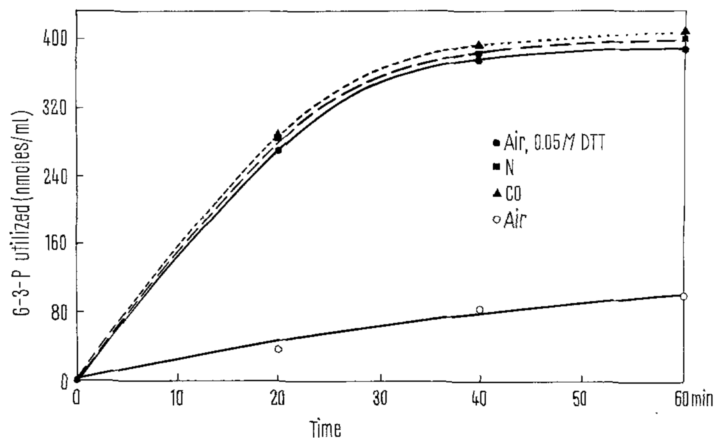

Fig. 2. Glyceraldehyde-3-phosphate dehydrogenase activity in incubation mixtures maintained under air, carbon monoxide and nitrogen. When 0.05 M dithiothreitol was added to the system, the differential effect of aerobic versus anaerobic incubation was abolished.

was measured under atmospheric conditions at varying concentrations of phosphate, and the enzyme was found to be proportionately more active at higher phosphate concentrations (Table).

When G-3-PD activity was measured under air, carbon monoxide and nitrogen, the enzyme was found to be nearly 300% more active under carbon monoxide and nitrogen. In the presence of 0.05 M dithiothreitol, G-3-PD activity was equivalent under aerobic and anaerobic conditions (Figure 2). Similarly, 2,3-DPG synthesis was equivalent under aerobic and anaerobic atmospheres when 0.05 M dithiothreitol was added to the incubation system. At a concentration of 7 mM phosphate, 147 and 154 nmoles/ml of 2,3-DPG were synthesized after 2 h under air and nitrogen, respectively.

G-3-PD is composed of 4 polypeptide subunits, each contributing 1 sulphydryl residue (cysteine 148) to the active center of the enzyme[11]. Oxidation of these sulphydryl residues is accompanied by inactivation of the enzyme. Recently peroxide has been shown to inactivate G-3-PD, presumably by the oxidation of the essential sulphydryl groups to sulphuric acid[12]. Enzyme inactivated in this manner may be reactivated by sulphydryl reagents.

The most likely explanation for the effect of anaerobic conditions upon G-3-PD activity reported in the current study would appear to be in the prevention of sulphydryl oxidation and subsequent inactivation of the enzyme. The effect of dithiothreitol would then derive both from the protection afforded sulphydryl residues and the reactivation of previously inactivated enzyme.

The accelerated rate of 2,3-DPG synthesis in an anaerobic atmosphere in the absence of hemoglobin would therefore seem to be dependent upon increased G-3-PD activity. Experiments employing artificial 2,3-DPG generating systems should guard against G-3-PD inactivation. What role this finding may play in the intact human erythrocyte remains to be demonstrated. With its high concentration of hemoglobin and reduced glutathione, the intact erythrocyte, under normal conditions, should not be vulnerable to G-3-PD inactivation.

It is of interest that when adult erythrocytes are incubated under nitrogen, 2,3-DPG levels increase[13]. This increase has been ascribed to relief of 2,3-DPG mutase from its product inhibition by 2,3-DPG as a consequence of its binding to deoxyhemoglobin[5,14]. The studies reported herein suggest that in an anaerobic atmosphere, at least in vitro, increased 1,3-DPG synthesis secondary to augmented G-3-PD activity may also contribute to increased 2,3-DPG synthesis[15].

Zusammenfassung. In Erythrozyten ist die Synthese von 2,3-Diphosphoglycerat aus Fructose-1,6-diphosphat in anaerobem Milieu um 18% höher als unter aeroben Bedingungen. Das anaerobe Milieu steigert die Aktivität der Glyceraldehyd-3-phosphat-dehydrogenase um 300%.

J. B. Weissberg, F. A. Oski[16] and A. J. Gottlieb

Departments of Pediatrics and Medicine, University of Pennsylvania School of Medicine, Philadelphia (Pennsylvania 19146, USA), 4 May 1970.

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[16] Address reprint requests to: Frank A. Oski, M.D., Children's Hospital of Philadelphia, 1740 Bainbridge Street, Philadelphia (Pennsylvania 19146, USA).

Autoradiographic Identification of Rabbit Retinal Neurons that take up GABA

GABA (γ-aminobutyric acid) is now widely believed to be one of the mammalian neurotransmitters. It has recently been shown that the substance is actively taken up and retained by the central nervous tissue[1,2], the mechanism somewhat resembling that for catecholamines in adrenergic neurons. The uptake of exogenous GABA into what might be presumed to be GABA neurons makes it possible to demonstrate these neurons autoradiographically, provided that diffusion can be controlled. The retina was chosen as test tissue because its regular structure permits simple classification of cells and

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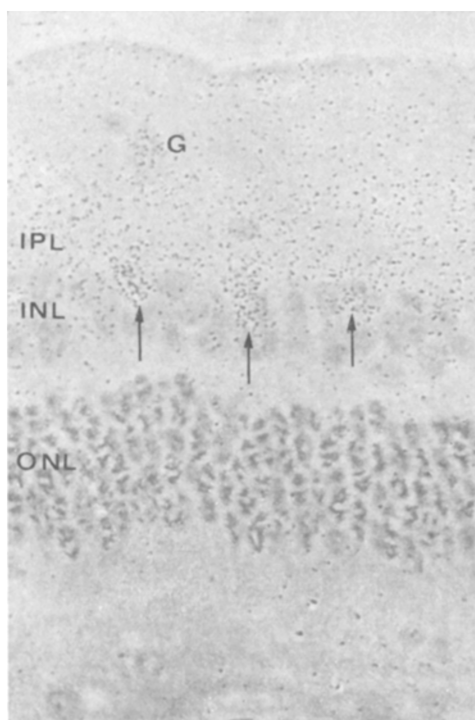
because it can very easily be exposed to exogenous substances by injecting these intravitreally.

GABA has been demonstrated in the dog, ox and rabbit retina[3,4], as has the enzyme synthesizing GABA[4]. Moreover, GABA has been shown to influence retinal nervous mechanisms[5–7].

Tritiated GABA (10–25 μC) was injected intravitreally into rabbit eyes. After 4 h, the retina was dissected out, frozen in a liquid propane-propylene mixture cooled by liquid nitrogen, freeze-dried, fixed with dry gaseous formaldehyde, embedded in vacuo directly in Durcopan ACM (Fluka), sectioned in an LKB Pyramitome, and covered with autoradiographic stripping film (Kodak AR 10). As judged by the excellently preserved monoamine fluorescence, which is highly sensitive to water, diffusion is negligible with this procedure.

The radioactivity of the retina (Figure) was mainly localized in the inner plexiform layer. No sublayering has yet been demonstrated. There was also some radioactivity in the innermost part of the inner nuclear layer and in the ganglion cell and nerve fibre layers. In addition, some cells occupying the same position as amacrine cells were radioactive, as were some nerve cells of the ganglion cell layer.



Autoradiogram, rabbit retina, intraocular injection of 25 μC GABA, 4 h. Radioactivity is seen over a ganglion cell (G) and over cells in the innermost part of the inner nuclear layer (arrows). There is also diffuse radioactivity in the inner plexiform layer (IPL), the ganglion cell layer, and the nerve fibre layer. × 700.

It has been shown that most of the GABA taken up into brain slices is retained as such[8]. Also, the distribution of exogenous and endogenous GABA in rat cerebral cortex is very similar in differential and gradient centrifugal fractions[9]. There is thus little reason to suppose that GABA metabolites interfere to any considerable extent, and, presumably, the radioactivity signifies GABA-containing neurons. This is further supported by the fact that the endogenous GABA of the rabbit retina has been found mainly in parts containing the ganglion cell layer and the inner plexiform layer[4] which agrees well with the present results.

It is of interest to note that there is now evidence suggesting 4 different transmitter substances in the inner nuclear – inner plexiform regions of retina: GABA (as judged from the present work), acetylcholine (as judged from acetylcholinesterase studies[10]), dopamine, and noradrenaline (as judged from fluorescence microscopy[11–13]).

Résumé. En employant la technique autoradiographique, on constate que l'acide γ-aminobutyrique est accumulé principalement dans la couche plexiforme interne et dans certaines cellules de la couche de cellules ganglionnaires et de la couche de cellules amacrines.

B. EHINGER

Departments of Experimental Ophthalmology and Histology, University of Lund, S-22362 Lund (Sweden),
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[13] With a similar technique, HÖKFELT and LJUNGDAHL (personal communication 1970) have recently demonstrated uptake of tritiated GABA in cells in rat cerebellar cortex.

'Rupture Sites' in Elastin During the Course of Organo-Alkaline Hydrolysis

We have studied the various rupture sites of the polypeptide chains produced during the organo-alkaline hydrolysis of elastin by labelling with dinitro-fluorobenzen (DNFB) according to the method of SANGER[1]. We have found 6 sites having identified dinitro-protein-(DNP)-aspartic acid, DNP-glutamic acid, DNP-glycine, DNP-alanine, DNP-valine and DNP-leucine[2].

The distinction between DNP-glutamic acid and DNP-serine is sometimes doubtful. To obtain more rigorous separations, we carried out a series of two dimensional chromatograms of the organo-alkaline effected elastin lysate labelled with DNFB (elastin Kp[2]) using the following solvents: 1. Toluene/Pyridine/2-chloroethanol/ 0.8 N Ammonium hydroxide (100:30:60:60). 2. Chloroform/Benzyl alcohol/Acetic acid (70:30:3). We were able to observe 4 poorly separated spots of Rf lower than that of DNP-glycine in the 2 solvent systems used (Figure 1). These spots were eluted with ether and