The Influence of Dyes on the Respiration of Baker's Yeast

In this paper we relate some observations on the influence of three types of dyes on the respiration of baker's yeast as measured by the Warburg technique in phosphate buffer ($p_H = 5 \cdot 9$) at 28° C. These three types of dyes are (1) the acridines, (2) the thiazines, (3) the dyes belonging to the triphenylmethane compounds.

BERNHEIM[1] has shown that acridines are inhibitors of bacterial respiration. We have observed that crystal violet readily diminishes the respiration of baker's yeast and that methylene blue in concentrations of $2 \cdot 10^{-3}M$ and $10^{-3}M$ causes a drop in the respiration of baker's yeast.

The following dyes were studied: trypaflavine as a representative of the acridines, methylene blue as a representative of the thiazines and crystal violet as belonging to the triphenylmethane dyes. The three dyes cause a progressive inhibition of the respiration of baker's yeast in glucose-phosphate medium, the most potent inhibitor being crystal violet.

ALBERT[2] and coll. have shown the importance of the basic dissociation constant in acridine compounds. MCILLWAIN proved that the toxicity of acridines towards the growth of *Bacterium coli* is reversed by nucleic acid and adenylic acid. This finding corresponds to the fact that WAGNER-JAUREGG[3] and MCILLWAIN[4] found that definite compounds are formed *in vitro* between nucleid acid and acridines.

We have found that the inhibition of baker's yeast respiration caused by trypaflavine, methylene blue and crystal violet is at least partially reversed by yeast nucleid acid, yeast adenylic acid and adenosine triphosphate. We give here some instances of these reversions The Warburg cups always contained 2 ml of fluid: 1 ml of a 1% yeast suspension in phosphate buffer p_H 5·9, 0·1 ml of glucose 10% and the necessary amounts of inhibitor and reversing agent in 0·9 ml.

Results expressed in mm^3 CO_2 per hour.

No adenylic acid No trypaflavine	No adenylic acid trypaflavine: $10^{-3}M$	1500 γ adenylic acid; trypaflavine: $10^{-3}M$
114	10	34
116	16	39

No A.T.P. no crystal violet	No A.T.P. crystal violet $2 \cdot 5 \cdot 10^{-4}M$	500 γ A.T.P. crystal violet $2 \cdot 5 \cdot 10^{-4}M$
74	26	47
90	29	43

No nucleic acid No methylene blue	No nucleic acid Methylene blue: $2 \cdot 10^{-3}M$	500 γ nucleic acid Methylene blue: $2 \cdot 10^{-3}M$
88	44	60
85	47	66

[1] BERNHEIM: cited by A. ALBERT, Brit. J. exper. Pathol. *26*, 185 (1945).

[2] A. ALBERT, S. D. RUBBO, R. J. GOLDACRE, M. E. DAVEY and J. D. STONE, Brit. J. exper. Pathol. *26*, 160 (1945).

[3] TH. WAGNER-JAUREGG, Z. physiol. Chem. *239*, 188 (1936).

[4] H. MCILLWAIN, Biochem. J. *35*, 1311 (1941).

Adenylic acid and nucleic acid were employed as Na-salt; A.T.P. as Ca-salt. These substances alone have no influence on the respirative rate.

The interpretation of this type of reversion seems rather easy as definite compounds between acridines on the one side, nucleic acid and nucleotides on the other have been described. BRACHET[1] described the fixation of toluidine blue (a thiazine) by nucleic acid and adenylic acid. It is likely that other nucleotides more especially of the co-enzyme type will react with dyes. So the inhibition of respiration by dyes might be considered as a diversion of co-enzymes. That acridines and methylene blue have a common or similar action is also proved by the experiments on cross adaptation by HINSHELWOOD[2] and coll.

Full details on these experiments and a discussion on quantitative relations and biological significance shall be publised elsewhere.

This research was aided by a grant of the Ella Sachs Plotz Foundation, by gifts of adenylic acid by Messrs. Hoffmann-La Roche (Basle) and of A.T.P. by J. Banga.

L. MASSART, G. PEETERS, J. DE LEY and R. VERCAUTEREN

Biological Department and Pharmacological Department of the Veterinary College University of Ghent, January 15, 1947.

Résumé

La respiration de la levure de boulangerie en milieu glucose-phosphate est inhibée par la trypaflavine, le bleu de methylène, le violet de cristal. Cette inhibition disparaît du moins partiellement par addition d'acide nucléique, d'acide adénylique et d'A.T.P.

[1] J. BRACHET and J. JEENER: Enzymologia *11*, 222 (1943–1945).

[2] J. M. G. PRYCE, D. S. DAVIS and C. N. HINSHELWOOD, Trans. Faraday Soc. *41*, 465 (1945).

PRO LABORATORIO

Ein Verfahren zur kolorimetrischen Bestimmung des Thyroxins

Im Rahmen einer entwicklungsphysiologischen Untersuchung über die Anurenmetamorphose stellte sich das Bedürfnis nach einer einfachen chemischen Bestimmungsmethode des Thyroxins. Das in dieser Untersuchung ausgearbeitete kolorimetrische Analysenverfahren beruht auf der von W. KOMANT[1] entdeckten Diazoreaktion des Thyroxins: Thyroxin reagiert in sodaalkalischer Lösung bei tiefen Temperaturen mit Diazobenzolsulfosäure unter Bildung eines roten Farbstoffes. Da die sodaalkalische Zersetzung der überschüssigen Diazobenzolsulfosäure die Stabilität des Thyroxinfarbstoffes stark beeinträchtigt und daher eine direkte kolorimetrische Bestimmung des Thyroxins verunmöglicht, wurde versucht, diese Zersetzung in hinreichendem Maße zu hemmen. Dies gelingt, wenn man der Farbstofflösung zwischen 1 und 6 min nach Beginn der Kupplungsreaktion Natronlauge zufügt. Werden 6,5 Volumteile Farbstofflösung mit 1 Teil 2n- oder 3n-NaOH versetzt, so beträgt die Extinktionsabnahme zwischen 5 und 15 min nach Kupplungsbeginn nur noch

[1] W. KOMANT, Arch. exper. Path. *158*, 116 (1930).