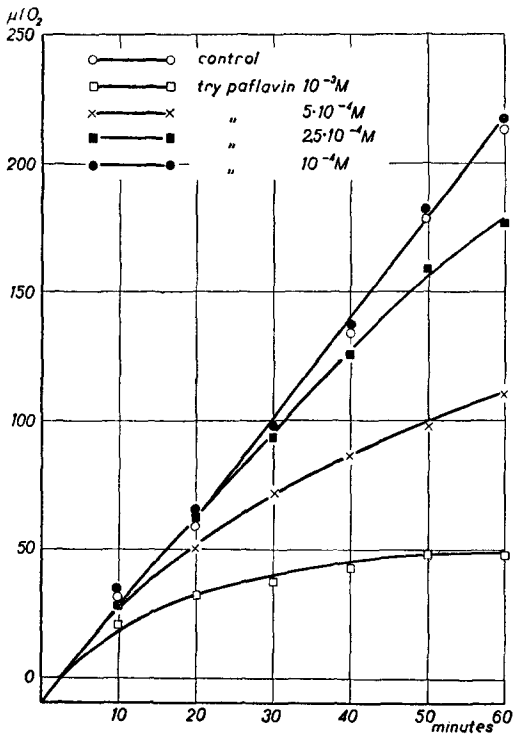

Fig. 1. Inhibition of the respiration of bakers' yeast by different trypaflavin concentrations

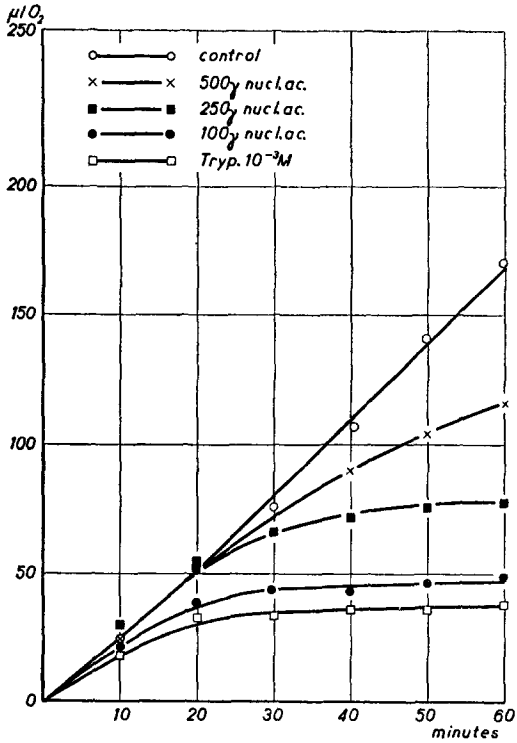

Fig. 2. Reversion of the inhibition by nucleic acid

(this is the commercial name for the lactate of 2-ethoxy-5,7-diaminoacridine) and yeast nucleic acid, containing 4 molecules of rivanol to 1 molecule of nucleic acid. The existence of these compounds suggests that the inhibition of the respiration might be due to the binding of nucleotides of the co-enzyme type such as adenylic acid, adenosine triphosphoric acid, co-enzymes of flavin nature, cozymase and so on. If this were really the case, one should expect such co-enzymes to inverse the inhibition due to acridines. We have shown that at least a partial reversion can be obtained by addition to the medium of adenylic acid (from yeast) and adenosine triphosphoric acid. As we had no other nucleotides of the co-enzyme type, we could not try them. We also obtained a reversion with yeast nucleic acid. It should be said that neither adenylic acid, adenosine triphosphoric

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acid, nor nucleic acid produced any effect on the respiration of yeast in glucose-phosphate medium in the absence of trypaflavin and under the conditions of our experiments. We give some of our reversion experiments in Tables I and II and in Fig. 2.

Reversion by nucleic acid:

The WARBURG cups were filled as follows:

A. 1 ml yeast suspension 1 % (Y)
0.1 ml glucose 10 % (G)
0.9 ml water (W)

B. 1 ml Y
0.1 ml G
0.5 ml W
0.4 ml trypaflavin $\frac{M}{200}$ (T)

C. 1 ml Y
0.1 ml G
0.5 ml nuc. ac. (100γ)
0.4 ml T

D. 1 ml Y
0.1 ml G
0.5 ml nuc. ac. (250γ)
0.4 ml T

E. 1 ml Y
0.1 ml G
0.5 ml nuc. ac. (500γ)
0.4 ml T

The nucleic acid was carefully neutralised.

Reversion by adenylic acid and adenosine triphosphoric acid

The adenylic acid from yeast was a HOFFMANN–LAROCHE product. It was carefully neutralised before use.

The adenosine triphosphoric acid was a 85 % pure Ca-salt, kindly given to us by J. BANGA. The contents of the WARBURG cups follow from the Tables.

TABLE I

REVERSION OF TRYPAFLAVIN INHIBITION BY ATP AND ADENYLIC ACID

Time in minutes	A Control	B Trypaflavin 10^{-3}M	C Trypaf.+ 500γ A. T. P.	D Trypaf.+ 1500γ Aden. acid.
10	30	20	22	24
20	60	32	44	46
30	98	38	66	68
40	136	44	78	82
50	188	48	88	86
60	212	48	92	90

TABLE II

REVERSION OF TRYPAFLAVIN INHIBITION BY ATP AND ADENYLIC ACID

Time in minutes	A Control	B Trypaflavin 5.10^{-4}M	C Trypaf.+ 500γ A. T. P.	D Trypaf.+ 1500γ Aden. acid.
10	30	28	28	26
20	60	50	62	60
30	98	72	98	94
40	142	86	134	128
50	192	98	166	154
60	248	110	190	182

Fig. 2 and Tables I and II show that 100γ nucleic acid causes practically no reversion, but that 250γ causes a marked reversion and 500γ a great reversion. It appears also that the reversion is practically the same in the presence of 500γ adenosine triphosphoric acid, 1500γ adenylic acid, and 250—500γ nucleic acid.

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3. THE INHIBITION OF GROWTH

Concentrations of trypaflavin which have no effect on respiration still show a definite influence on growth. Thus concentrations of 10^{-4}M stop growth without inhibiting respiration. To prove this we have measured respiration and at the same time have made cell counts.

The experiments were carried out as follows. To 1 ml of a 0.2 % yeast suspension in water were added 0.8 ml of beer wort and in the control 0.2 ml of water, whilst in the experiment itself these 0.2 ml of water were replaced by 0.2 ml of trypaflavin in order to ensure an end concentration of 10^{-4}M. The temperature was 28° C, the duration of the experiment three hours. This means that only the initial phase of growth was measured. Systematic investigations on further phases are being continued by one of us (J. D. L.).

After three hours, bud formation was very intense in the control. In the cell counts big buds were considered as new cells and small buds were not taken in account. In the trypaflavin experiments the buds had a characteristic elongated form, which reminds us of the experiments of HINSHELWOOD *et al.*[3].

TABLE III

INFLUENCE OF 10^{-4} MOL TRYPAFLAVIN ON RESPIRATION AND GROWTH

Time in minutes	Results expressed in μl O_2	
	Control	Trypaflavin 10^{-4}M
20	20	22
5	44	42
60	62	56
80	78	74
100	98	94
120	116	110
180	166	144

Number of cells: at the beginning of the experiment: 155 per μl;
at the end of the experiment: control 686 per μl;
at the end of the experiment: trypaflavin 394 per μl.

This experiment was run at different times with the same results. It appears therefore that although respiration was normal, there was an inhibition of growth: bud formation was not so intense and the shape of the buds was abnormally long. (See Table III).

DISCUSSION

1. From our experiments it appears that acridine compounds cause a progressive inhibition of the respiration of bakers' yeast. This may indicate a competition for an enzyme component (co-enzyme) between the acridines and the substrate of the enzyme or enzymes in question. A similar conclusion was reached by MARTIN and FISHER[8], who found that the bacteriostatic effect of proflavin on staphylococci could be reversed by adenylic acid, diphosphopyridine-nucleotide, and several intermediary metabolites. It must be stressed, however, that low concentrations, without doing any harm to respiration, stop growth.

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2. As adenylic acid and adenosine triphosphoric acid cause a reversion of the inhibition and are at the same time physiological substances (co-enzymes of transphosphorylation), it appears that the inhibition of respiration is due to the diversion of co-enzymes, adenylic acid and diphosphopyridine-nucleotide (MARTIN and FISHER[8].)

3. From our experiments it appears that nucleic acid also causes a reversion of the inhibition, far greater even than that caused by A. T. P. or adenylic acid. So far as we know nucleic acid has nothing to do with respiration. Still, our observation presents some interest, as it proves that in the cell nucleic acid will have a greater affinity for acridines than the co-enzymes of the nucleotide type have; so in presence of increasing concentrations of acridines the nucleic acid will be the first affected, and later on the co-enzymes and respiration.

4. This last conclusion is in good agreement with our experimental results, as we have seen that at low concentrations only growth ond not respiration is affected; that is to say, inhibition concerns the nucleic acids which play a rôle in protein synthesis and thus in growth (CASPERSSON[10], BRACHET[11].)At higher concentrations respiration is inhibited because the co-enzymes are bound.

SUMMARY

Acridines exert a progressive inhibition on the respiration of bakers' yeast. This inhibition is reversed by adenylic acid, adenisine triphosphoric acid, and nucleic acid. At low concentrations of acridines only the growth of yeast is affected; at higher concentrations both growth and respiration are inhibited. A discussion is given of the bearing of these results.

RÉSUMÉ

Les acridines exercent une inhibition progressive de la respiration de la levure de boulangerie. Cette inhibition disparaît en présence d'acide adénylique, d'acide adénisinetriphosphorique, et d'acide nucléique. A basse concentration en acridine la croissance seule des levures est arrêtée; à de plus fortes concentrations la respiration aussi bien que la croissance sont inhibées. Une discussion sur la portée de ces résultats a été ajoutée.

ZUSAMMENFASSUNG

Akridine üben eine progressive Hemmung auf die Atmung von Bäckerhefe aus. Diese Hemmung wird durch Adenylsäure, Adenosintriphosphorsäure und Nukleinsäure aufgehoben. Durch niedrige Akridinkonzentrationen wird nur das Wachstum der Hefe herabgesetzt. Die Bedeutung dieser Befunde wird besprochen.

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