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Endogenous fermentation in intact yeast cells

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The cells of fresh baker's yeast *(S. cerevisiae)* usually have a glycogen content which varies between 7 and 12 %. Under anaerobic conditions, (a suspension of these cells in distilled water, 15 mg/ml., dry weight) they present a very reduced capacity of utilizing this glycogen. During the first hour, the polysaccharide which can be hydrolized (1) and the glycogen which can be titrated by the anthrone method decrease by some 60 mg/100 g yeast. Simultaneously there takes place a very small liberation of CO_2, an average of 15 to 20 ml/h/100 g of yeast (table I). Although various authors (1,2,3,4) have considered this fermentation as negligible, a simple calculation allows the apreciation that it justifies the decrease in glycogen observed, without the necessity of searching for other final products, for their anaerobic utilization, other than CO_2 and ethanol.

TABLE I

CO_2 (μl)produced in anaerobiosis by 60 mg (wet weight) of baker's yeast in 1 h. (30° C, pH 3,5)

Samples	I	II	III	IV
	7	7	10	8
	7	11	9	7
	9	9	11	10
Mean	8	9	10	8

CO_2 Average Production, 16 ml/h/100 g.

It is a known fact that both during the alcoholic fermentation and in the aerobic utilization of glucose there takes place an increase in the polysaccharide reserves of the yeast. Nevertheless, we have observed that the cells freshly fed by aerobic incubation in glucose (10 % glucose, 1h.) obtain the capacity of subsequently utilizing their carbohydrate reserves at a rate 30 times greater than normal (1900 mg/h./100 g) (Table II). In this case there takes place a much greater liberation of CO_2 (average of 546 ml/h/100 g.) Here also the utilized glycogen corresponds with considerable aproximation to the produced CO_2.

TABLE II

Relation between the mobilized polyssaccharide and the CO_2 produced in endogenous fermentation

Endogenous fermentation	Polyssaccharide decrease (mg./h/100 g.)		CO^2 production (ml./h/100 g.)	
	Exper.	Calcul.	Exper	Calcul.
Spontaneous	60	56	15	16
After incubation in glucose.	1900	2047	546	507

Fig. 1.- The utilization of glycogen by baker's yeast in anaerobiosis

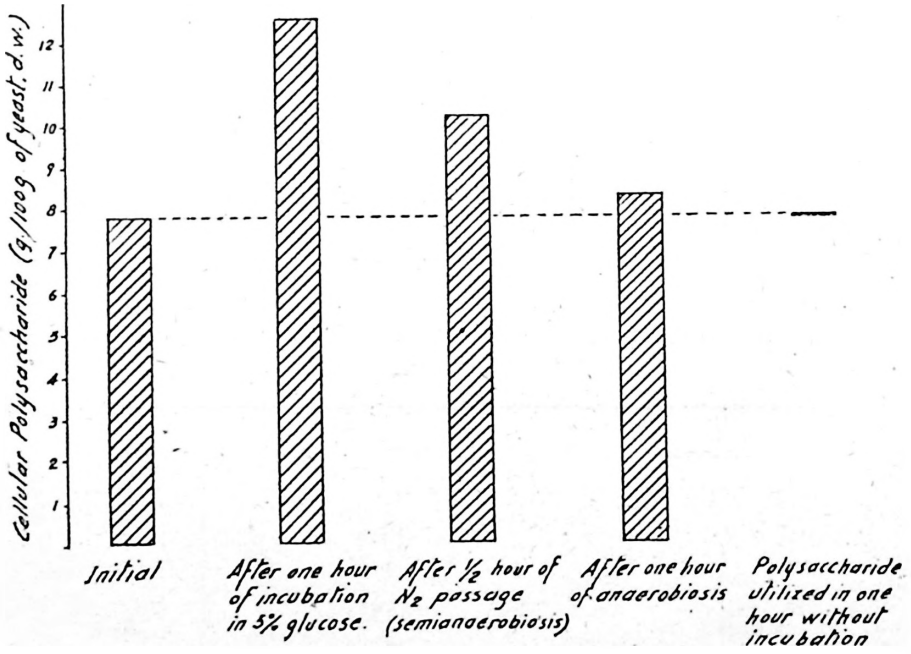
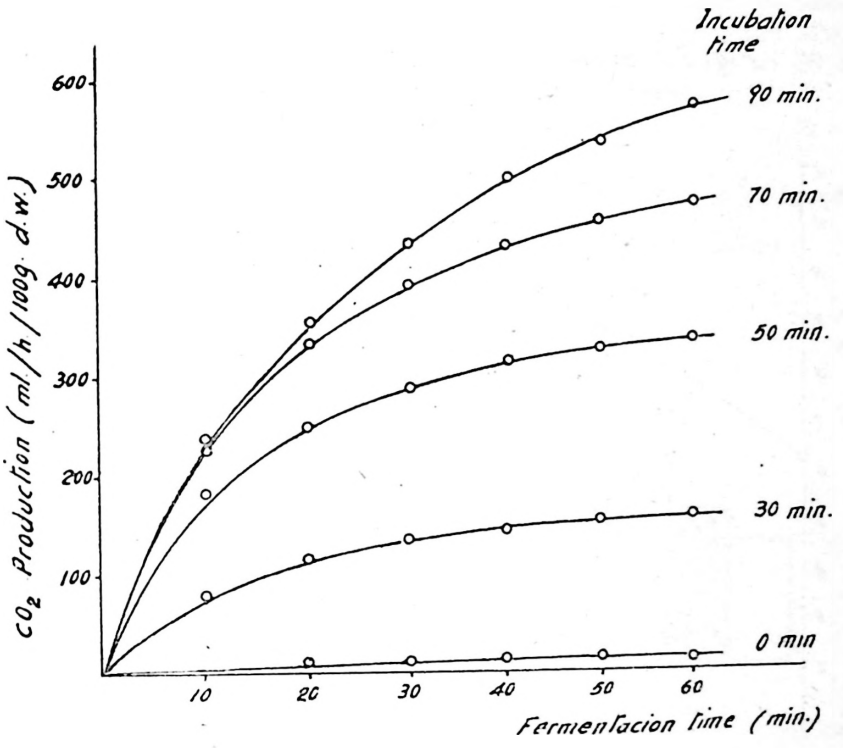


Fig. 2.- Endogenous fermentation intensity according to the incubation time.

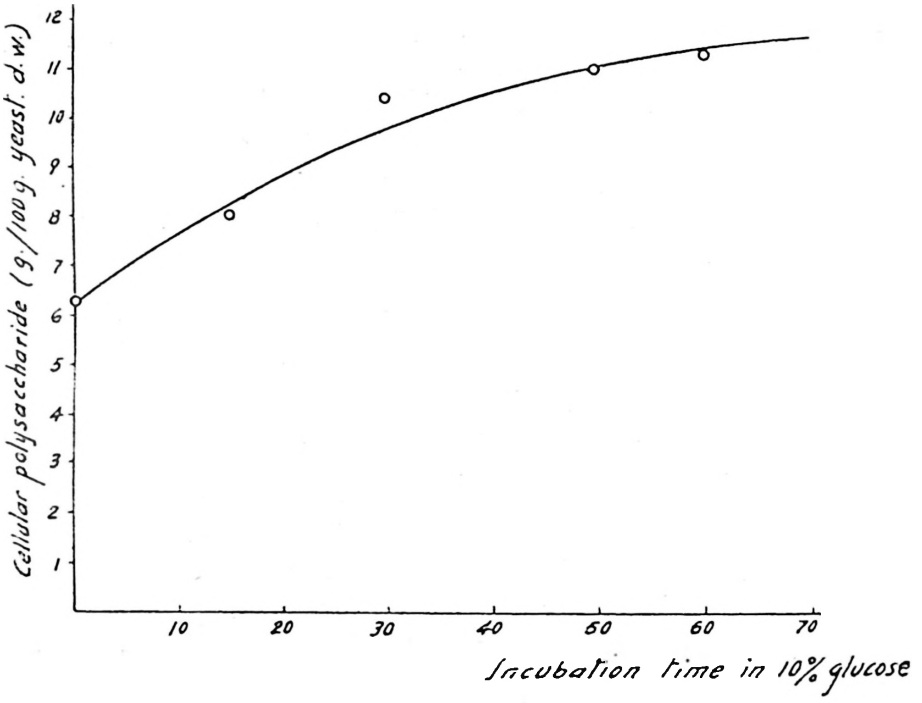


The consumed glycogen is lower than that which the cells had stored during the recent previous incubation in glucose (5 g/h/100 g) (Fig. 1). As can be observed in the series of curves on figure 2, the intensity of the endogenous metabolism increases with the time of incubation and the induced fermentation decreases rapidly reaching after the first hour an intensity similar to that of the spontaneous fermentation. The increase in the intensity of fermentation with the time of previous glucose assimilation seems to show a limit after which the initial intensity increases very little, but the rate of fermentation lasts longer.

An incubation of 15 minutes in glucose produces an increase in glycogen of 1.8 g/100 g of yeast. During the 30 minutes of nitrogen passage, this glycogen is probably reduced in half. But it is enough to increase the rate of CO_2 production from 5 to 10 times, during the next first hour. We find ourselves before the fact that an increase in glycogen of aproximately 10 to 12 % due to assimilation, considerably activates its uti-

lization, while the basal reserves are utilized without sensible intensity variations even with differences of up to 50 % (fig. 3).

Fig. 3.- Growth of the glycogen of yeast by aerobic incubation in glucose.



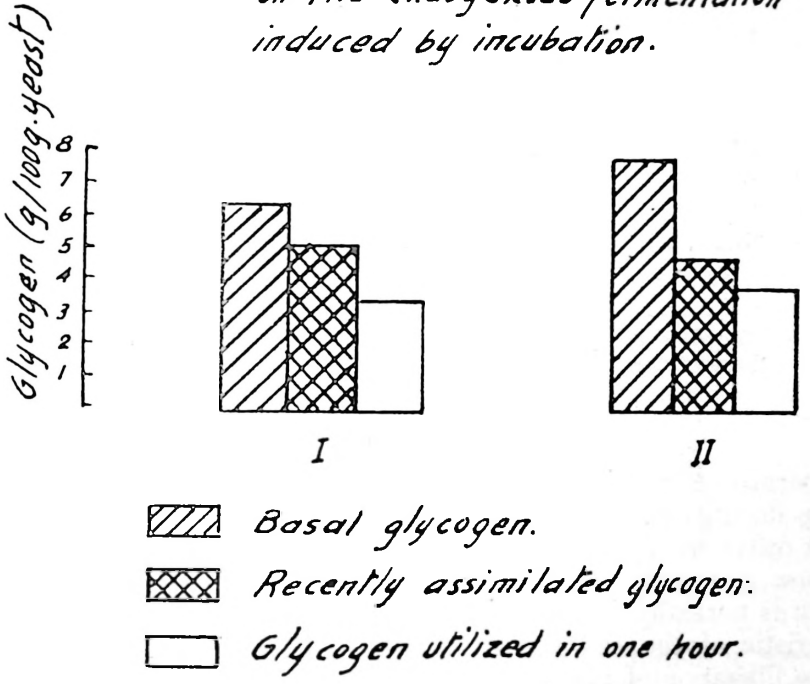
On figure 4 the relations between the basal glycogen, recently assimilated glycogen, and the glycogen fermented in one hour are expressed. This last one seems to be little influenced by the amount of basal glycogen.

As a whole, the expressed facts seem to indicate the existence of two types of carbohydrate reserves, before which the yeast cells present a deep difference in capacity of utilization.

The possibility of producing a reserve which can be easly utilized in the same manner as the exogenous alcoholic fermentation, has been used by us as a base of a method discriminating from the inhibitors which act specificaly or selectively on the penetration of glucose (5,6,7). The endogenous fermentation induced by this previous incubation of the yeast in glucose is sensitive to fluoride, as well as the proper utilization of external glucose (5,6) Both processes are also equally

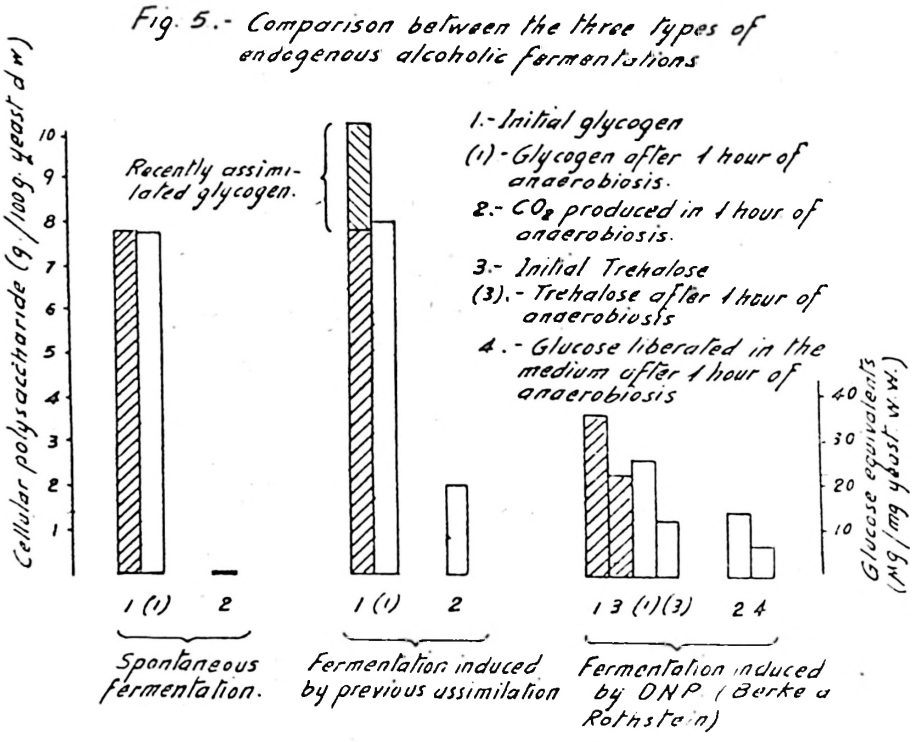
affected by the extracellular *p*H variations (8). On the other hand, the endogenous fermentation is not modified by the uranyl ion, non penetrating agent which inhibits the utilization of external glucose up to 80 %, in concentrations as small as 10^{-5} M (5,6). It is not affected either by the Mg^{++} and Mn^{++} ions which activate the exogenous fermentation up to 20 to 25 % (10^{-1} M) (9).

Fig. 4.- Influence of the basal glycogen on the endogenous fermentation induced by incubation.



If the previous incubation in glucose takes place in the presence of 2,4 dinitrophenol (DNP) 3×10^{-4} M, the subsequent endogenous fermentation does not take place. This concentration of DNP does not inhibit the exogenous fermentation. On the other hand BERKE and ROHTSTEIN (10) have observed that at these concentrations of DNP, the exogenous utilization of glucose is not followed by an increase in the carbohydrate reserves. In this manner, the assumption that during the endogenous fermentation induced by previous incubation only the recently formed reserves are utilized, is confirmed once again.

ROHTSTEIN and BERKE (11) found that apropiate amounts of DNP can increase the anaerobic endogenous metabolism of



the normal cells (up to producing some 300 ml of CO_2/h/100 g yeast during the first hour). This endogenous fermentation must differ from the induced by previous glucose assimilation because it rapidly movilizes a type of carbohydrate reserve which is normally difficult to utilize. Another differential characteristic of the endogenous fermentation induced by DNP is the liberation of glucose in the medium, which is ulteriously fermented. In the fermentation induced by previous assimilation, this liberation of glucose can not take place, because the presence of uranyl would inhibit it, giving a decrease in the global production of CO_2. As we have pointed out before, this inhibitor turns out totally inefective and the utilized glycogen and produced CO_2 are equivalent.

In accordance with the results obtained by BERKE and ROHTSTEIN (10), during the endogenous fermentation induced by DNP there takes place a decrease in trehalose of 1200 mg/h/100 g of yeast (dry weight), a decrease of 600 mg/h/100 g of yeast in total glycogen, and a liberation of glucose into the medium of 700 mg/h/100 g of yeast (Fig. 5). The production of CO_2 is aproximately equal to the one resulting from the exclusive utilization of trehalose. It seems that the utilization of endocellu-

lar glycogen is without doubt activated, but the characteristic note of the fermentation induced by DNP must consist in the movilization of trehalose.

Trehalose does not seem to play any important role in the fermentation induced by previous assimilation. By the anthrone method, used by us, it is separated in the soluble fraction in alcohol. Thus, the decrease in the carbohydrate reserves which we measure must refer exclusively to glycogen and, by the facts previously pointed out, to recently assimilated glycogen. We do not have exprimental evidence of a possible relation between the two glycogens separated by their different movilization ability and the fractions of glycogen alcali- insoluble and alcali- soluble of TREVELYAN (12). Paying attention to the fact that during the assimilation of glucose the alcali-insoluble glycogen is formed in a grater proportion and more rapidly, there exists the possibility that this type of glycogen corresponds to the one which the yeast cell can utilize with greater ease.

Thus it can be concluded :

1. The endogenous fermentation of the normal yeast cells is a very slow process in which the CO_2 produced keeps a proportion with the utilized glycogen according to a normal alcoholic way. This fermentation can be stimulated by DNP.

2. By previous incubation of the cells in glucose solution, an endogenous fermentation much greater than normal is obtained. The CO_2 produced corresponds well with the utilized glycogen. The rate of this fermentation depends principally on the glycogen which is assimilated during the incubation and becomes as slow as in normal cells when this recent glycogen is exhausted. The DNP inhibits the assimilation of glycogen during the incubation and so the subsequent fermentation does not take place.

3. The yeast cell, in basal conditions, must possess glycogen in some way different to the one with is formed during the incubation, by assimilation of external glucose. The basal glycogen is characterized by its slow spontaneous fermentation and it can be mobilized by DNP. The recently assimilated glycogen is of fast fermentation without necessity of DNP.

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