In another series of experiments we were able to show: (a) that the same laws as those stated above apply also to the taking up of other basic dyes by dried and by living yeast cells; (b) that cations have the same action upon the taking up of acridines by other basophilic substances, such as mucines.

It is to be remembered here that BANK and BUNGENBERG DE JONG[1] showed that the metachromasy of chondroitine sulfate and other negative colloids disappears or diminishes in the presence of cations and admit the existence of electro-adsorption complexes.

From our experiments it follows that basophily is due to the formation of adsorption complexes such as:

$$(BS^{x-})\ (xD^{+})$$

in which BS represents the basophilic substance and D the dye.

In the light of our researches and taking into account the well-known hypothesis that ribonucleoproteids are necessary for synthesis of proteins (BRACHET-CASPERSSON), it is logical to admit that basic dyes inhibit the growth of micro-organisms because they take the place of a necessary cation in the electro-adsorption complexes existing in the cell between the nucleoproteids and physiological cations. From their experiments, ALBERT *et al.*[2] conclude that the toxic action of acridines can be explained by a competition between acridine ions and hydrogen ions. Our researches indicate that this competition takes place in an adsorption complex; we can represent this competition as follows

$$(NP^{x-})\ (xH^{+}).$$
$$\uparrow$$
$$acri^{+}$$

The hypothesis that antibiotics optimally active in an alkaline medium (e. g. streptomycine) exert their activity through a competition between the antibiotic and hydrogen ions has been advanced[3]. We represent this competition as follows:

$$(NP^{x-})\ (xH^{+})$$
$$\uparrow$$
cation of the anti-biotic.

In a preceding paper[4] we proved that cations are able to reverse the inhibition of the respiration of baker's yeast caused by acridines and other basic dyes. We admit that this reversal is also due to competition between the dye and cations, or what is the same, the inhibition of respiration caused by basic dyes is due to the fact that they replace certain ions in a catalytically active electro-adsorption complex. Of course here we must not think of nucleoproteids, as they do not play any role in respiration, but rather of enzymes activated by a dissociable metallic ion. Here we think more especially of phosphopherases, because they are basophilic and are activated in an aspecific way by Mg-ions. They might be inactivated by a competition between the Mg-ions and the basic dyes:

$$(\text{Phosphopherase}^{x-})\ \left(\frac{x}{2}\text{Mg}^{++}\right).$$
$$\uparrow$$
$$acri^{+}$$

It is tempting to surmise that enzymes activated in an aspecific way by Mg^{++} and other bivalent ions owe their activity to the formation of electro-adsorption complexes, such as:

$$(\text{Enzyme}^{x-})\ \left(\frac{x}{2}\text{Mg}^{++}\right).$$

It is important to state that at least two enzymes, known to be activated by Mg-ions, are optimally active in an alkaline medium and are inhibited by acridines. This is the case for alkaline phosphatase[1] and for cholinesterase[2]:

$$(\text{Enzyme}^{x-})\ \left(\frac{x}{2}\text{Mg}^{++}\right).$$
$$\uparrow$$
$$acri^{+}$$

The fact that the adsorption complex containing Mg^{++} or other bivalent ions shows an enzymatic activity might be explained by a well-known hypothesis of alkaline phosphatase activity, so that Mg-ions are responsible for the binding of the substrate. The adsorption complex containing the acridine ion would be an enzymatic inactive one, as the large organic ion does not possess the same properties as the small anorganic ion[3].

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Biochemical Laboratory and Pharmacological Laboratory of the Veterinary College, University of Ghent, Mai 12, 1947.

Résumé

Les acridines forment avec les nucléoprotéides des levures, des complexes électro-adsorptifs. Le pouvoir bactéricide des acridines s'exerce par une compétition entre les ion H^{+} de ce complexe et l'ion acridine. Différents enzymes activés par des ions métalliques doivent leur activité catalytique au même type de complexes. Les acridines inhibent ces enzymes parce qu'ils déplacent le cathion métallique.

[1] R. IWATSURI and K. NANGO, Bioch. Z. *301*, 15 (1939).
[2] L. MASSART and R. DUFAIT, Enzymologia *9*, 364 (1941).
[3] Full details of our experiments will be published elsewhere. This research was aided by a grant of the Ella Sachs Plotz Foundation.

[1] O. BANK and H. G. BUNGENBERG DE JONG, Protoplasma *32*, 489 (1939).
[2] A. ALBERT, S. RUBBO, R. GOLDAERE, and Y. STONE, Brit. J. exp. Path. *26*, 160 (1945).
[3] E. CHAIN, Lecture at the University of Ghent on April 27th, 1947.
[4] L. MASSART, G. PEETERS, J. DE LEY, and R. VERCAUTEREN, Exper. *3*, 154 (1947).

Acridines and Streptomycine

In a preceding paper[1], we have shown that acridines interfere with the growth of micro-organisms because they compete with physiological cations, more especially hydrogen ions, in electro-adsorption complexes. These complexes, when saturated with hydrogen ions, can be written:

$$(NP^{x-})\ (x\,H^{+}),$$
$$\uparrow$$
$$acri^{+}$$

where NP stands for nucleoproteids.

[1] L. MASSART, G. PEETERS, J. DE LEY, R. VERCAUTEREN, and A. VAN HOUCKE, Exper. *3*, 288 (1947).

In the same paper[1], we have remarked that the hypothesis has been advanced[2] that the activity of streptomycine might be due to a competition between H^+ ions and this antibiotic.

We have now proved that this is really the case, and that this competition takes place in an electro-adsorption complex of the type already mentioned.

In fact, it is very easy to show, by methods similar to those we have already described, that in dead and in living yeast cells a competition takes place for the same linkage between acridines and streptomycine, when those two substances are added together to yeast cells.

1st method: On a series of watch glasses we put 0,1 ml of a 1% suspension of baker's yeast, dry it below 70° C, fix it with alcohol and dry it again; then we stain during 10 minutes with trypaflavine 10^{-3} M or with trypaflavine 10^{-3} M containing definite concentrations of streptomycine; we wash with alcohol until the washing fluid is colorless- then we wash the acridine out with HCl (normal solution) and determine by colorimetry (Stufenphotometer).

Here follow the results of such an experiment:

Table I

Concentration of Trypaflavine	Concentration of Streptomycine	Colorimeter Reading
10^{-3} M	0	0·26
10^{-3} M	$\frac{1}{100}$	0·05
10^{-3} M	$\frac{1}{1{,}000}$	0·10
10^{-3} M	$\frac{1}{10{,}000}$	0·125

In a concentration of 10^{-3} M trypaflavine and 10^{-4} streptomycine, the trypaflavine has been expelled from the electro-adsorption complex to the extent of 50%.

2nd method: In a series of centrifuge tubes we put 8 ml of fluid composed of 2 ml of a 3% suspension of baker's yeast and 6 ml containing known amounts of trypaflavine and streptomycine; then we centrifuge. The color of the *centrifuged yeast cells* gives a first qualitative idea of the competition between trypaflavine and streptomycine. The supernatant fluid is examined in the stufenphotometer as to its content of trypaflavine.

Here follow the results of such an experiment. The colorimeter reading for the trypaflavine solution used (10^{-4} M) is 1·40.

We see that for a concentration of 10^{-4} M trypaflavine and 10^{-5} streptomycine, about half of the linkages are occupied with acridine.

3rd method: We proceed as in the first method, but stain all the suspensions with trypaflavine, wash with alcohol until the washing fluid is colorless, and then add to the suspensions different concentrations of streptomycine solutions. Through competition the trypaflavine is driven out.

[1] L. MASSART, G. PEETERS, J. DE LEY, R. VERCAUTEREN, and A. VAN HOUCKE, Experientia, *3*, 288 (1947).

[2] DR. E. CHAIN, Lecture at the University of Ghent on April 24th, 1947.

Table II

Concentration of		
Trypaflavine	Streptomycine	Colorimeter Reading
10^{-4} M	0	1·22
10^{-4} M	$1 \cdot 10^{-2}$	1·40
10^{-4} M	$1 \cdot 10^{-3}$	1·40
10^{-4} M	$1 \cdot 10^{-4}$	1·355
10^{-4} M	$1 \cdot 10^{-5}$	1·295
10^{-4} M	$1 \cdot 10^{-6}$	1·225

All our results indicate that acridines (and basic dyes in general) interfere with the metabolism of the nucleoproteids. They do this by displacing physiologically active ions, especially hydrogen ions, from an electro-adsorption complex. So does streptomycine. This explains why acridines, which are strong bases, and streptomycine all are more active inhibitors when the hydrogen ion concentration is decreased Streptomycine and basic dyes have a common effect, but of course it is not impossible that they have still a specific action as well.

During the course of our experiments we had the good fortune to obtain a copy of the paper of COHEN[1,2]. This author has found that streptomycine combines with nucleic acids to produce polymeric compounds. He suggests that the size of these compounds depends on the combining ratios of the bivalent base (streptomycine contains the diguanido base streptidine) to multivalent nucleates. He concludes that it is not unlikely that the *in vitro* reactivity of streptomycine with nucleic acid is related to the *in vivo* activity of this antibiotic.

Our experiments were performed with a $^1/_{10}$ solution of streptomycine Merck put kindly at our disposal by Prof. REGNIER, Director of the Clinic for Internal Medicine of the University of Ghent.

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L. MASSART, G. PEETERS, and A. VAN HOUCKE

Biochemical Laboratory and Pharmacological Laboratory of the Veterinary College, University of Ghent, May 17, 1947.

Résumé

La streptomycine et les acridines ont une propriété commune, en ce qu'ils forment avec les nucléoprotéides des levures de complexes électro-adsorptifs.

[1] S. S. COHEN, J. biol. Chem. *166*, 393 (1946).

[2] We thank DR. H. VELDSTRA (Amsterdam) for sending us a copy of this interesting paper.

Action *in vitro* de la pénicilline sur la virulence du Tréponème pâle

Le problème n'a été que peu étudié jusqu'ici. Pour DUNHAM et RAKE[1], qui travaillèrent avec la souche Nichols, la pénicilline n'est virulicide *in vitro* qu'à partir de 1 100 unités par centimètre cube. Nos premières re-

[1] W. DUNHAM et G. RAKE, Am. J. Syph., Gon. a. ven. Dis. *29*, 214 (1945).