his experimental model of photosynthesis, used JB as electronic shunt between chlorophyll as acceptor and OH^- as electron donor.

A $FADH_2$-JB-O_2 electronic shunt accounts for an uncontrolled depletion of the cell of $NADPH_2$ and $NADH_2$, which means lack of energy and of anabolic capacity. The underdevelopment of the embryos injected with JB may be explained by this.

ONCHI and SALVAREJ[7] found that macrophage reaction (in rabbits) is conditioned by an intense biological oxidation. Inhibitors of the tricarboxylic cycle, or general inhibitors of respiration such as amytal or KCN, do not affect this process. Electron acceptor dyes like methylene blue, menadion bisulfite or phenazin methosulfate stimulate respiration and inhibit phagocytosis.

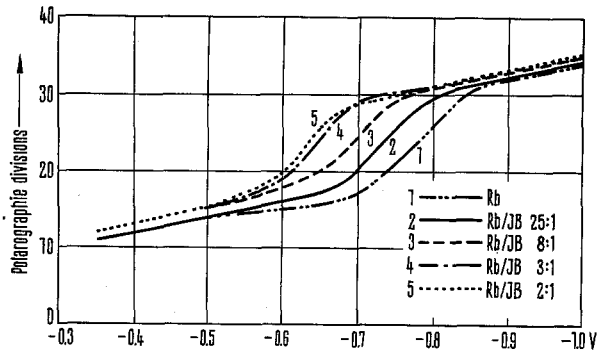


Fig. 1. Polarographic reduction wave of riboflavine + JB.

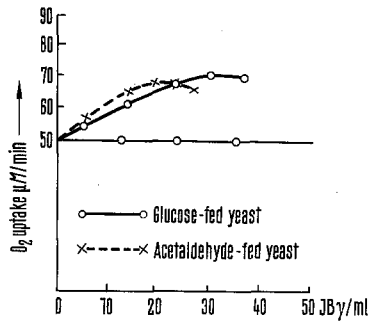


Fig. 2. Yeast respiration activated by JB.

Therefore, syndactylism induced by JB may be explained by the impediment on macrophagic transformation of the mesenchymal cells and diminished activity of the highly aerobical macrophages upon the interdigital tissue. This agrees with the finding that limb buds transplanted on the allantois[8] grow but develop syndactylism. Lack of vascularization and inadequate energy support prevents macrophagic reaction and phagocytosis in this case.

Syndactylism produced by JB and its reduction derivatives

Test series	No. of eggs	Treated with	Survivors at 10 days (%)	Syndactylism in survivors (%)
O	35	Controls	92	0
A	65	JB	57	100
B	65	DES + DMA	50.7	0
C	65	DES + DMA	53.8	0

In series A, eggs were injected in the 29 HH stage with 10 µg JB. In series B, the equivalent amount of DES + DMA injected resulted from the reduction of 10 µg JB by catalytical hydrogenation (with Pd on $BaSO_4$). In series C, the same amount of JB was used, but reduction was performed by boiling with excess ascorbic acid.

Zusammenfassung. Janusgrün B mit oxidativen Fermenten, insbesondere mit dem FAD, geht eine Komplexverbindung ein, die eine Syndaktylie zur Folge hat. Die Abbauprodukte des Janusgrün B (Diäthylsafranin und *p*-Dimethylanilin) wirken zwar gleich toxisch wie das Janusgrün B, haben aber keinen Syndaktylie-Effekt.

E. STEIN, CORNELIA IDITOIU and M. DELEANU[9]

University of Timişoara, Chemistry Department, Timişoara (Romania), 20 February 1969.

[7] E. ONCHI, R. J. SALVAREJ and A. J. SBARRA, Expl. Cell. Res. *40*, 457 (1965).
[8] M. DELEANU, Revue roum. Embryol. cyt. Embryol. *4*, 85 (1967).
[9] We are indebted to Prof. B. MENKES for his advice and generous aid in our problems.

Inhibition of Phosphoglucomutase by Galactose 1,6-Diphosphate

Phosphoglucomutase (α-D-glucose-1,6-diphosphate: α-D-glucose-1-phosphate phosphotransferase, EC 2.7.5.1) catalyzes the interconversion of glucose-1-phosphate and glucose-6-phosphate. A two step mechanism has been proposed[1]:

$$\text{Glc-1-P} + \text{EP} \rightleftarrows \text{Glc-1,6-P}_2 + \text{E}$$
$$\text{Glc-1,6-P}_2 + \text{E} \rightleftarrows \text{Glc-6-P} + \text{EP}$$

where EP is the phosphorylated enzyme and E is the unphosphorylated enzyme. RAY and ROSCELLI[2] have reexamined the role of glucose 1,6-diphosphate in the phosphoglucomutase reaction and have suggested that glucose 1,6-diphosphate is an abortive product of the enzyme-P-substrate complex and is not an obligatory intermediate in the reaction, but it serves to prevent the depletion of EP, the active enzyme species, by phosphorylating the dephosphoenzyme. GOUNARIS et al.[3] have confirmed the results of RAY and ROSCELLI[2].

It would seem reasonable that an analog of glucose 1,6-diphosphate such as galactose 1,6-diphosphate might be an inhibitor of the phosphoglucomutase reaction. The use of such an inhibitor may be useful for elucidating

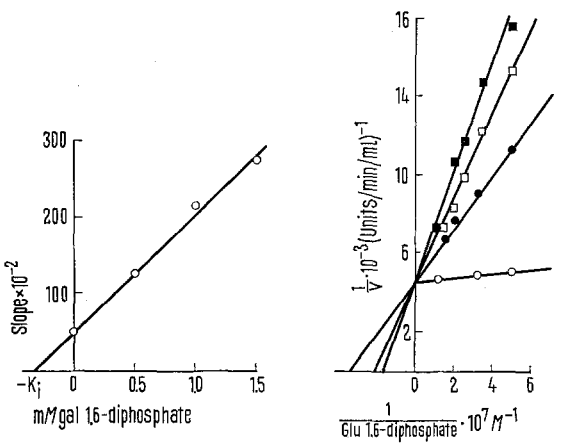
[1] V. A. NAJJAR and M. E. PULLMAN, Science *119*, 631 (1954).
[2] W. J. RAY and G. A. ROSCELLI, J. biol. Chem. *239*, 1228 (1964).
[3] A. D. GOUNARIS, H. R. NORTON and D. E. KOSHLAND, Biochim. biophys. Acta *132*, 41 (1967).

the role of glucose 1,6-diphosphate in the phosphoglucomutase reaction. Galactose 1,6-diphosphate was synthetized chemically and tested as a possible inhibitor of the phosphoglucomutase reaction.

Galactose, glucose-6-phosphate dehydrogenase and glucose-1-phosphate (Grade 5) were from Sigma Chemical Company, Anhydrous phosphoric acid was from Matheson, Coleman and Bell. Other chemicals were of reagent grade.

Galactose-6-phosphate was prepared according to the procedure described by LEVENE[4,5] except for the preparation of the diacetone galactose[6]. Galactose-6-phosphate was acetylated to form the tetraacetate by the same procedure used to acetylate glucose-6-phosphate[7]. The 1,2,3,4-tetraacetyl galactose-6-phosphate was phosphorylated with anhydrous phosphoric acid by a slightly modified MACDONALD's procedure[8] as used for the preparation of glucose-1-6-diphosphate[7] except that the phosphorylation temperature was 56 °C. The product was purified as previously described for glucose 1,6-diphosphate[7] and 204 mg (0.28 mmoles) were obtained from 487 mg (1.08 mmoles) of the tetraacetate. Inorganic phosphate was removed[7] and 168 mg (0.24 mmoles) of galactose 1,6-diphosphate as the tetracyclohexylammonium salt was obtained. The ratio of total phosphate to acid labile phosphate (7 min at 100 °C in 1 N H_2SO_4) was 1.8:1. No reducing sugars or inorganic phosphate were found in the product. The product migrated as a single spot (7 min phosphate spray) on paper chromatography on Whatman No. 3 with methanol-formic acid-water 80:15:5 v/v containing 0.2 g of EDTA/100 ml as the mobile phase.

The galactose 1,6-diphosphate inhibition of the phosphoglucomutase reaction was determined by the coupled enzymatic assay described by RAY and ROSCELLI[2]. It was necessary to use glucose-1-phosphate free of glucose 1,6-diphosphate (Sigma Type V) since inhibition was not observed with glucose-1-phosphate (Type 1) which contains sufficient glucose 1,6-diphosphate for the reaction to proceed normally. It was also observed that low levels of galactose 1,6-disphosphate stimulated the phosphoglucomutase reaction slightly until 2 mM was reached and at higher concentrations it was inhibitory. The initial stimulation was probably due to traces of glucose 1,6-diphosphate in the galactose 1,6-diphosphate.



Inhibition of galactose 1,6-diphosphate with respect to glucose 1,6-diphosphate in the phosphoglucomutase reaction. Double reciprocal plot of velocity versus glucose 1,6-diphosphate concentrations at galactose 1,6-diphosphate concentrations of ○—○, Zero; ●—●, 0.5 mM; □—□, 1.0 mM; ■—■, 1.5 mM. The K_i for galactose 1,6-diphosphate was determined from the secondary plot of slope versus galactose 1,6-diphosphate and was 0.34 mM. Galactose 1,6-diphosphate was added prior to the addition of substrate and cofactor. Glucose 1,6-diphosphate was added to start the reaction and the low rate observed with galactose 1,6-diphosphate was subtracted from the rate in the presence of glucose 1,6-diphosphate to correct for the small amounts of glucose 1,6-diphosphate presumably in the synthetized galactose 1,6-diphosphate.

This initial rate with galactose 1,6-diphosphate was subtracted from the rate when glucose 1,6-diphosphate was added to the reaction (see Figure for details). The double reciprocal plots of velocity versus glucose 1,6-diphosphate at varying galactose 1,6-diphosphate concentrations were linear. Galactose 1,6-diphosphate was a linear competitive inhibitor as evidenced by the fact that the secondary plot of the slope versus galactose 1,6-diphosphate was linear (Figure). From the Figure, the Km for glucose 1,6-diphosphate was $2.6 \times 10^{-8} M$ which is similar to the reported value[2] and the K_i for galactose 1,6-diphosphate was 0.34 mM.

The data presented in the Figure shows that galactose 1,6-diphosphate is a competitive inhibitor with respect to glucose 1,6-diphosphate in the phosphoglucomutase reaction. The probability exists that the chemically synthetized galactose 1,6-diphosphate contained traces of glucose 1,6-diphosphate which would explain the low rates observed with galactose 1,6-diphosphate. The solvent system used does not separate glucose 1,6-diphosphate from galactose 1,6-diphosphate and even if it did it would be doubtful if the small amounts of glucose 1,6-diphosphate could be detected. Commercial sources of galactose contain trace amounts of glucose and if the galactose used for synthesis contained 0.01% glucose and since the chemical yields of glucose 1,6-diphosphate[7] and galactose 1,6-diphosphate are similar, the concentration of glucose 1,6-diphosphate at mM galactose 1,6-diphosphate would be $1 \times 10^{-7} M$ which is sufficient to give an appreciable enzymatic rate.

The kinetics of the inhibition by galactose 1,6-diphosphate are linear competitive which suggests that galactose 1,6-diphosphate is binding at the glucose 1,6-diphosphate binding site which would argue against galactose 1,6-diphosphate acting as a poor substrate. Galactose 1,6-diphosphate is a relatively poor inhibitor (K_i = 0.34 mM) though the K_i may be slightly lower since it is probable that galactose 1,6-diphosphate is not the pure α isomer. MACDONALD[9] has shown that galactose-1-phosphate synthetized by a similar procedure is about 50% α and 50% β isomer[10].

Zusammenfassung. Chemisch synthetisiertes Galaktose-1,6-Diphosphat wird als Hemmer des Glukose-1,6-Diphosphats in der Phosphoglukomutase-Reaktion bestimmt.

Y. L. HUANG and K. E. EBNER

Department of Biochemistry,
Agriculture Experiment Station,
Oklahoma State University,
Stillwater (Oklahoma 74074 USA), 12 May 1969.

[4] P. A. LEVENE and G. M. MEYER, J. biol. Chem. *92*, 256 (1931).
[5] P. A. LEVENE, J. biol. Chem. *92*, 765 (1931).
[6] C. S. HUDSON and J. M. JOHNSON, J. Am. chem. Soc. *37*, 1270 (1915).
[7] W. A. KHAN, T. BALDWIN, U. BRODBECK and K. E. EBNER, Analyt. Biochem. *20*, 58 (1967).
[8] D. L. MACDONALD, J. org. Chem. *27*, 1107 (1962).
[9] D. L. MACDONALD, Carbohyd. Res. *6*, 376 (1968).
[10] This research was supported in part by NSF Grant No. GM 5000 and a NIH Career Development Adward No. 1 KO4 GM 42396 to KURT E. EBNER.