Pregnant cases

month	IInd–IIId month		
1. IInd	4·92	6·88	1·96
2. IInd	4·05	5·68	1·63
3. IIId	4·12	6·92	2·80
	IIId–IXth month		
4. IIId	6·90	7·77	0·87
5. IIId	5·45	5·45	0
6. IVth	5·12	5·95	0·77
7. Vth	3·44	3·44	0
8. Vth	5·06	5·06	0
9. VIth	6·20	7·87	1·67
10. VIth	7·06	7·51	0·45
11. VIth	7·21	6·45	−0·76
12. VIIth	4·69	5·45	0·76
13. VIIth	4·94	5·86	0·76
14. VIIth	5·06	5·57	0·51
15. VIIth	5·45	6·32	0·83
16. VIIth	7·84	7·97	0·13
17. VIIIth	1·40	2·16	0·76
18. VIIIth	4·78	4·78	0
19. IXth	4·10	4·10	0
20. IXth	6·95	6·95	0
21. IXth	7·72	7·20	−0·52
Average (IIId–IXth month): 0·31			

action of the fœtal pancreas contributing with its insulin production to the sugar metabolism of the mother. This explanation cannot be regarded as satisfactory. Our results speak in favour of the assumption that the secretion of diabetogenic hormone is greatly diminished in pregnancy after the third month, and that this is the cause of amelioration of diabetes in pregnancy.

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Résumé

6 heures après l'absorption de graisse et d'albumine, on constate dans le sang une augmentation de la concentration des substances qui fixent le bisulfite. Cette augmentation, due à des combinaisons cétoniques, ne se produit pas pendant la grossesse à partir du 3me ou du 4me mois. Elle fait aussi défaut, dans certains cas, pendant la lactation. Du moment que l'augmentation de la teneur du sang en bisulfites ne se produit pas non plus lors d'une déficience du lobe antérieur de l'hypophyse, il semble que l'on en peut conclure que durant la grossesse la sécrétion de l'hormone diabétogénique du lobe antérieur est fortement diminuée ou que l'action de celle-ci est enrayée.

The Mechanism of the Biochemical Activity of Acridines

In two preceding papers[1] we have shown: (1) that acridines (and basic dyes in general) inhibit the respiration and the growth of baker's yeast; (2) that the inhibition of respiration can be reversed not only by means of nucleic acid and of nucleotides, but also by means of salts. This paper offers an explanation of the above stated facts.

In first instance we have now proved that acridines are bound by the nucleoproteids of the yeast cell; indeed, we were able to show that dried and alcohol-fixed yeast cells are no longer colored by acridines after treatment with cristalline ribonuclease.

The binding of acridines (and other basic dyes) is based upon the formation of an electro-adsorption complex between the negatively charged nucleoproteids and the acridine ions. We can represent such complexes as follows:

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

That such complexes are really built can be shown readily by desorption experiments by means of different cations, such as H^+, Na^+, Ng^{++}, Al^{+++}. In our experiments we proceeded in three different ways:

(1) On a series of watch glasses we put 0,1 ml of a 1% suspension of baker's yeast; we dry it at a temperature below 70° C, fix it during 10 minutes by means of alcohol and stain with acridines (trypaflavine); then we wash with alcohol until the washing fluid is colorless. The preparations thus stained lose their color partially or completely when treated with different salt solutions, in which the cation is the active agent. We have found that the power of the cations to wash out the acridines depends on their valence. The position of the hydrogen ion is, however, an outstanding one.

We admit that when Mg-ions are employed to wash out the acridines, a new complex is built such as:

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

This means that in the adsorption complexes we admit the possibility of a competition between cations, and that mixed complexes exist such as:

$$(NP^{x-})\ \left(\begin{matrix}H^+\\ Mg^{++}\\ Al^{+++}\\ A\end{matrix}\right)$$

x positive charges.

It is to be recalled that here MAC CALLA[1] proved the existence of a competition between different cations in complexes formed between negatively charged bacterial cells and cations.

(2) We have stained dried and alcohol-fixed yeast cells on the one side with acridines (or other basic dyes) and on the other with acridine solutions containing different concentrations of different cations (H^+, Na^+, Mg^{++}, Al^{+++}); we have then measured the amount of acridine fixed in the two cases. We have found that the same laws apply as in the first series of experiments; that is to say, in the presence of cations no or less acridine is bound, and the valence of the cation (except for H^+) is the determining factor.

(3) With living yeast cells the same facts were observed. We have treated living yeast cells with acridines in the presence and in the absence of different cations. After centrifuging the color of the supernatant fluid was determined.

[1] L. MASSART, G. PEETERS, J. DE LEY, and R. VERCAUTEREN, Exper. *3*, 119 (1947); *3*, 154 (1947).

[1] MAC CALLÁ: stated by G. B. WISLOCKI, Physiol. Prev. *26*, 3 (1946).

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

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

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:

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

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:

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

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:

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

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]:

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

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:

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

where NP stands for nucleoproteids.

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