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Influence of Certain Dye-Stuffs on Fermentation and Respiration of Yeast Extract.

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An aqueous extract of yeast can be prepared which is free of cells but which will actively ferment glucose to alcohol and CO_2. Such an extract consumes only a small amount of oxygen and this small amount exerts no measurable effect on the rate of fermentation. Consequently the extract exhibits no Pasteur reaction. If, however, a dyestuff, which is reversible with respect to oxidation and reduction and which can be reduced by the extract and oxidized by oxygen, is added to the extract, an appreciable oxygen consumption ensues. According to Lipmann[1] this oxygen consumption may or may not inhibit fermentation. He concludes that the deciding factor is the oxidation-reduction potential which the dyestuff imposes on the fermenting mixture. If the potential range of the dye added is relatively negative no inhibition occurs. If it is relatively more positive and oxygen is supplied at a rate sufficient to keep a part of the dye in the oxidized form, inhibition does occur and, according to his results, may be reversed if the potential is lowered by making the experiment anaerobic and adding a reductant (ascorbic acid) plus diphosphate. He regards this as an example of the Pasteur reaction and considers the mechanism of this reaction to be the reversible oxidation of some component of the fermentation system which is active only in the reduced form. If this explanation of the Pasteur reaction is true, and it seems to have found a ready acceptance by some investigators,[2, 3] it would have a most important bearing on the fundamental aspects of respiration and fermentation. It seemed to us desirable to try and check this general idea.

The yeast used was a bottom yeast kindly supplied to us by Ruppert's brewery. It was pressed at 800 lb. pressure, dried in the air at 25°C., ground to a fine powder and stored in the ice box. The extract was prepared from a portion of this powder with 3 volumes of distilled water according to Lebedev.[4] Various samples had a

1 Lipmann, F., *Biochem. Z.*, 1934, **268**, 205.

2 Kluyver, A. J., and Hoogerheide, J. C., *Biochem. Z.*, 1934, **273**, 197.

3 Morgulis, S., and Munsell, J. D., *Biochem. Z.*, 1935, **278**, 89.

4 Lebedev, A. v., *Z. Physiol. Chem.*, 1911, **73**, 447.

pH of 6.10, varying no more than ±0.05 unit, measured with the glass electrode; and were slightly less than 0.1 m. in inorganic phosphate. All the extracts prepared exhibited strong reducing properties. Anaerobically methylene blue and indigo disulfonate were rapidly and completely reduced. Even rosindulin G G was completely, though very slowly reduced. As judged by the iodine titration, oxidized glutathione was reduced at an appreciable rate. Benzyl viologen was not reduced.

The general effect of small amounts of dyes on fermentation is to shorten the induction period and accelerate the beginning stages. Under anaerobic conditions this is the only effect of small amounts of dye, but larger amounts may distinctly inhibit although there may be an initial period of acceleration. Under aerobic conditions oxygen is consumed and fermentation is usually inhibited after an initial period of marked acceleration, but the rate at which the inhibition sets in varies considerably from dye to dye. Thus in agreement with Lipmann we have found that 1 naphthol 2 sulfonate indophenol inhibits much more strongly than thionine. The concentration range tested was from 7×10^{-4} m. to 4.9×10^{-3} m. We have also found that pyocyanin inhibits about as strongly as the indophenol. Indigo disulfonate also exerts an appreciable inhibition, while phenosafranine does not inhibit although it brings about a considerable oxygen consumption.

A consideration of these results together with the potential range of the above dyes indicates that with our material the potential range is not the factor that determines the inhibition of fermentation. A comparison of the oxygen consumed by the different dyes and at different temperatures (experiments were done at 37°, 28°, and 24°C.) shows that there is no correlation between the amount of oxygen consumed and the degree of inhibition. The inhibition obtained with all the dyes except naphthol sulfonate indophenol is always irreversible and is due to the destruction of enzymes. Thus the carboxylase activity of the extracts is completely destroyed. Under the proper conditions a partially purified preparation of carboxylase[5] will restore fermentation. The carboxylase activity of this purified preparation is readily destroyed by treatment with dye plus oxygen. Carboxylase is, however, not the only enzyme inactivated. Phosphorylation and phosphate hydrolysis also cease. The enzymes responsible for the reducing action of the yeast remain active after fermentation has ceased, but they are also gradually in-

[5] Axmacher, Fr., and Bergstermann, H., *Biochem. Z.*, 1934, **273**, 259.

activated. Naphthol sulfonate indophenol also brings about this irreversible inhibition, particularly at 37°, but in addition it can inhibit fermentation in such a way that making the experiment anaerobic and adding ascorbic acid and hexose-diphosphate as Lipmann reports restores the fermentation. This inhibition is, according to our experiments, due to the removal of the diphosphate or the normal intermediates that result from it and the restoration is due to the addition of more diphosphate and not to the reduction of the dye. The addition of sufficient diphosphate at the beginning diminishes this inhibition. The reduction of the dye after it has inhibited fermentation does not restore the process, but the addition of diphosphate without reducing the dye does restore it.

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Experiments on Purification of Bacteriophage, and a Respiratory Pigment in Escherichia Coli Communis.

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During a study of the bacteriolytic enzyme, lysozyme,[1] we wished to compare its properties with those of bacteriophage. The method used for the purification of lysozyme was not applicable to bacteriophage because of the extreme sensitivity of phage to the common organic solvents.

Previous efforts at purification of bacteriophage have usually employed physico-chemical methods: ultrafiltration through membranes of graded porosity,[2] adsorption,[3] and more lately centrifugation.[4] In the present work the removal of undesired culture medium and bacterial components was attempted by chemical methods and the behavior of phage toward some chemical agents was observed.

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1 Meyer, K., Thompson, R., Palmer, J. W., and Khorazo, D., *Science*, 1934, **79**, 61; complete report in press.

2 Elford, W. J., and Andrews, C. H., *Brit. J. Exp. Path.*, 1932, **13**, 446.

3 Gildemeister, E., and Herzberg, K., *Zentralbl. Bakt.*, 1924, **91**, 228; **93**, 402; Kligler, I. J., and Olitzki, L., *Brit. J. Exp. Path.*, 1931, **12**, 172; Clifton, C. E., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 32.

4 Schlesinger, M., *Z. Hygiene*, 1932, **114**, 161.