albumin-γ-globulin mixtures. This unusual protein fraction electrophoretically resembles the peak formed when γ-globulin alone is sonicated.

The electrophoretic data and other evidence suggest that ultrasound causes the formation of an albumin-globulin complex. Moreover, while the complex retains certain solubility properties of γ-globulin, those of albumin seem obscured. The total protein content of salt precipitable γ-globulin fraction has been shown to increase after whole serum is sonicated[3]. The sonically formed complex probably contains an outer layer of γ-globulin surrounding the albumin molecules. Hence, only a single new fraction residing in the β-globulin region can be distinguished electrophoretically after exposure of albumin-γ-globulin mixtures to ultrasound.

The atypical electrophoretic patterns observed in some diseases probably denote the presence of unusual proteins. Changes in electrophoretic behavior following sonic treatment likely depends upon the quantitative and qualitative nature of the proteins in serum. Ultrasound might be useful for detecting the presence of certain abnormal serum proteins.

Zusammenfassung. Normale und abnorme Seren sowie gereinigtes menschliches Serumalbumin und Serumglobulin, wurden vor und nach Ultraschalleinwirkung auf elektrophoretisches Verhalten untersucht. Die Unterlagen deuten auf eine Bildung aussergewöhnlicher Komplexe zwischen gewissen Serumproteinen, wahrscheinlich γ-Globulin und Albuminfraktionen, durch ultrasonische Frequenzen hin.

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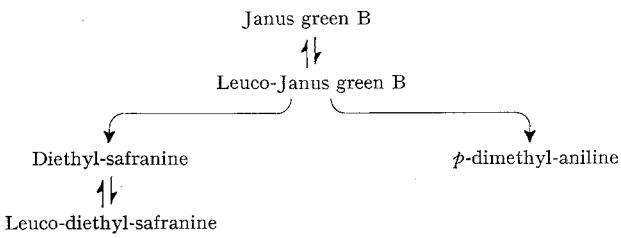
Janus Green B and Experimental Syndactyly in Chick Embryos

Very small amounts of Janus green B (JB) injected into the amniotic fluid of incubated eggs (in the 29 Hamburger-Hamilton (HH) stage) lead to syndactyly in all survivors[1,2].

According to HAVEMANN[3], JB (diethyl-safranine-azo-*p*-dimethylaminobenzene) can be reversibly reduced to leuco-Janus green B, a hypothetical intermediary compound, which on further reduction splits by an irreversible process into pink diethyl-safranine (DES) and *p*-dimethyl-aniline (DMA). This is the final reduction stage in the living cell, while a leuco-DES, which can be obtained by catalytical hydrogenation, is instantly oxidized into pink DES by O_2.



As JB electively taints in green living mitochondria, an interference of the dye with biological oxidation has been assumed[4]. We actually found an interaction between JB and riboflavine, the active site of FAD: (1) JB reacts with dihydroriboflavine and forms a water-insoluble greenish compound. No interaction between JB and riboflavine could be observed in the visible range of the spectrum. (2) The reduction of JB with ascorbic acid is accelerated by small amounts of riboflavine. (3) The polarographic reduction wave of riboflavine shifts to more positive values on the addition of JB. In Figure 1 the molar ratio of the compounds is presented.

In an experimental model, we observed that very small amounts of JB exert stimulation on yeast oxygen uptake, as determined by polarography in paraffin-oil sealed media. Glucose has been added to yeast suspensions in 7.2 pH phosphate buffer (Dulbecco isotonic medium for tissue cultures), until further addition of glucose did not increase the rate of oxygen uptake. Then, progressive amounts of JB were added, increasing respiration rate up to 45% over the maximal level attained by saturation with glucose.

The same experiment was repeated with acetaldehyde as food instead of glucose, for starved yeast. The results obtained were most similar to those with glucose (Figure 2). As acetaldehyde is directly oxidized by the respiratory chain[5], this similitude suggests an action of the dye upon this common metabolic pathway.

As long as oxygen concentration could be maintained by constant air bubbling, above approximately 50% of saturation, JB was not reduced by the yeast and the respiration stimulus persisted. In lack of aeration, O_2 concentration of the medium rapidly fell and JB was reduced to pink DES, while oxygen uptake sank to very low values, assumedly by toxic action. JB rapidly turns into pink DES in the allantoic fluid of the chick embryos, so it had to be investigated whether the dye itself or some of its reduction products is actually responsible for the syndactylism induced.

A comparative testing of JB and the equivalent amounts of the reduction compounds (DES + DMA) proved that only the dye itself is teratogenetical. However, a toxic effect of the reduction products, as concluded from the high lethality, can be admitted.

It can be assumed that JB has an action upon the respiratory chain (at least in yeast) forming a FAD-JB type complex. The respiration stimulus observed suggests an electronic shunt role of this complex. METZNER[6], in

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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.

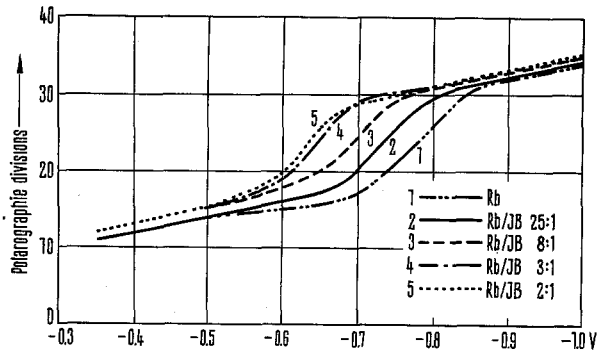

Fig. 1. Polarographic reduction wave of riboflavine + JB.

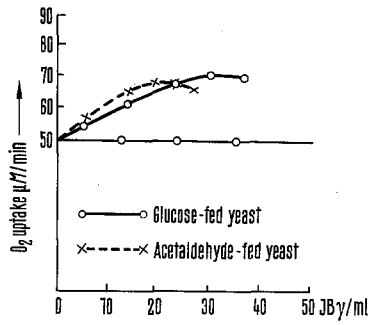

Fig. 2. Yeast respiration activated by JB.

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.

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[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

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