

## The Bacteriostatic Action of Benzoic and Salicylic Acids

### III. The Effect on Pyruvate and Acetate Oxidation by Different Organisms

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The influence of benzoic and salicylic acids on pyruvate and acetate oxidation by resting cells of different organisms was investigated.

Both inhibitors blocked pyruvate oxidation at the acetate level in experiments with washed cells of *Proteus vulgaris* and *Escherichia coli* harvested from a medium containing amino acids and additional carbon sources. A similar but less marked effect was observed with cells pre-grown without added amino acids, but not in experiments with cells cultivated in a medium with acetate as the sole carbon source.

Benzoic and salicylic acids did not produce a corresponding effect on pyruvate oxidation by *Pseudomonas fluorescens* or baker's yeast in a similar investigation with these organisms. In the presence of benzoic acid washed cells of *Ps. fluorescens* oxidized acetate completely to CO<sub>2</sub> and H<sub>2</sub>O, if the cells were pre-grown in a medium with acetate as the sole carbon source.

The experiments with baker's yeast were performed at three different pH-values and included an investigation of the influence of benzoic and salicylic acids on growth and on endogenous respiration. It was shown that all effects could be ascribed solely to the undissociated molecules of the acids. With both inhibitors there was close correspondence between inhibition of acetate oxidation and inhibition of growth at all pH-values.

Previously in this investigation<sup>1,2</sup> the influence of benzoic and salicylic acids on the oxidation of glucose, pyruvate and acetate by washed cells of *Proteus vulgaris* was studied. It was observed that both inhibitors specifically blocked glucose and pyruvate oxidation at the acetate level in experiments with cells grown in a medium containing glucose and amino acids. When the cells were grown with acetate as the sole carbon source no such inhibition was noticed — on the contrary, in such experiments, both benzoic and salicylic acids caused an increase in the total CO<sub>2</sub>-output, especially from pyruvate.

These results clearly indicate that the influence of benzoic and salicylic acids on the metabolism of resting cells is largely determined by the pre-history of the cells. It seemed important to investigate this relationship somewhat further and at the same time include other organisms in the study. For this purpose *Escherichia coli*, *Pseudomonas fluorescens* and baker's yeast were selected.

It was suggested previously in this work that the results obtained with *P. vulgaris* might be associated with the presence of a special type of pyruvate oxidase in this organism. This enzyme, which transforms pyruvate directly into acetate without intermediate formation of any metabolically active C<sub>2</sub>-compound (Moyed and O'Kane<sup>3</sup>), has also been shown to occur in *E. coli* (Razzell and Gunsalus<sup>4</sup>). It was therefore considered of interest to compare the effects of benzoic and salicylic acids on pyruvate oxidation by these organisms.

*Ps. fluorescens* and baker's yeast were selected as test organisms for essentially two reasons. Firstly, it was considered of importance to study the effects of the inhibitors under investigation on organisms essentially different from *P. vulgaris* and *E. coli*. Secondly, it was desirable to choose organisms capable of growing with acetate as the sole carbon source and with fairly known pyruvate metabolism.

#### EXPERIMENTAL

*Organisms.* The strain of *P. vulgaris* used in this investigation was the same as before. *E. coli* (NCTC 8196) and *Ps. fluorescens* (NCTC 950) were obtained from the National Collection of Type Cultures, London. Baker's yeast was supplied by Svenska Jästfabriks AB.

*Media and growth conditions.* The liquid media employed for cultivation of *P. vulgaris* and *E. coli* in this investigation were essentially the same as those previously<sup>2</sup> described (medium A containing glucose and casein hydrolysate and medium B with acetate as the sole source of carbon). In some experiments, however, medium A was modified by replacing glucose by sodium pyruvate and sodium succinate (final concentration, 0.5 g per liter of each), and in others casein hydrolysate was omitted.

In all experiments using liquid culture media, the inoculations were made with heavy amounts of washed cells (generally grown in the same media), and the cultures were adequately aerated during growth. The cells were harvested early in the logarithmic growth phase (generally 3–4 h after inoculation), which was ascertained by turbidity measurements. This procedure made it unnecessary to sterilize the growth medium.

*Ps. fluorescens* was grown for 24–48 h on Petri dishes containing substrates with the same composition as above (nicotinamide omitted) and solidified by addition of 1.5 % agar. In experiments where *Ps. fluorescens* was inoculated in liquid media, aerated or non-aerated, the growth rate was generally low, and the total amount of cell mass obtained was small.

*Preparation of cell suspensions.* *P. vulgaris* and *E. coli* were harvested from the liquid media by centrifugation, washed and suspended in phosphate buffer at appropriate pH as described previously<sup>1</sup>.

*Ps. fluorescens* was harvested by washing off the colonies from the agar surface with 1/15 M phosphate buffer. The resulting cell suspension was centrifuged, and the cells were washed twice in 1/15 M phosphate buffer. In the work with baker's yeast, the yeast cells were suspended in buffer and aerated for 1 h prior to the experiments in order to reduce endogenous respiration. Some of the studies were performed at pH-values, where phosphate was unsuitable as buffer substance. In these cases 1/15 M potassium biphthalate or a mixture of 1/15 M potassium biphthalate and 1/15 M potassium phosphate was used. It was ascertained by experiments that the biphthalate ion had no influence on the rates of oxygen consumption recorded in the respiration studies.

*Respiration studies.* The procedure followed was essentially the same as that previously described<sup>1</sup>. The values recorded in figures and tables are, as before, in appropriate cases corrected for endogenous respiration.

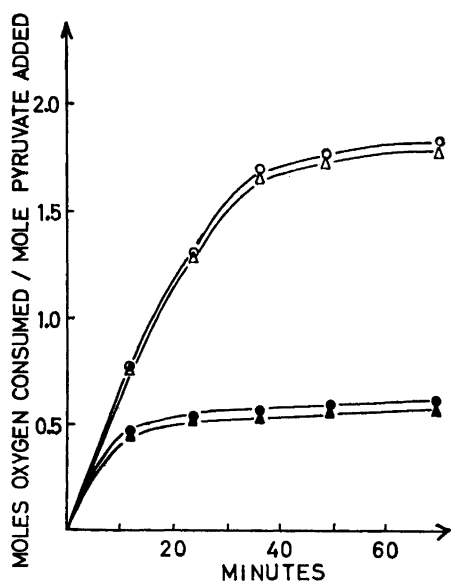
## RESULTS

*Experiments with Proteus vulgaris.* Respiration studies described in the first two parts of this series show, that the influence of benzoic and salicylic acids on the metabolism of resting cells varies considerably with conditions prevailing during cultivation of the cells. Especially the composition of the growth medium seems to be of importance. This is demonstrated by the fact that these inhibitors block glucose and pyruvate oxidation by resting cells of *P. vulgaris* at the acetate level, if the cells are pre-grown in glucose-casein hydrolysate medium, but not if the cells are grown in acetate medium. In this work some additional experiments were performed in order to emphasize the validity of these results.

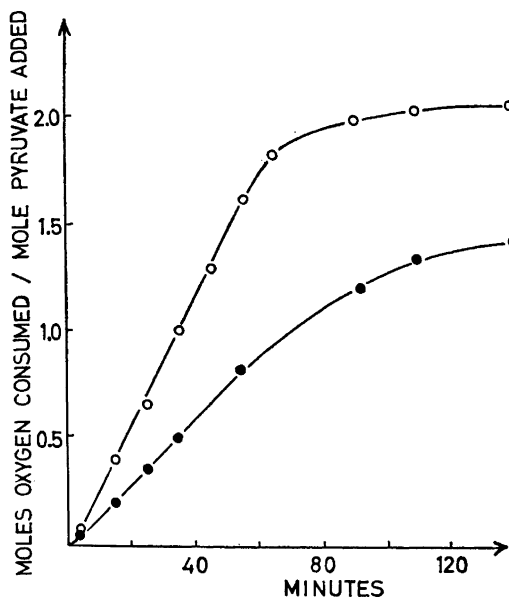
The glucose-casein hydrolysate medium previously employed (medium A) was modified by exchanging glucose for pyruvate and succinate. This supplied the growth medium with an excess of two easily utilizable carbon sources, which as well could give rise to large amounts of starting material for the operation of the TCA cycle. The medium was inoculated with a large amount of cells, which were harvested as soon as the logarithmic growth phase was established. This procedure, besides insuring reproducible conditions of growth, was thought to give rise to cells with a high ability to oxidize acetate. It is evident from Fig. 1 A, however, that benzoic acid, even with such cells, completely inhibited pyruvate oxidation at the acetate level (similar results were obtained with salicylic acid). This inhibition was not affected by simultaneous oxidation of succinate added together with the pyruvate. Previously in this work<sup>2</sup> small amounts of succinate were shown markedly to reduce inhibition of acetate oxidation by benzoic acid.

Fig. 1 B illustrates an experiment with washed cells pre-grown in medium A with omission of casein hydrolysate leaving  $\text{NH}_4^+$  ions as the sole source of nitrogen. The presence of benzoic acid during pyruvate oxidation by such cells neither inhibits the oxygen consumption at the acetate level nor increases the total amount of oxygen consumed per mole of pyruvate (as in experiments with cells grown with acetate as the sole carbon source) but seems to produce some intermediary form of effect.

*Experiments with E. coli.* Figs. 2 A and 2 B illustrate the influence of benzoic acid on pyruvate oxidation by washed *E. coli* cells. The cells were pre-grown in medium A with pyruvate and succinate instead of glucose (Fig 2 A) or in medium B (Fig 2 B) and harvested in the logarithmic growth phase. It is evident, that the results are very similar to those obtained with *P. vulgaris*. Even with *E. coli* pyruvate oxidation is blocked at the acetate level by benzoic acid, when the cells have been cultivated in a fairly rich medium, whereas no such inhibition is observed in studies with cells grown in the simple acetate medium. Corresponding experiments with salicylic acid gave essentially the same results (not shown here). Benzoic and salicylic acids were required at concentrations of about 7 and 1.5 mM, respectively, in order completely to



*Fig. 1 A.* Effect of benzoic acid on pyruvate oxidation by washed *P. vulgaris* cells. The cells were previously grown with aeration in a medium containing pyruvate, succinate and casein hydrolysate and harvested in the logarithmic growth phase. The vessels contained besides 4.5 mg dry wt. of cells, 133  $\mu$ moles of potassium phosphate and 7  $\mu$ moles of sodium pyruvate:  $\Delta$  no further additions;  $\circ$  0.2  $\mu$ moles of sodium succinate;  $\blacktriangle$  8.3 mM benzoic acid;  $\bullet$  0.2  $\mu$ moles of sodium succinate and 8.3 mM benzoic acid. Final volume, 2.4 ml. Experimental conditions: gas phase, air; pH 6.0; temp. 37°C.



*Fig. 1 B.* Effect of benzoic acid on pyruvate oxidation by washed *P. vulgaris* cells. The cells were previously grown with aeration in a medium containing glucose and ammonium chloride as carbon and nitrogen sources and harvested in logarithmic growth phase. The vessels contained besides 2.0 mg dry wt. of cells and 133  $\mu$ moles of potassium phosphate:  $\circ$  7  $\mu$ moles of sodium pyruvate;  $\bullet$  7  $\mu$ moles of sodium pyruvate and 9.7 mM benzoic acid. Final volume, 2.4 ml. Experimental conditions: gas phase, air; pH 6.0; temp. 37°C.

inhibit pyruvate oxidation at the acetate level. In the experiment with acetate grown cells shown in Fig. 2 B benzoic acid did not increase the total amount of oxygen consumed per mole of substrate in contrast to what was observed in corresponding experiments with *P. vulgaris*. Such an increase was, however, occasionally obtained even in respiration studies with *E. coli* in the presence of slightly lower concentrations of benzoic acid.

*Experiments with Pseudomonas fluorescens.* In another series of experiments the effect of benzoic and salicylic acids on the pyruvate and acetate oxidation by washed *Ps. fluorescens* cells was investigated. The cells were grown on agar plates, as described in the experimental part. The solid media were either pyruvate-succinate-casein hydrolysate agar or acetate agar. In a few experiments the cells were grown on succinate agar (acetate agar with acetate

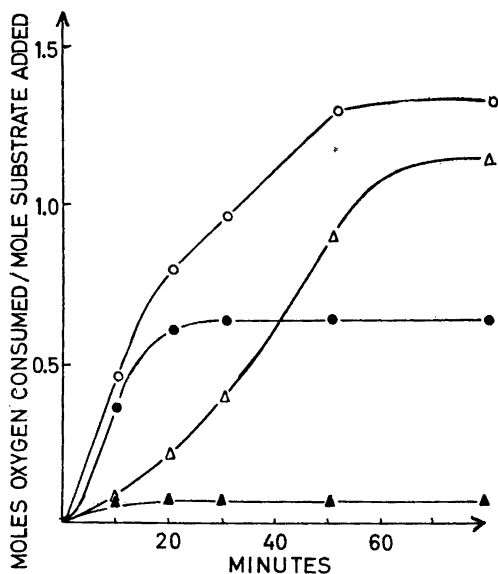


Fig. 2 A. Effect of benzoic acid on pyruvate and acetate oxidation by washed *E. coli* cells. The cells were previously grown with aeration in a medium containing pyruvate, succinate and casein hydrolysate and harvested in the logarithmic growth phase. The vessels contained besides 4.0 mg dry wt. of cells and 133 μmoles of potassium phosphate: ○ 7 μmoles of sodium pyruvate; ● 7 μmoles of sodium pyruvate and 7.5 mM benzoic acid; Δ 7 μmoles of sodium acetate; ▲ 7 μmoles of sodium acetate and 7.5 mM benzoic acid. Final volume, 2.4 ml. Experimental conditions: gas phase, air; pH, 6.0; temp., 37°C.

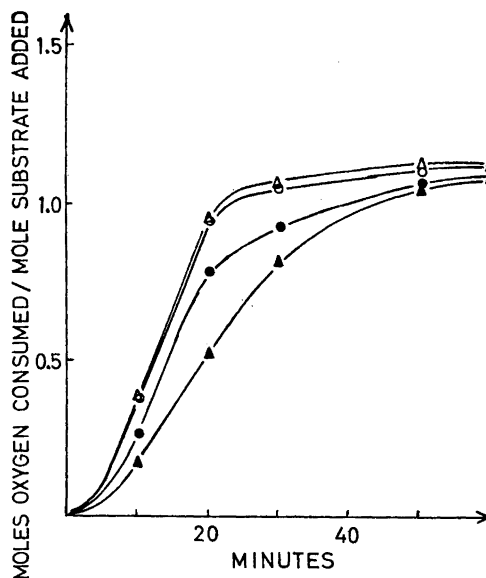


Fig. 2 B. Effect of benzoic acid on pyruvate and acetate oxidation by washed *E. coli* cells. The cells were previously grown with aeration in a medium containing acetate as the sole carbon source and harvested in the logarithmic growth phase. The vessels contained besides 2.5 mg dry wt. of cells and 133 μmoles of potassium phosphate: ○ 7 μmoles of sodium pyruvate; ● 7 μmoles of sodium pyruvate and 7.5 mM benzoic acid; Δ 7 μmoles of sodium acetate; ▲ 7 μmoles of sodium acetate and 7.5 mM benzoic acid. Final volume, 2.4 ml. Experimental conditions: gas phase, air; pH 6.0; temp. 37°C.

replaced with an equal mM amount of succinate). The results of these studies can be summarized as follows:

1. The rate of oxygen consumption during oxidation of acetate was greatest with cells pre-grown on acetate agar, intermediate with cells pre-grown on succinate agar and smallest with cells pre-grown on pyruvate-succinate-casein hydrolysate agar. The  $Q_0$ -values recorded with such differently grown cells were roughly the same as those obtained in experiments with *P. vulgaris* and *E. coli* pre-grown in liquid media of analogous composition.

2. The inhibiting effect of benzoic acid on the rate of acetate oxidation was strongest in experiments with cells oxidizing acetate at a low rate. Accordingly inhibition was greatest in experiments with cells pre-grown on pyruvate-

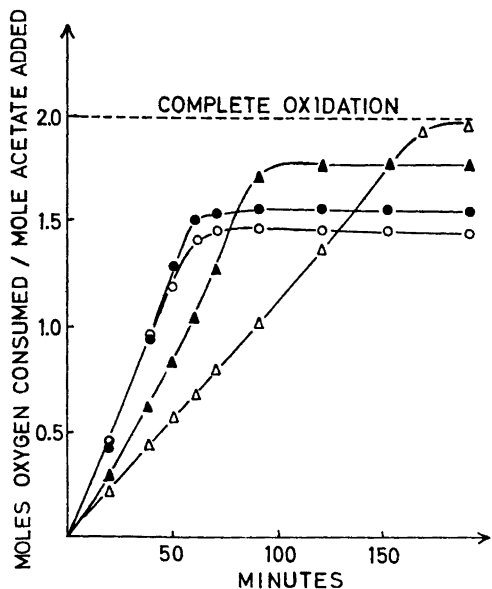


Fig. 3. Effect of benzoic acid on acetate oxidation by washed *Ps. fluorescens* cells. The cells were previously grown on acetate agar. The vessels contained besides 1.2 mg dry wt. of cells, 133  $\mu$ moles of potassium phosphate and 7  $\mu$ moles of sodium acetate: ○ no further additions; ● 2 mM benzoic acid; ▲ 4 mM benzoic acid; △ 6 mM benzoic acid. Final volume, 2.2 ml. Experimental conditions: gas phase, air; pH 6.0; temp. 25°C.

succinate-casein hydrolysate agar (50 % inhibition at about 2 mM benzoic acid) and smallest in the logarithmic growth phase (50 % inhibition at about 15 mM benzoic acid). These results are analogous to those previously obtained with *P. vulgaris*.

3. No sign of a specific inhibiting effect on pyruvate oxidation at the oxidation level of acetate in presence of benzoic or salicylic acid was observed in any experiment with washed cells regardless of the composition of the medium on which the cells had been grown. In this respect there exists a clear difference between *Ps. fluorescens* on one hand and *P. vulgaris* and *E. coli* on the other.

4. As with *P. vulgaris*, benzoic acid increased the total amount of oxygen consumed per mole of acetate in experiments with cells previously grown with acetate as the sole carbon source. This increase was most significant if the cells had passed the logarithmic growth phase at the time of harvesting. In respiration studies with such cells the acetate oxidation — in presence of 4–6 mM benzoic acid — proceeded, until the theoretical amount of oxygen required for complete oxidation of the acetate to  $\text{CO}_2$  and  $\text{H}_2\text{O}$  had been consumed. At these concentrations the rate of oxygen consumption was inhibited to about 50 % by benzoic acid (Fig. 3). If the cells were grown on succinate agar, a similar but smaller increase was observed in presence of benzoic acid. A corresponding increase in oxygen consumption was recorded in respiration studies with pyruvate as substrate.

Some remarks concerning these experiments may be added to the above summary. Washed cells of *Ps. fluorescens* prepared from cultures grown on yeast extract agar can be adapted to oxidize benzoic acid by incubating them in the

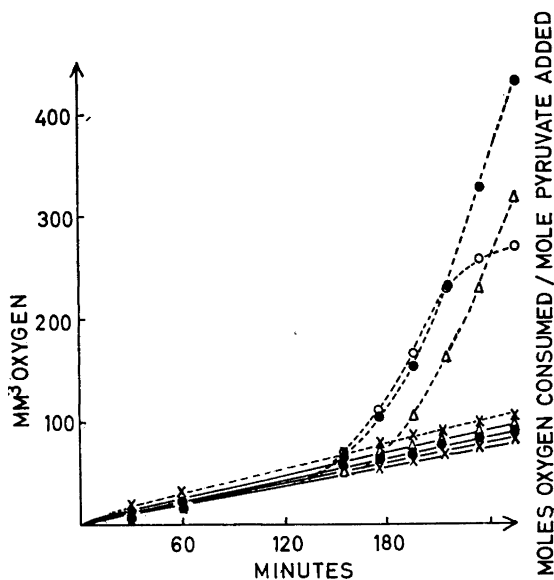


Fig. 4. Adaptation by washed *Ps. fluorescens* cells to oxidation of benzoic acid. The cells were previously grown on yeast extract agar. The vessels contained besides 1.0 mg dry wt. of cells and 33  $\mu$ moles of potassium phosphate:  $\times$  no further additions;  $\circ$  1.75  $\mu$ moles of sodium benzoate;  $\bullet$  2.50  $\mu$ moles of sodium benzoate;  $\Delta$  4.78  $\mu$ moles of sodium benzoate. Dashed lines, pH 7.0; solid lines, pH 6.0; gas phase, air; temp. 25°C.

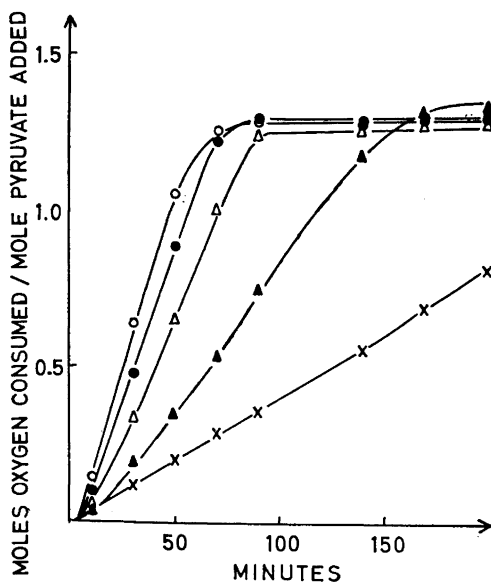


Fig. 5. Effect of benzoic acid on pyruvate oxidation by baker's yeast. The vessels contained 40 mg wet wt. of yeast, 7  $\mu$ moles of sodium pyruvate and benzoic acid at the following concentrations:  $\circ$  no benzoic acid;  $\bullet$  1.4 mM;  $\Delta$  2.8 mM;  $\blacktriangle$  4.2 mM;  $\times$  8.4 mM. Final volume, 2.4 ml. Experimental conditions: gas phase, air; pH 6.0; temp. 25°C.

presence of this substance, as shown by Stanier<sup>5</sup>. It may be argued that some of the oxygen consumed in experiments where benzoic acid is added can be ascribed to such oxidation. This possibility can, however, be ruled out in the present investigation by the following facts: a) in all experiments an aliquot of the cells was incubated with benzoic acid as the sole substrate in a separate vessel, and in no case was any increase of the oxygen consumption observed in these vessels during the experimental time; b) the exponential increase of the rate of oxygen consumption during the adaptation process (Fig. 4) should be clearly revealed, even during simultaneous acetate oxidation, if it actually occurred. The adaptation experiment shown in Fig. 4 was made at pH 7.0 with washed cells grown on yeast extract agar as described by Stanier. It is noteworthy that no adaptation occurred at pH 6.0 — the pH value maintained in the actual experiments in this study; c) the uptake in presence of benzoic acid of almost exactly the amount of oxygen required for complete oxidation of the acetate to  $\text{CO}_2$  and  $\text{H}_2\text{O}$  was obtained irrespectively of the amount of acetate added or the length of the experiment.

*Table 1.* Effect of benzoic acid on pyruvate oxidation by baker's yeast. Each vessel contained 40 mg wet wt. of yeast in 1/15 M biphthalate-phosphate buffer and 7  $\mu$ moles of sodium pyruvate. Final volume 2.2 ml. Experimental conditions: gas phase, air; temp. 30°C; pH 5.0.

Time, min.	Without inhibitor			With 0.5 mM benzoic acid		
	O <sub>2</sub> consumed, $\mu$ l	CO <sub>2</sub> evolved, $\mu$ l	R.Q.	O <sub>2</sub> consumed, $\mu$ l	CO <sub>2</sub> evolved, $\mu$ l	R.Q.
10	52	58	1.12	37	42	1.14
20	131	150	1.15	95	107	1.13
30	200	232	1.16	152	177	1.17
50	266	312	1.17	255	302	1.18
70	288	338	1.17	298	351	1.18

*Table 2.* Effect of benzoic and salicylic acids on oxidative processes and growth in experiments with baker's yeast.

Effect studied	Inhibitor concentration required for the effect studied											
	Benzoic acid						Salicylic acid					
	Total concn., mM			Calculated * concn. of undissociated acid, mM			Total concn., mM			Calculated * concn. of undissociated acid, mM		
	pH 4.1	pH 5.1	pH 6.0	pH 4.1	pH 5.1	pH 6.0	pH 4.1	pH 5.1	pH 6.0	pH 4.1	pH 5.1	pH 6.0
50 % inhibition of the rate of acetate oxidation	1.1	4.0	35	0.62	0.45	0.55	0.6	5.0	45	0.044	0.039	0.045
50 % inhibition of the rate of pyruvate oxidation	0.4	0.6	5.0	0.22	0.067	0.078	0.2	1.5	15	0.015	0.012	0.015
Complete inhibition of growth in glucose-casein hydrolysate medium	—	5.0	60	—	0.56	0.94	—	12	70	—	0.095	0.070
Maximal increase in endogenous respiration	—	—	—	—	—	—	0.55	6.1	43	0.040	0.048	0.043

\* In this calculation  $10^{-4.20}$  was used as dissociation constant for benzoic acid and  $10^{-3.00}$  for salicylic acid.

In studies on cell respiration, where the amount of oxygen consumed per mole of exogenous substrate (substrate added to the medium) is determined, it is of importance that the simultaneous endogenous respiration (oxygen consumption due to oxidation of cell material) is low, since it is a matter of controversy whether this latter oxygen consumption is suppressed during oxidation of exogenous substrate or not. If it is suppressed, it is clearly not correct to subtract the value of the endogenous respiration from the experi-



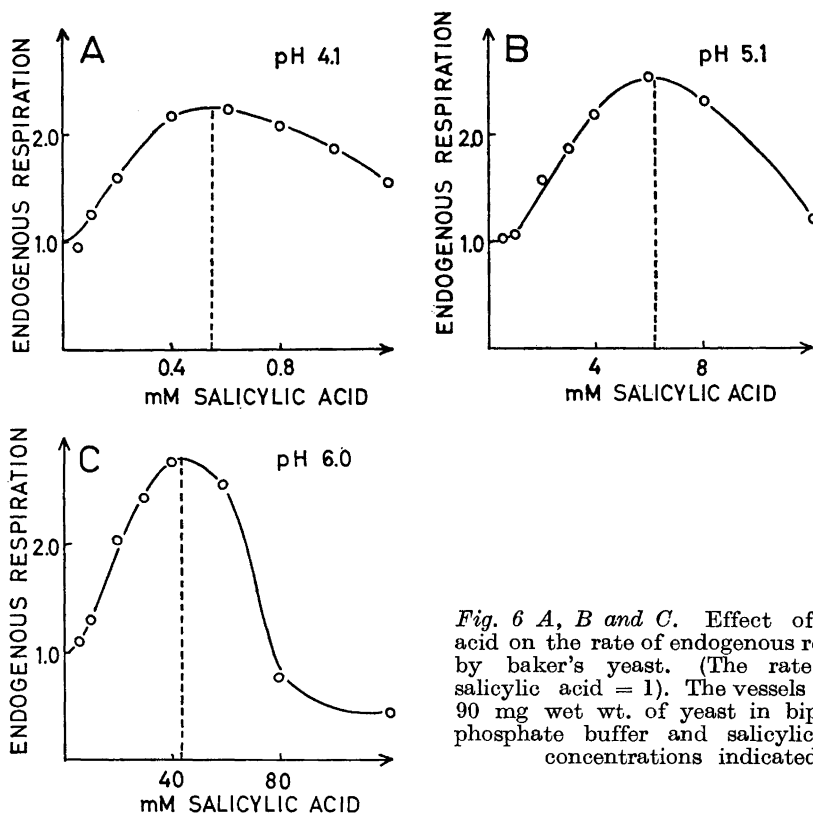


Fig. 6 A, B and C. Effect of salicylic acid on the rate of endogenous respiration by baker's yeast. (The rate without salicylic acid = 1). The vessels contained 90 mg wet wt. of yeast in biphthalate-phosphate buffer and salicylic acid at concentrations indicated.

mentally determined oxygen consumption when calculating the oxygen uptake per mole of exogenous substrate. In the present investigation this problem was of minor importance, since endogenous respiration in *Ps. fluorescens* cells, grown and washed as described, is practically zero.

*Experiments with baker's yeast.* In this study the effect of benzoic and salicylic acids on pyruvate and acetate oxidation and on endogenous respiration was investigated at three different pH-values. In addition the growth inhibiting effect in glucose-casein hydrolysate medium was determined at the same pH-values. Fig. 5 shows the influence of various concentrations of benzoic acid on pyruvate oxidation by baker's yeast at pH 6.0. It is evident, that there is no sign of a specific inhibiting effect in the presence of benzoic acid at the oxidation level of acetate. This is also apparent from Table 1, which shows the *R.Q.*-values during oxidation of pyruvate in the presence and absence of benzoic acid. Similar results were obtained with salicylic acid. In no case there was any significant increase found in the amount of oxygen consumed per mole of pyruvate or acetate in presence of the inhibitors studied. The results of respiration studies at pH 4.1, 5.1 and 6.0 are shown in Table 2,

which gives the concentrations of benzoic and salicylic acids required for 50 % inhibition of the rates of pyruvate and acetate oxidation. The results can be summarized in the following way:

1. Irrespective of the experimental pH-values, the same concentration of undissociated inhibitor is required for a certain effect.

2. The oxidation of pyruvate and glucose (not shown here for the latter substance) is more sensitive, especially to benzoic acid, than the oxidation of acetate. This is contrary to the results obtained in experiments with *P. vulgaris* and *E. coli* previously reported.

3. There is good agreement between inhibition of acetate oxidation and growth with both benzoic and salicylic acids. The concentrations necessary for complete suppression of acetate oxidation is approximately twice those required for 50 % inhibition and thus closely corresponding to those preventing growth at pH 6.0 and 5.1. At pH 4.1 no growth occurred, not even in the absence of inhibitor.

During this investigation it was noticed that salicylic acid significantly stimulated the endogenous respiration of the yeast cells. The appearance of such an effect generally indicates that the stimulating substance affects the energy conservation process in the cells. Since interference with this process and inhibition of acetate oxidation previously in this work have been placed in relation to each other, the increase in endogenous respiration in the presence of salicylic acid was determined at pH 4.1, 5.1, and 6.0. The result of this study is shown in Fig. 6 A, B, and C. Maximal increase in the rate of endogenous oxygen consumption occurred at 0.55, 6.1 and 43 mM salicylic acid at the three pH-values investigated. These figures correspond to a constant value of undissociated salicylic acid in the three experiments, as calculated in Table 2.

No similar stimulation of endogenous respiration was caused by benzoic acid.

#### DISCUSSION

The role of pyruvate as a key intermediate in the metabolism of micro-organisms has been recognized and studied for a long time. Special attention has been paid to the mechanism by which it is converted to acetyl-CoA, a metabolically active compound, which can be used by the cell as a building stone in the synthesis of cell material or as an energy source. The latter function is fulfilled by its oxidation to CO<sub>2</sub> and H<sub>2</sub>O through the TCA cycle. This cycle is now accepted as the chief pathway of terminal respiration in most aerobic organisms. The oxidative decarboxylation of pyruvate may, however, occur by other pathways without the intermediate formation of acetyl-CoA, e.g. the direct formation of free acetate from pyruvate by the pyruvate oxidase system in *P. vulgaris*.

The presence of benzoic and salicylic acids during pyruvate oxidation by resting cells of *P. vulgaris* leads to an accumulation of large amounts of acetate in the medium as previously<sup>1</sup> reported. These results were tentatively explained by assuming that some step in the pathway leading from pyruvate to acetyl-CoA was inhibited, while the acetate producing pyruvate oxidase system

was insensitive to the inhibitors investigated. This interpretation was supported by further experiments <sup>2</sup> with *P. vulgaris* grown under different conditions.

The results reported in this work are principally concerned with the effect of benzoic and salicylic acids on pyruvate and acetate metabolism in *Escherichia coli*, *Pseudomonas fluorescens* and baker's yeast. The similarity of the pyruvate metabolism in *P. vulgaris* and *E. coli* was pointed out by Razzell and Gunsalus <sup>4</sup>. They reported that extracts from *E. coli* yield one particulate and one soluble fraction, which combine to generate acetate from pyruvate with oxygen consumption. The two fractions are mutually replaceable with analogous fractions obtained from *P. vulgaris*. Likewise, *P. vulgaris* was shown to possess an acetyl-CoA generating system apparently identical with that in *E. coli*. It is consistent with these findings that benzoic and salicylic acids affect pyruvate oxidation by these two organisms in the same way.

The terminal respiration of *Ps. fluorescens* and baker's yeast has been studied extensively, and no oxidase system analogous to that present in *P. vulgaris* and *E. coli* producing acetate directly from pyruvate has been reported. This may explain the absence of a specific inhibiting effect of benzoic and salicylic acids on the acetate level during pyruvate oxidation by *Ps. fluorescens* and baker's yeast (Fig. 5).

Irrespective of the inhibition pattern, however, the terminal respiration of all the organisms investigated is inhibited by benzoic and salicylic acids at concentrations which suppress the growth rate in a medium containing glucose and amino acids. The close correspondence between these effects is especially apparent in the experiments with baker's yeast (Table 2).

Previously in this work results have been reported indicating that under certain conditions benzoic and salicylic acids may increase both the rate and the extent of substrate breakdown. In particular it was shown in experiments with acetate grown cells of *P. vulgaris* that the assimilation of acetate was more sensitive to benzoic acid than its oxidation. This finding is confirmed by the respiration studies with acetate grown cells of *Ps. fluorescens* reported in this paper (Fig. 3). It is evident that benzoic acid may cause a complete oxidation of acetate to CO<sub>2</sub> and H<sub>2</sub>O and thereby prevent utilization of this substance in synthesis of cell material. The effect of benzoic acid in these experiments is quite analogous to that obtained by Clifton <sup>6</sup> in his early investigation of the prevention of acetate assimilation in *Pseudomonas calcoacetica* by 2,4-dinitrophenol, sodium azide and iodoacetate. The two first mentioned substances are now well known as specific inhibitors of oxidative phosphorylation.

It is evident that benzoic and salicylic acids may inhibit both respiration and assimilation at concentrations affecting growth. Inhibition of terminal respiration may well be the primary cause of the growth inhibiting effect in a medium containing large amounts of readily utilized substrates such as glucose and amino acids. Both inhibitors are, however, more effective in a poor medium and it is possible that interference with the synthesis of cell material is a more important factor in this case. This question is now under investigation.

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