2-DEOXYGLUCOSE AS METABOLIC SUBSTRATE AND INHIBITOR OF GLYCOLYSIS IN FUNGI

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Since the discovery that 2-deoxyglucose (2DG), an unnatural glucose analogue, is a strong inhibitor both of glucose fermentation and growth in yeast (Woodward, Cramer and Hudson, 1953), considerable interest has been aroused for its use as an important tool in the study of various kinetic aspects of glycolysis in animal tissues (see Niremberg and Hogg, 1958, Landau et al., 1958 and Kipnis and Cori, 1959) and in cell free preparations (Eys and Warnock, 1959). Throughout these researches the widespread assumption that 2DG is metabolically inert except as an inhibitor is apparent. We have found in certain fungi that inhibition of glycolysis is compatible with utilization of 2DG as a metabolic substrate.

The rate of growth of Neurospora crassa in a basal mineral medium (that of Medina and Nicholas, 1957, without the tartrate) with glucose as source of carbon and energy, is decreased in the presence of a similar concentration of 2DG [*]. Nevertheless, if the amount of glucose is limiting, the addition of 2DG increases the total yield. A typical result is shown in Fig. 1 A. With fructose instead of glucose the inhibitory effect is much more pronounced, but still is accompanied by an increase in maximum

[*] Aldrich Chemical Company.

yield (Fig. 1 B). Something more than a possible sugar sparing effect is involved. N. crassa can grow on 2DG as sole source of carbon and energy (Fig. 1 C). The observed yield, although small, is quite significant since with the basal medium alone there is no detectable growth at all. At the end of the 16 days incubation time, 2DG had disappeared from the medium (glucose oxidase reagent of Sols and Fuente, 1957). Transfer to new medium resulted again in the same low rate of growth with 2DG and in normal rate with glucose and fructose.

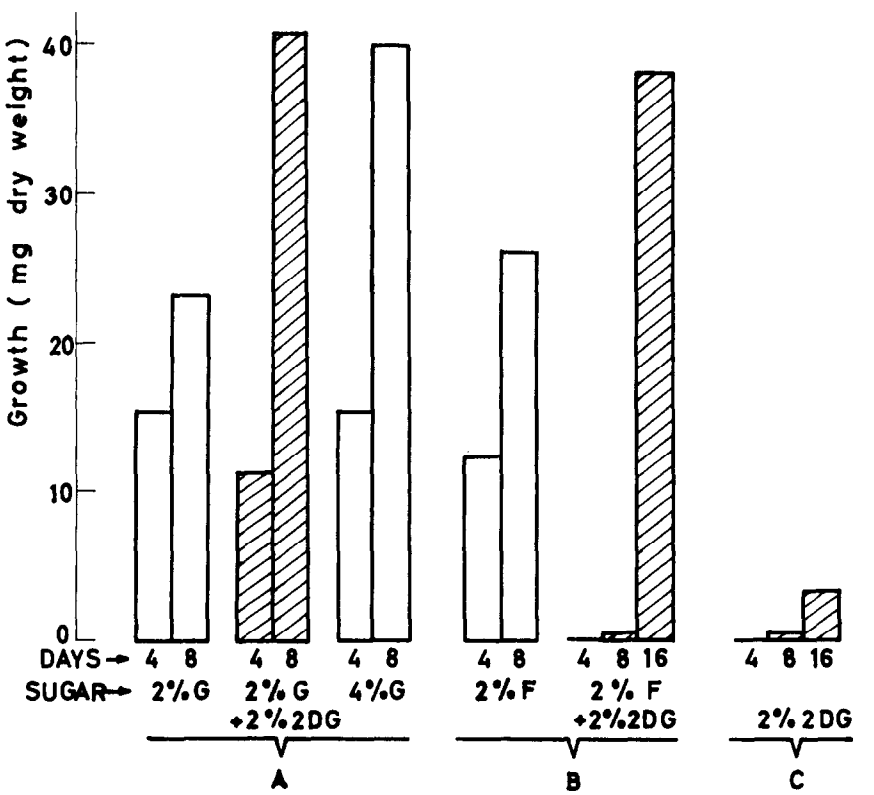

Fig. 1. 2-Deoxyglucose and Neurospora crassa.

Duplicate tubes containing 4 ml of mineral medium plus sugars and 2DG as indicated, were inoculated with a suspension of spores of N. crassa and incubated at 25º C. After intervals of time as indicated, mycelia were harvested and dried for 4 hours at 80º C. Average dry weights are plotted.

The rate of growth of Aspergillus oryzae in a basal mineral medium (Mulder, 1939) with glucose, fructose or mannose is also inhibited by 2DG, while it can grow on 2DG as sole source of carbon and energy. The rate of growth on 2DG was about 1/5 and the total yield about 1/2 of that with glucose (at an initial concentration of carbohydrate of 3.75 %). Here also it has been observed that when growth on 2DG had leveled off (24 days), 2DG had disappeared from the medium. And again the 2DG grown A. oryzae grew normally upon transfer to a glucose medium. Growth of A. oryzae on 2DG had previously been observed in a study on precursors of kojic acid formation (Barnard and Challenger, 1949).

Fungi tend to be rather omnivorous. This is the case of A. oryzae (Tamiya, 1932) and N. crassa (Heredia and Medina, 1959). There might be many others able to metabolize 2DG.

Typical hexokinases readily phosphorylate 2DG. This is the case of the constitutive hexokinase of A. oryzae (Ruiz-Amil, 1959). The same has been found to occur in N. crassa. The relative affinities of N. crassa hexokinase for glucose, 2DG and fructose are in the proportions 1 : 0.25 : 0.025. These relative affinities could account for the fact that the rate of growth in mixtures of fructose and 2DG is considerably smaller than in mixtures of glucose and 2DG, through the differences in ability to compete for the hexokinase. Phosphorylation may also be the first step in the utilization of 2DG by these fungi. Nevertheless, in A. oryzae an inducible glucose dehydrogenase can readily oxidize 2DG (Ruiz-Amil, 1959). On the other hand, extracts of 2DG grown N. crassa which readily catalyzed the reduction of TPN by glucose-6-P did not act on 2DG-6-P, although the latter activity has been reported as constitutive in Leuconostoc mesenteroides (De Moss and Happel, 1955).

Further work on the pathway(s) of utilization of and

inhibition by 2DG is in progress.

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