however [1] and [2]) causes an inhibition of about 40 to 60 % depending on the yeast examined. This inhibition disappears at least up to 50 % in the presence of $MgCl_2$ 10^{-2}M. We give here some examples of our results on different samples of baker's yeast.

mm³ O_2 per hour		
Without Trypaflavine	Trypaflavine $5 \cdot 10^{-4}$ M	Trypaflavine $5 \cdot 10^{-4}$ M, $MgCl_2$ 10^{-2} M
264	152	270
250	164	246

$MgCl_2$ alone has no influence on the respiration of baker's yeast. The inhibition by other acridines such as 5-aminoacridine and 1-methyl-5-aminoacridine is also reversed by $MgCl_2$.

Inhibition by methylene blue and crystal violet: Under the same conditions as for trypaflavine, $MgCl_2$ offers also a protection against the inhibitory activity of methylene blue and crystal violet. It is however important to state that the protection against crystal violet is not so effective as for methylene blue and trypaflavine.

So far, we were not able to prove that salts offer also a protection against the bacteriostatic action of basic dyes. All the experiments stated above concern only the inhibition of respiration, which occurs at concentrations higher than those necessary to stop growth of yeast.

We have observed that the same facts apply when alcohol is used as substrate instead of glucose, and that once the inhibition is established it is not reversed by $MgCl_2$.

We have proved also that there is a correlation between this protective action and the fact that living yeast cells are notably less colored by trypaflavine in the presence of salts than in their absence.

A systematic investigation on the role of salts is run at this moment and full details will be published elsewhere.

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Biochemical Laboratory and Pharmacological Laboratory of the Veterinary College, University of Ghent, March 11, 1947.

Résumé

$MgCl_2$ et d'autres sels à des concentrations définies protègent la respiration de la levure de boulangerie contre l'action inhibitrice de colorants basiques.

[1] Gaillot, Quart. J. Pharmacy and Pharmacology *7*, 63 (1934).
[2] H. Berry, Quart. J. Pharmacy and Pharmacology *14*, 143 (1941).

On the Existence of Intermediate Compounds between Hemoglobin (wholly ferrous) and Methemoglobin (wholly ferric)

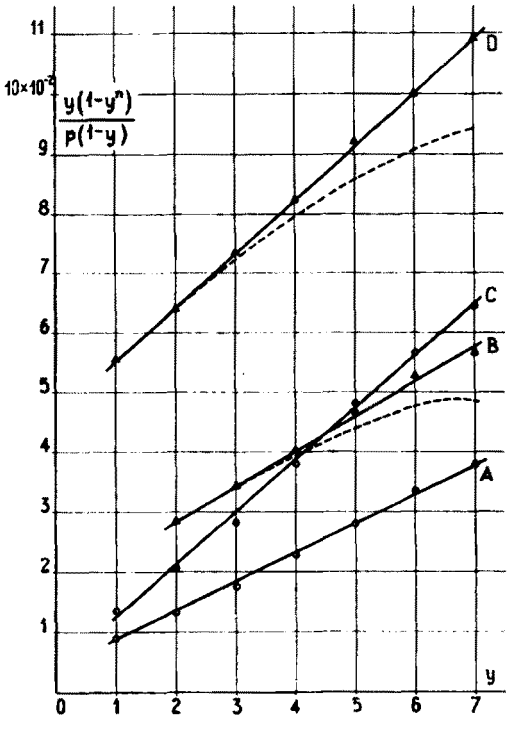
In 1942 Darling and Roughton[1] in order to explain the shift to the left and the change of the shape of the oxygen dissociation curves in mixtures of methemoglobin and ordinary hemoglobin, proposed the interesting hypothesis of the existence of intermediate compounds between hemoglobin (wholly ferrous) and methemoglobin (wholly ferric). These compounds should contain ferrous and ferric iron atoms in the same molecule. If this hypothesis is correct, the n number of ferrous atoms which are able to bind oxygen in a reversible way at varying oxygen pressures, should decrease from 4 per molecule to lower values, and the new value of n should appear from a mathematical evaluation of the experimental data by means of the stoichiometric equation which has recently been proposed by one of the writers[1]. We have submitted to this control the data from the figures 1 and 2 of Darling and Roughton's work (page 59).

We remind that the stoichiometric equation, which determines the n number of intermediate stages of the reaction of a protein P with a substance A, is the following

$$\frac{y(1-y^n)}{p(1-y)} = \frac{1}{c\,p_m} + \frac{c-1}{c\,p_m}\,y$$

where y is the degree of saturation of P with A (from zero to unity); p is the active concentration of A (in the present case the oxygen pressure is used, being a proportional magnitude) and c and p_m are constants whose meanings will be found in the original article.

The following tracings are reproduced in figure 1, worked out, as already explained, on the data of Darling and Roughton: A, for $n = 4$ from the oxygen dissociation curve of an ox hemoglobin solution in the



absence of methemoglobin; B, for $n = 4$ (continuous line) and $n = 3$ (dotted line) of the same solution in the presence of methemoglobin amounting to 60 % of the total pigment; C, for $n = 4$ of a human hemoglobin solution; D, for $n = 4$ (continuous line) and $n = 3$ (dotted line) of the same solution in the presence of methemoglobin amounting to 54 % of the total pigment.

[1] R. C. Darling and F. J. W. Roughton, Amer. J. Physiol. *137*, 56 (1942).

[1] E. Boeri, Enzymol. *12*, nr 2 (1947).

The concavity downwards of the curves for $n = 3$ means that $n > 3$, and the straight line for $n = 4$ means that $n = 4$.

The present considerations do not support therefore the hypothesis of DARLING and ROUGHTON and point out that in this case (hemoglobin plus methemoglobin by ferricyanide) only wholly ferrous and wholly ferric molecules are present in the solution.

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Physiological and Biochemical Laboratories, University of Naples, January 25, 1947.

Zusammenfassung

Experimentelle Befunde von DARLING und ROUGHTON wurden mathematisch mittels der stöchiometrischen Gleichung analysiert. Entgegen der Meinung jener Versuche sind der vorgenannten Gleichung nach in einer Lösung von Hämoglobin plus Methämoglobin (durch Ferrizyanid) nur Moleküle anwesend, deren vier Eisenatome alle in dem Ferro- bzw. in dem Ferrizustand sind.

Beeinflussung der Antennendifferenzierung durch Colchicin bei der *Drosophila*mutante *Aristopedia*[1]

Nachdem nähere Untersuchungen (VOGT[2]) ergeben hatten, daß bei der *Drosophila*mutante *Aristopedia* (ss^a) die Fühler-Imaginalscheibe während des letzten Larvenstadiums lediglich ein im Vergleich zur Wildform *verstärktes Wachstum* der *Aristenanlage* aufweist, schien die Frage von Interesse, ob seinerseits eine *künstliche* Wachstums*hemmung* der Aristenanlage von ss^a die Entwicklung einer Arista anstelle eines Antennenfußes zur Folge haben würde. Eine solche Möglichkeit erschien um so wahrscheinlicher, als bei der gleichzeitig zu Antennenmehrbildungen führenden Genkombination *scute-echinus-cut / Deformed-recessive-Lüers* (*sc ec ct / Dfd^r-Lss^a*) sowie der Genkombination *Antennaless / Aristopedia* (*Ant / ss^a*) wiederholt das Auftreten einer kleinen Arista in allen den Fällen beobachtet wurde, in denen sich das (im ersteren Fall überzählige) dritte Antennenglied durch eine besondere *Kleinheit* auszeichnete (Fig. 1 und 2).

Zur Erzielung einer künstlichen Wachstumshemmung der Aristenanlage wurden Augen-Antennen-Imaginalscheiben von 44–48 Stunden alten *Aristopedia*larven für die Dauer von 30 Minuten in Colchicinlösungen verschiedener Konzentrationen übergeführt und anschließend in die Leibeshöhle gleich alter Larven verpflanzt. Tabelle I bringt die Ergebnisse. Während alle Imaginalscheiben, die 30 Minuten in 0,75% Kochsalzlösung (Kontrollserie) bzw. in unwirksamen Colchicinkonzentrationen (an der Ausbildung eines normal großen Auges erkennbar) gehalten worden waren, fast ausnahmslos zwei ausgebildete Krallen (Fig. 3*a*) oder zumindest deutliche Tarsalglieder ohne Anzeichen einer Arista aufwiesen, zeigten die Fälle, in denen die Toxizität der Colchicinlösung nicht zu einer völligen Degeneration des Implantats geführt hatte, alle Übergänge zwischen einer eben angedeuteten Aristenspitze (Fig. 3*b*) und

Tabelle I

Einfluß verschiedener Colchicinkonzentrationen auf die Differenzierung der ss^a-Antennenanlage

Konzentration der angewandten Colchicinlösung	Differenzierung der Implantate						
	Krallen	Tarsalglieder ohne Krallen	Aristenspitze	kurze Arista	lange Arista	Degeneration	n
$1:10^4$						11	11
$1:2\cdot10^4$						7	7
$1:5\cdot10^4$	1			6	1	36	44
$1:8\cdot10^4$				2		30	32
$1:10^5$			2	1	1	34	38
$1:2\cdot10^5$	5	5				10	20
Kontrollserie	83	6	—	—	—	—	89

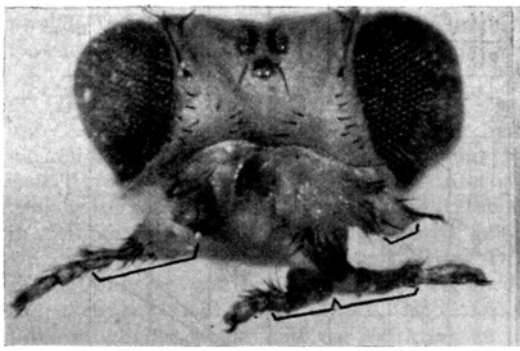


Fig. 1.

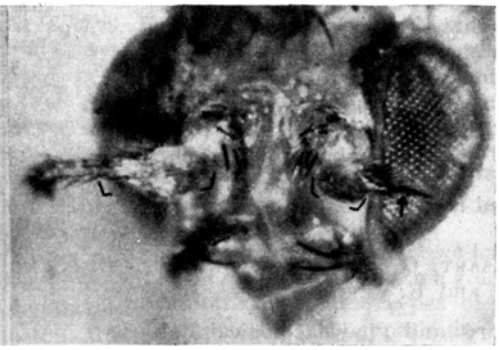
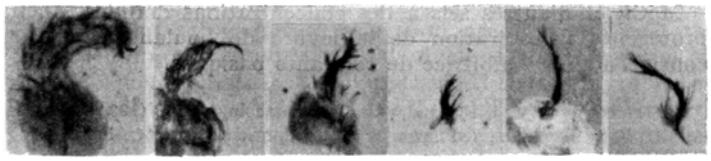


Fig. 2.

Fig. 1. Kopf einer *sc ec ct / Dfd^r-Lss^a*-Fliege. Pfeil: Arista. Klammern: Grenzen des dritten Antennengliedes. 63/1.

Fig. 2. Kopf einer *Ant / ss^a*-Fliege. Zeichen, siehe Fig. 1. 63/1.



a *b* *c* *d* *e* *f*

Fig. 3. Antennenimplantate der Mutante ss^a, in *a* aus der Kontrollserie: Krallenbildung, in *b*–*f* nach Colchicinbehandlung: verschiedene Stufen einer Aristenbildung. 86/1.

[1] Die *Drosophila*mutante *Aristopedia* unterscheidet sich von der Wildform durch die Umwandlung der Fühlergeißel (= Arista) in einen Fuß.

[2] M. VOGT, Exper. *2*, 313 (1946); Biol. Zbl. (im Druck).