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Membrane inhibitors of glucose transport in yeast

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In many cases has been arrived the conviction that the transport of sustances across cellular membrane was enzymatically controlled. The glucose uptake has been specially studied in this respect.

One of the methods employed to inquire if one transport is enzymatically controlled consists in studying the effect of inhibitors. The diffficulty is in the little specificity of a inhibitor and specially in differentiating if it influences an endogenous process that rebounds in the penetration or if it properly inhibits an enzymatic reaction in the cell surface.

We have elaborated a method that allows the separation in yeast of the inhibition of the sugar transport across the cell membrane from all other actions of the inhibitor upon metabolism.

Our method is founded on the comparison of the effect of one inhibitor upon the sugar uptake with the effect of the same inhibitor upon the endogenous alcoholic fermentation.

The spontaneous endogenous alcoholic fermentation in yeast is extraordinarily little and practically non-measurable. An endogenous alcoholic fermentation in yeast was induced through the addition of appropriate amounts of azide or dinitrophenol (ROTHSTEIN and BERKE[1]). We have induced one well measurable

(1) ROTHSTEIN, A., and H. BERKE, 1952: *Proc. Soc. Exptl. Biol. Med.*; 81, 559, 63.

* Con una beca del Patronato Juan de la Cierva.

endogenous fermentation without effectors by previous incubation of the cells in glucose solution. The CO_2 production was measured by means of the manometric technique of Warburg (Table I).

TABLE I

μl. of CO_2 produced in nitrogen atmosphere in 30 min. at 30° C (averages). 4 ml. of suspended cells in H_2O. Washed centrifuged yeast. Yeast concentration, 0,03 ml/ml. pH, 3-4.

Non-incubation	After 60 min. incubation in glucose 5 %	
	anaerobic incubation	aerobic incubation
8	—	41
9	—	41
10	—	40
8	—	38
—	29	34

Several authors have arrived at the conclusion that uranyl inhibits glucose penetration in yeast through an exclusive action upon cell surface[2,3]. Applying the uranyl nitrate to the induced endogenous fermentation by incubation it was found that it was totally non-efective up to a concentration as great as 10^{-3} M., while as it is known the sugar uptake is strongly inhibited from as little as 10^{-6} M. concentrations.

On the other hand the sodium fluoride is a well known inhibitor of fermentation that easily penetrates into the cell, and truly at a concentration of 10^{-2} M. inhibits 81 % of the induced endogenous fermentation.

The induced fermentation increases with the length of previous incubation, it is not affected by the uranyl and it is very sensible to fluoride. This allows one to assert that it is really an endogenous alcoholic fermentation.

(2) BARRON, E. S., MUNTZ, J. A., and GASVODA, B., 1948 : *J. Gen. Physiol.*; **33**, 163, 78.

(3) ROTHSTEIN, A., and LARRABEE, 1948 : *J. Cell. and Comp. Physiol.*; **32**, 247, 61.

Having obtained an endogenous alcoholic fermentation in yeast without using effectors, then we were in conditions to applying the discrimination criterion for the action site of a determined inhibitor. As can be seen in fig. 1, in anaerobic conditions, a sugar uptake inhibitor can act upon the glucose transport across cell membrane or upon glucose metabolic utilization into the cell or in both simultaneously. If the inhibitor

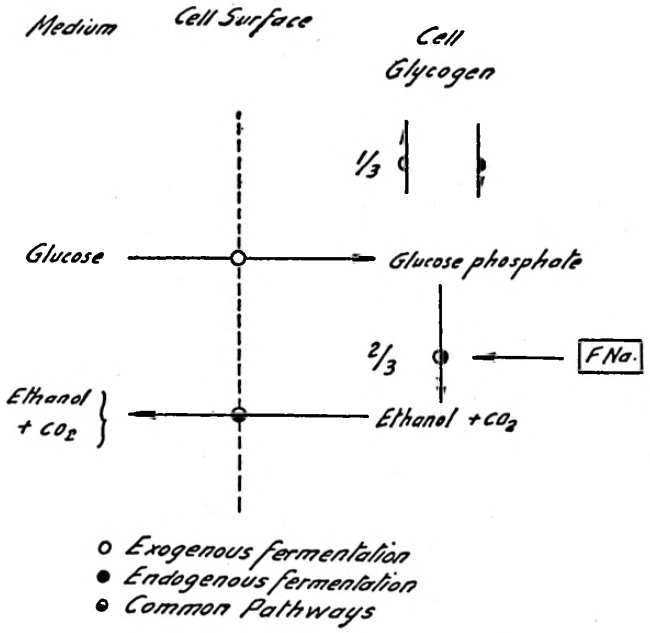


Fig. 1

only acts upon the glucose transport, it will only inhibit the sugar uptake. If the sugar uptake is affected by inhibitor concentrations smaller than those that affect the endogenous fermentation, the method reveals that inhibitors act a these concentrations exclusively upon the cell surface and that in general exercises a selective but not exclusive inhibition of the glucose transport. It has always been operated in anaerobic conditions to reduce to a minimum the metabolic pathways that could give place to non-glucidic CO_2. Also it has been performed at pH between 3 and 4 to avoid that the glucose utilization could produce other sustances different from ethanol and CO_2.

The selective membrane inhibition is expressed by the

greatest inhibition of the sugar uptake obtained with the highest inhibitor concentration that does not affect the endogenous fermentation. This value is referred to percentages of the maximum degree, of the sugar uptake inhibition, that could possibly be gotten by the said inhibitor.

In the table II is represented the application of this criterion to three substances : Uranyl nitrate, Sodium fluoride and the Di-isobutyl-phenoxy-ethoxy-ethyl-dimethyl-benzil-ammonium

TABLE II

Selective membrane inhibition

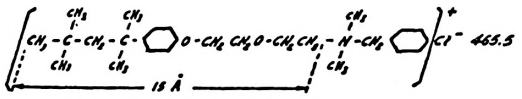
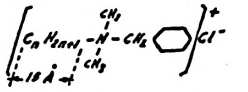
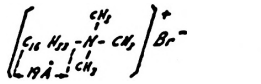
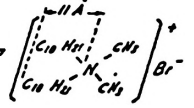
Inhibitor	Maximal inhibition of sugar uptake	Maximal inhibition without effect on endogenous fermentation	Selective membrane inhibition
Sodium fluoride	100	0	0
Di-isobutyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl ammonium chloride (Compound I)	100	82	82
Uranyl Nitrate	86	86	100

chloride (compound I), a cationic germicide detergent. Sodium fluoride does not inhibit sugar uptake at concentrations lower than that concentration in which the endogenous fermentation is inhibited ; consequently it does not show selective membrane inhibition. Uranyl nitrate produces the greatest inhibition of the sugar uptake at concentrations that do not affect the endogenous fermentations : It presents a 100 % selective membrane inhibition. Ammonium quaternary compound I inhibits up to 82 % the sugar uptake without affecting endogenous fermentation, yet at greater concentrations it arrives to inhibit up to 100 % the sugar uptake. Its selective membrane inhibition is 82 %.

We have studied by this method the action upon the glucose transport in yeast of four ammonium quaternary compounds (table III).

TABLE III

Tested Ammonium Quaternary Compounds

Compound	Chemical Name	Formula	W.M.
I	Diisobutyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl-ammonium chloride		465.5
II	Alkyl-dimethyl-benzyl-ammonium chloride		353.5
III	Cetyl-trimethyl-ammonium-bromide		364.
IV	Didecyl-dimethyl-ammonium bromide		406.

The relationship between yeast concentration and sugar uptake

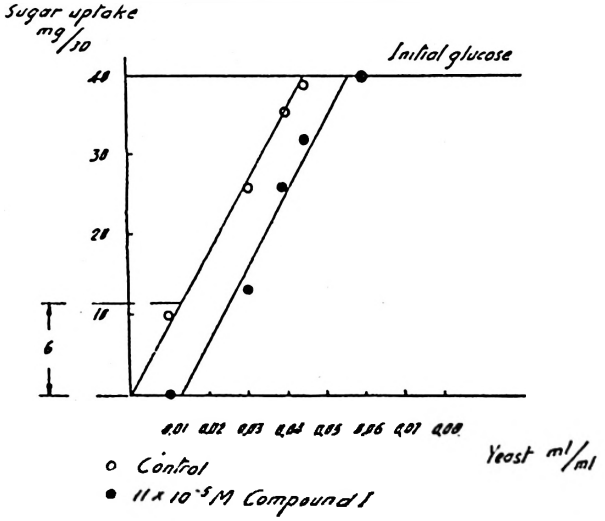


Fig. 2

The relationships of glucose concentration and sugar uptake

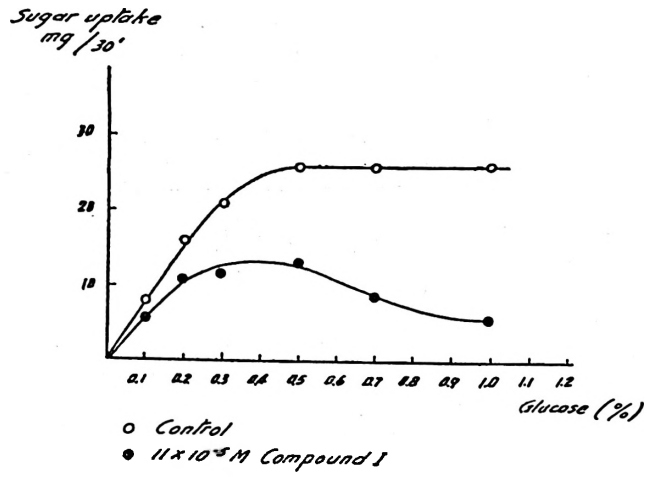


Fig. 3

Kinetics of inhibition of sugar uptake

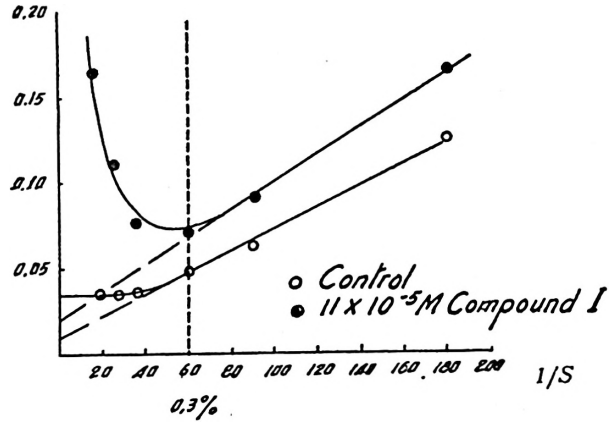


Fig. 4

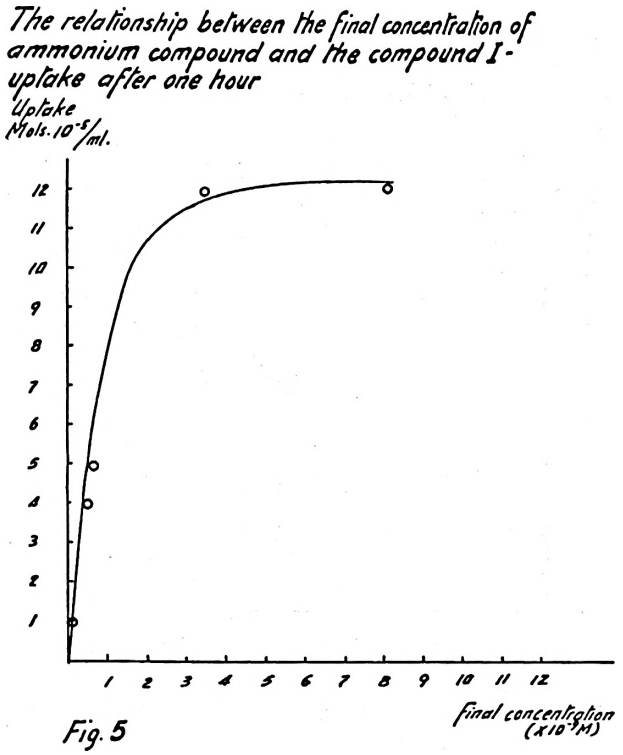


Fig. 5

Previously was studied the relationship betwen cellular concentration and sugar uptake inhibition, for compound I. In presence of a constant amount of inhibitor (fig. 2), the anaerobic sugar uptake continues to be sensibly proportional to cell concentration in the same manner as without inhibitor. Consequently, the inhibitions result inversely proportional to the cellular concentrations. These relationships only hold for short time experiences in wich the cell sugar uptake comes to be worthless in comparison to the total amount of sugar in the medium.

In fig. 3 is represented the relationships between sugar concentration and glucose uptake. There is found a maximum absorption rate for low concentrations of glucose without inhibitor. There is also a maximum take-up for this same level of sugar concentration in presence of a determined amount of inhibitor. Increasing the amount of glucose diminishes the take-up rate in this last case.

Compound I behaves like a non-competitive inhibitor up to

The relationship the final concentration of ammonium compound and the Compound I-uptake after one hour.

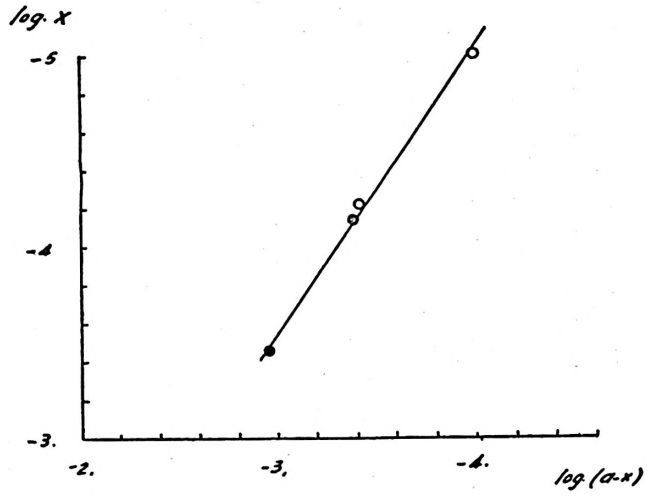


Fig. 6

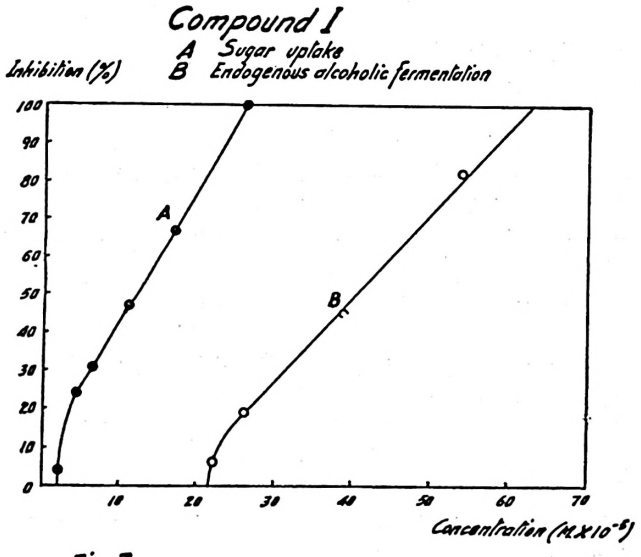


Fig. 7

the saturation level in accordance to the Michaelis-Menten theory. (Fig. 4).

In fig. 5 is pictured the uptake of compound I by yeast. The data represent experiments at yeast concentrations of 9 and 20 ml/liter. There exists a saturation level for initial concentrations of inhibitor relatively high. The general form of the curve shows the existence of an equilibrium between cellular concentration, inhibitor concentration in the medium and inhibitor uptake by the cells. This equilibrium can be described

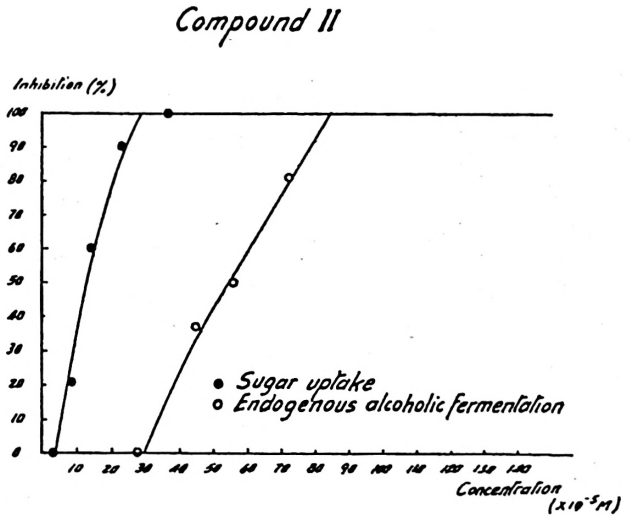


Fig. 8

by the Freundlich-Langmuir isotherms. Certainly, with logarithmic co-ordinates is obtained a straightening of the obtained data (fig. 6). This result suggests that below the saturation level, the amount of inhibitor that penetrates in the cell must be very little in comparison to that fixed in the cell membrane.

Compound I (fig. 7) presents a clear selective membrane inhibition and it is the one of the greatest absolute activity, as much for the sugar uptake inhibition as for the endogenous alcoholic fermentation inhibition.

Compound II (fig. 8) is the one of the greatest selective membrane inhibition, and its absolute activity is a little inferior to compound I.

Compound III (fig. 9) presents a low membrane selective inhibition. The inhibition curves of the sugar uptake and the

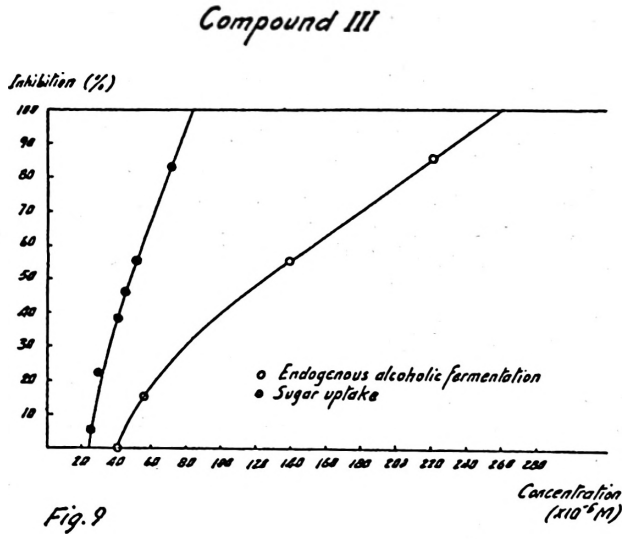


Fig. 9

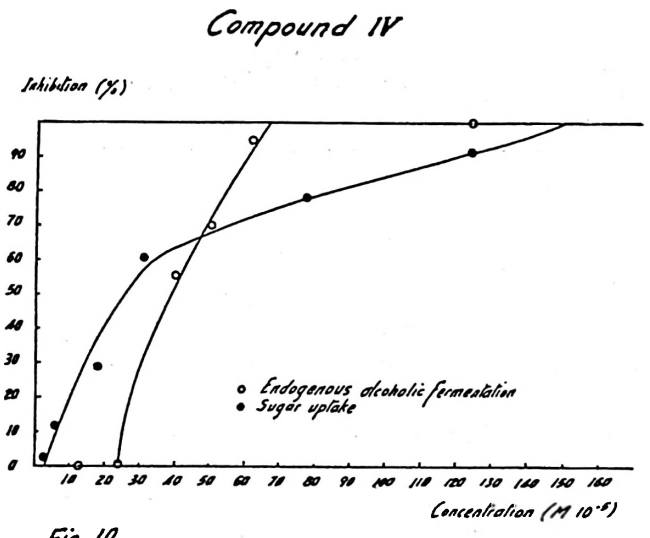


Fig. 10

endogenous fermentation are here between themselves more different than in the two former compounds It looks as if the innermost inhibition mechanism must be different in the endogenous and exogenous metabolism.

Compound IV (fig. 10) has an action upon anaerobic endo-

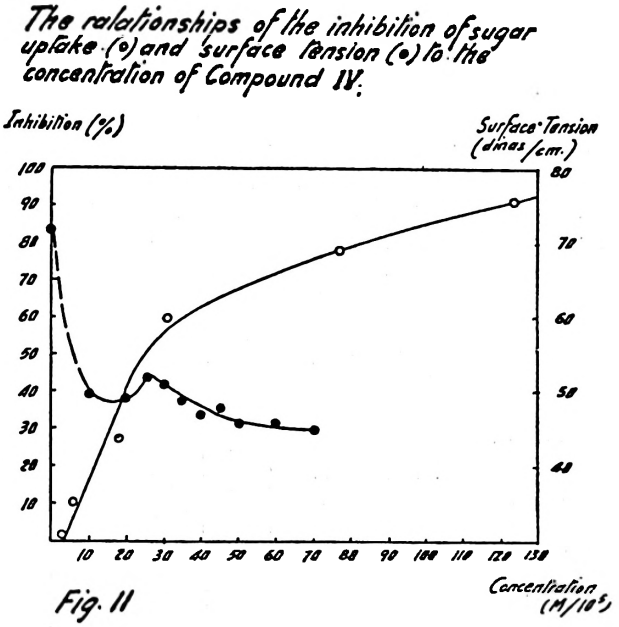


Fig. 11

genous metabolism very similar to those of compounds I and II. It possesses a selective membrane inhibition rather low. Referring to the sugar uptake inhibition, it presents two very interesting anomalies. Firstly it allows the yeast cells to continue some exterior sugar uptake in concentrations that completely cut out endogenous fermentation. It is suggested that this behaviour is the consequence that compound IV. shows a selective action upon glycogenolysis. On the other hand the inhibition curve shows in this case a very remarkable inflexion for concentrations of the order that begin to affect endogenous metabolism. Just at this inflexion level also exists another bending on the surface tension and inhibitor concentration diagram (fig. 11). It is suggested that at this concentration there takes place a micelles' formation and for it a different adsorption isotherm must be fulfilled than that corresponding to free molecules of the inhibitor.

In table IV are pointed out the values of the selective membrane inhibition and absolute activity of the four studied compounds. It can be appreciated that their surface activity has relationship neither with the selective membrane inhibition, nor with the absolute activity upon the sugar uptake, nor even with the endogenous fermentation inhibition. Strictly speaking

the values referred of the germicide power can not be compared with those of the selective membrane inhibition ; however, on the whole, there is likely a relationship between them.

The selective membrane inhibition looks to be very much influenced by the peculiarities of the molecular structure. The more active compounds (I and II) are the only ones that have the benzyl group and a lateral chain of the same length, approximately 15 A. For the moment it has not been possible to find out if for the selective membrane inhibition what it is

TABLE IV

Comp.	Surface Tension 0,002 M. 19°C dynes/cm.	Phenol Coefficient S. aureus 20°C.	Selective membrane inhibition	Sugar uptake 100 % inhi.	Endogenous Fermentation 100 % inhi.
I	46.5	323 (Lawrence)	82	$26 . 10^{-5}$ M	$63 . 10^{-5}$ M
II	45.5	250 (Lawrence)	98	$29 . 10^{-5}$ M	$85 . 10^{-5}$ M
III	51.1	300 (Hoogerheide)	38	$83 . 10^{-5}$ M	$258 . 10^{-5}$ M
IV	39.5	330 (Ressuggan)	48	$150 . 10^{-5}$ M	$67 . 10^{-5}$ M

really important is the benzyl group of the lateral chain. It is interesting to recall that RAWLINS, SWSST and JOSYLN[4] too, pointed out the importance of the benzyl group for the germicide power and that BAKER, HARRISON and MILLER[5] obtained a greater activity of the ammonium quaternary compounds with benzyl in the respiration and fermentation inhibition of glucose in some microorganisms.

RAWLINS, SWEET and JOSLYN[4] also pointed out that exists a critical length of the alkylic chain above and below which diminishes the germicide power. The obtained results oblige us to believe that the ammonium quaternary compounds are non-specific enzymatic inhibitors. The data of several authors about the action of those compounds upon free enzymes are in accordance with our conclusion.

It is true that what makes these compounds inhibitors, is probably the general character of its molecule, but the inten-

(4) RAWLINS, A. L., SWEET, L. A., and JOSLYN, D. A., 1943 : *Jour. of the Amer. Pharmaceutical Assoc., Scient E.;* **32**, 11, 16.

(5) MILLER, B. F., BAKER, Z., and HARRISON, R. W., 1939 : *Proc. Soc. Exptl. Biol. Med.;* **42**, 705, 706.

sity of their action is really influenced by small variations of its molecular structure.

Its selective inhibition upon the glucose transport across the cell surface is a consequence of these compounds being strongly fixed upon the membrane.