point by laboratory computer and plotted.

A typical result obtained when isosbestic fluorometry is employed in the study of cerebral cortical anoxia in dogs is shown in Fig. 2A, which simultaneously depicts the NADH redox state and capillary hemoglobin oxygenation state (the blood volume trace is omitted for clarity). The behavior of NADH during the anoxic transition is multiphasic and includes an unexpected initial early oxidation. The details of the experimental model and our interpretation of the behavior of the mitochondrial respiratory chain during anoxia will be described elsewhere (*11*).

For comparison, Fig. 2B illustrates the simultaneous measurement of 448 minus 366 nm (traditional method), 448/366 nm (traditional method, but imposing ratio analysis), and 448/549 nm (isosbestic fluorometry). During the early stages of the hypoxic response, the 448/366-nm trace resembles a reciprocal of the 560/549-nm (HbO → Hb) transition depicted in Fig. 2A; it may be inferred that the traditional method is compromised both by inadequate blood volume compensation and by high sensitivity to oximetric artifact, resulting in an apparent monotonic reduction of NAD far in excess of that observed by isosbestic fluorometry.

We also observed maximal oxidation of mitochondrial NADH in canine cortex during insulin-induced profound hypoglycemia (plasma glucose < 20 mg per 100 ml; cerebrospinal fluid glucose < 10 mg per 100 ml). These experiments simulate Chance and Williams's (*12*) mitochondrial state 2—that is, substrate depletion in vivo—and together with studies using uncouplers and selected respiratory inhibitors, indicate that in the basal state (before anoxia) mitochondrial NADH is ~ 66 percent reduced and that ~ 77 percent of the basal fluorescence signal at 448 nm is metabolically inert (non-NADH) contaminant fluorescence (*11*).

Our experience suggests that tissue fluorometry provides unique information for the study of mitochondrial behavior in situ and, when employed in the technical configuration that we have, called isosbestic fluorometry, may ultimately permit quantitative interpretation of the fluorescence signal.

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4-Aminobutyrate:2-Oxoglutarate Aminotransferase in Blood Platelets

Abstract. *Platelet lysates obtained from blood of humans, dogs, and rats catalyzed the transamination of 4-aminobutyrate with 2-oxoglutarate as cosubstrate. Human platelet 4-aminobutyrate:2-oxoglutarate aminotransferase (36.5 ± 3.2 picomoles per minute per milligram of platelet protein) resembled the brain enzyme in kinetic properties and in response to cofactors and inhibitors.*

The enzyme 4-aminobutyrate:2-oxoglutarate aminotransferase (E.C. 2.6.1.19) is principally responsible for catabolism of the putative inhibitory neurotransmitter γ-aminobutyric acid (GABA) in mammalian brain (*1*). This enzyme catalyzes the transfer of an amino group from GABA to 2-oxoglutarate, yielding glutamic acid and succinate semialdehyde. The latter may then be oxidized to succinic acid by mitochondrial succinate-semialdehyde dehydrogenase. Although GABA and GABA aminotransferase were formerly thought to be localized in central nervous system (CNS) tissue, more recent work has indicated that GABA metabolism does occur in some peripheral organs, including liver and kidney (*2, 3*). We report the presence of substantial amounts of the catabolic enzyme GABA aminotransferase in blood platelets and compare the properties of the human platelet enzyme with those of the human brain enzyme. If the enzyme activity of platelet GABA aminotransferase can be assumed to reflect brain enzyme activity, assays of the easily obtainable platelet enzyme may

Table 1. Comparison of human brain and platelet GABA aminotransferase.

Property	Human brain*	Human platelets
K_m (GABA)†	$3.1 \times 10^{-4} M$	$3.1 \times 10^{-4} M$
K_m (2-oxoglutarate)	$1.6 \times 10^{-4} M$	$0.70 \times 10^{-4} M$
Effect of pyridoxal phosphate	Stimulates	Stimulates
Inhibition (in vitro) by‡		
Aminooxyacetic acid ($10^{-8} M$)	69 percent	79 percent
Aminooxyacetic acid ($10^{-6} M$)	100 percent	97 percent
Ethanolamine *O*-sulfate (0.3 m*M*)	39 percent	41 percent
Inhibition (in vivo) by§		
Aminooxyacetic acid (10 mg/kg)	89 percent	100 percent
Ethanolamine *O*-sulfate (250 mg/kg)	62 percent	95 percent
Activity in crude tissue	167 nmole/min per gram of wet tissue	3.4 nmole/min per gram of platelets

*Extract partially purified from human cortex as described earlier (*3*). †For kinetic analysis, the GABA concentration was varied from 0.1 to 1 m*M* and the 2-oxoglutarate concentration from 0.04 to 0.4 m*M*. ‡Inhibitors were incubated with extract for 10 minutes at 37°C before addition of substrates. §Sprague-Dawley male rats (160 to 200 g) were given the indicated doses of aminooxyacetic acid or ethanolamine *O*-sulfate intraperitoneally in saline solution (two rats per dose). Controls were treated with saline solution. Rats were killed approximately 24 hours after dosing. The GABA aminotransferase activity was assayed in platelets and in whole brain homogenates from each rat.

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provide a convenient new approach in studies of a variety of syndromes or behaviors in which GABA has been implicated.

For preparation of platelet extracts, blood was freshly drawn into Vacutainers containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant and centrifuged at 200*g* for 10 minutes at 20°C to obtain platelet-rich plasma (PRP). The PRP was then centrifuged at 2450*g* for 15 minutes at 4°C. The platelet pellet was washed once in a buffer composed of 0.1*M* potassium phosphate, 0.1 m*M* EDTA, 0.5 m*M* dithiothreitol, 0.2 m*M* pyridoxal phosphate, *p*H 8.0 (at a volume of 10 percent of original blood volume); centrifuged at 10,000*g* for 15 minutes at 4°C; and finally resuspended in the same buffer to give a volume equal to 5 percent of the original blood volume. Lysis was accomplished by three freeze-thaw cycles, using a Dry Ice–acetone bath. This extract retained full activity during storage at −70°C for at least 5 days. Protein concentrations were determined by the Bradford method (*4*) with bovine albumin as a standard.

The GABA aminotransferase was assayed by using [^{14}C] GABA (New England Nuclear, adjusted to a specific radioactivity of 10 Ci/mole) and sodium 2-oxoglutarate as substrates. Components of a typical assay mixture (total volume, 100 μl) were 0.05 m*M* EDTA, 0.25 m*M* dithiothreitol, 0.1 m*M* pyridoxal phosphate, 50 m*M* potassium phosphate (*p*H 8.0), 0.67 m*M* [^{14}C]GABA, 0.17 m*M* 2-oxoglutarate, and 0.1 to 0.2 mg of platelet protein (30 to 50 μl of the above extract). Assay mixtures were incubated for 30 minutes at 37°C and acidified with 10 μl of 2*N* HCl, and products were eluted through Bio-Rad AG 50X8 resin as described earlier (*3*). All results were corrected by subtracting disintegrations per minute obtained in blank assays in which only 2-oxoglutarate was omitted.

The enzyme activity in human platelets from three normal individuals (two males and one female) was 36.5 ± 3.2 pmole/min per milligram of platelet protein (mean ± standard error). Assays of blood samples from a particular subject did not vary significantly over a 1-month period. Blood from Sprague-Dawley rats gave lower values (9.5 ± 0.7 pmole/min-mg), and values for beagle dog blood platelets appeared much higher (353 ± 28 pmole/min-mg). Product formation was linear with the extract protein concentration up to about 2 mg/ml (Fig. 1A) and for at least 60 minutes at 37°C (Fig. 1B). For optimal results each assay mixture contained platelet lysate extracted from 0.6 to 1 ml of whole blood. Plots of the reciprocal of enzyme activity against the reciprocal of substrate concentration are shown in Fig. 1C, where the GABA concentration was varied at two different concentrations of 2-oxoglutarate. Parallel lines in this type of analysis are typical of other transaminase reactions, including that of human brain GABA aminotransferase (*3*).

No GABA aminotransferase activity was detected in platelet-poor plasma, in lysed red blood cells, or in the layer of white cells that accumulated on the surface of the red cells during separation of platelets. This indicates that the GABA aminotransferase observed in platelet fractions was not due to contamination by other components of blood.

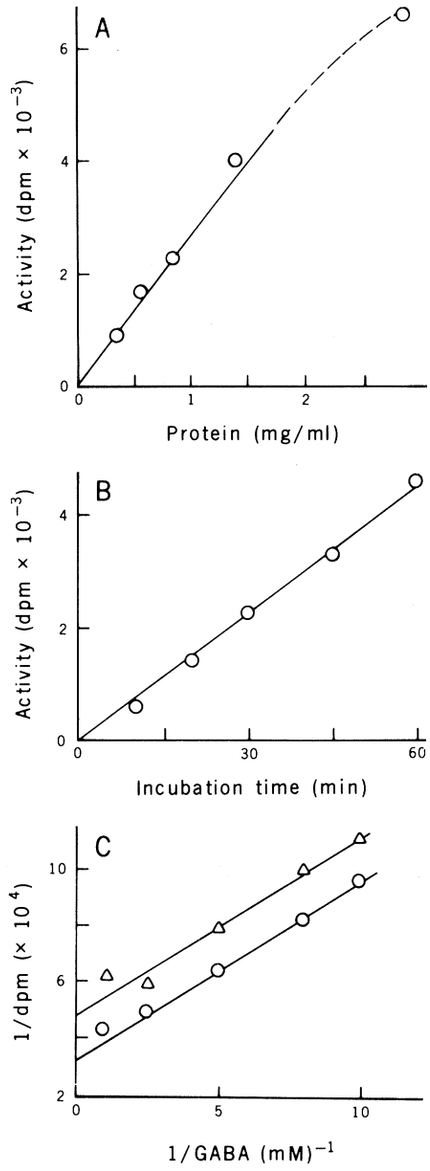

Fig. 1. Dependence of platelet GABA aminotransferase activity on (A) extract protein concentration, (B) time of incubation at 37°C, and (C) substrate concentration, with the GABA concentration varied from 0.1 to 1 m*M* at 2-oxoglutarate concentrations of (△) 40 and (○) 80 μ*M*. Other assay conditions are described in the text.

Because the importance of the platelet enzyme depends on its relationship to brain GABA aminotransferase, additional experiments were designed to compare the GABA transaminating activity of human platelets with that of human brain. The partially purified brain extract was obtained from autopsy tissue after death from Hodgkin's disease and has been described earlier (*3*). Assay mixtures containing either platelet or brain extracts, after incubation, were chromatographed on silica gel thin-layer plates (Whatman LK5D), using as the eluting solvent *n*-butyl alcohol, water, and acetic acid (14 : 8 : 1, top layer). Autoradiographs were prepared with Kodak SB-5 x-ray film. Unmetabolized [^{14}C]GABA remained near the origin of the chromatograms, and the same two radioactive products, having R_F values of 0.86 and 0.57 were observed with both brain and platelet extracts. The latter R_F corresponds to that of succinic acid. The product with the higher R_F was assumed to be succinic semialdehyde, because in the presence of 2 m*M* diphosphopyridine nucleotide (DPN), a cofactor for conversion of succinic semialdehyde to succinic acid, it markedly decreased while the product at R_F 0.57 increased proportionately. With platelet extracts in the absence of this cofactor the product ratio of succinate semialdehyde to succinic acid was approximately 60 to 40, whereas with the more highly purified brain extract the ratio was 74 to 26. This experiment showed that the platelet enzyme was capable of catalyzing the same enzyme reaction as brain enzyme.

A comparison of substrate kinetics provided further evidence for the similiarity of the two enzymes. The concentration of each substrate was varied at several fixed concentrations of the other substrate. Slopes and intercepts of reciprocal plots were used to obtain extrapolated values of the Michaelis constant K_m at saturating substrate concentrations (*5*). Table 1 shows these K_m values for both human extracts. The K_m for GABA, extrapolated to a saturating concentration of 2-oxoglutarate, was 0.31 m*M* in both cases. However, the K_m for 2-oxoglutarate in platelet extracts was about half of that for brain enzyme. This suggests that the platelet enzyme may have a higher affinity for 2-oxoglutarate, but such K_m differences could result from differences in extract purity.

Mitochondrial localization of brain

GABA aminotransferase has been well documented (*6*). Subcellular fractions of human platelet lysates were prepared by centrifugation at 280*g* for 10 minutes and then at 10,000*g* for 30 minutes at 4°C. The aminotransferase activity of the fraction that pelleted between 280 and 10,000*g* contained 77 percent of the activity of the original platelet lysate, with a fourfold higher specific activity. Most of the remaining activity was recovered in the 280*g* pellet. Although the 10,000*g* fraction could be expected to contain platelet granules as well as mitochondria, this preliminary experiment indicates that the platelet enzyme, like brain GABA aminotransferase, may be localized in mitochondria.

Pryidoxal phosphate, the cofactor that stimulates GABA aminotransferase activity in other tissues, also stimulated the activity in human platelets. When platelet lysates were prepared in the absence of pyridoxal phosphate, the addition of 0.1 m*M* pyridoxal phosphate to assay mixtures gave a 2.7-fold increase in product formation, similar to the cofactor stimulation observed with human brain GABA aminotransferse in comparable experiments (*3*).

Another comparison between human brain and platelet enzymes was based on their susceptibility to known GABA aminotransferase inhibitors. Aminooxyacetic acid at 0.01 μM and ethanolamine *O*-sulfate at 0.3 m*M* produced partial inhibition (Table 1), and aminooxyacetic acid at 1 μM completely inhibited activity in both extracts. When rats were pretreated with these irreversible inhibitors, the platelet enzyme was strongly inhibited at doses that also affected brain GABA aminotransferase in the same animals.

Table 1 summarizes and compares properties of GABA aminotransferase from human platelets and brain. Although the enzyme activity in platelets is much lower than that in brain, other properties suggest a similarity of the enzymes in the two tissues. This is consistent with earlier studies (*3*) showing that GABA aminotransferase of human brain resembles that of liver and kidney with respect to kinetic and molecular properties and susceptibility to inhibitors. Electrofocusing experiments indicated that purified active enzyme of human brain, liver, and kidney occurs as a single molecular form (*3*).

GABA aminotransferase is analogous to monoamine oxidase (MAO) in that both are mitochondrial enzymes which mediate the catabolism of putative neurotransmitters in the CNS. Likewise, both enzymes occur in human platelets. Although platelet MAO differs in some important respects from that of brain (*7*), the feasibility of MAO assays in human blood has stimulated studies by many investigators who are searching for useful biochemical correlates between this platelet enzyme and various clinical syndromes. Similar studies may now be possible with platelet GABA aminotransferase, particularly with reference to illnesses in which aberrations in GABA metabolism have been implicated, such as schizophrenia, Huntington's chorea, epilepsy, and other convulsive disorders (*8*). Although at this time there is insufficient evidence that the activity of platelet GABA aminotransferese can be used as a measure of the brain enzyme, the potential value of such a correlation justifies further investigation.

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Development of the Rat's Uncrossed Retinotectal Pathway and Its Relation to Plasticity Studies

Abstract. *In the normal newborn rat the retinotectal pathway from each eye distributes across the whole area of both the ipsilateral and contralateral superior colliculus. Most of the ipsilateral projection retracts during the first ten postnatal days to produce the normal adult pattern, but retraction fails to occur if one eye is removed at birth.*

Each side of the vertebrate brain responds to stimulation of the contralateral visual field. In mammals, in which there is overlap of the visual fields of the two eyes, this rule is satisfied by a distribution of retinal axons at the optic chiasm such that axons originating in the retina temporal to the region which views a line directly ahead of the animal (vertical midline) generally project ipsilaterally while axons originating nasal to this region project only contralaterally. This precise segregation of fibers is achieved before the animal ever uses its visual system (*1*-*3*), but it can be substantially modified by removing one eye early in development, and variations from the normal pattern also occur in genetic mutants such as albinos (*4*). Little is known about how optic axons are directed to one or the other side of the brain during development, however, or how factors generating anomalous distributions relate to those controlling normal development.

We have investigated this problem both in normal rats and in rats with one eye removed at birth, restricting our attention to the retinal projection to the superior colliculus. There is normally only a small uncrossed retinotectal projection in the adult, but after an eye is removed at birth, this pathway enlarges to cover all areas of the superior colliculus. Whereas the normal uncrossed pathway originates from ganglion cells of the lower temporal retina, the expanded uncrossed pathway arises, in addition, from cells throughout the rest of the retina. Many of these are situated nasal to the point of representation of the vertical midline (*5*, *6*).

We examined the retinotectal projections in 28 albino and 6 pigmented rat pups ranging in age from 1 to 10 days. Fourteen of the albino animals had the left eye removed at the time of birth. The right eye of each animal was injected with 1 to 4 μl of a 30 percent solution of horseradish peroxidase (HRP) 18 to 24 hours before being killed. Animals were perfused with phosphate buffer (*p*H 7.4) containing 5 percent sucrose (weight to volume) followed by 2 percent glutaraldehyde in phosphate buffer. Frozen sections through the brains were reacted with tetramethyl benzidine (TMB) and hydrogen peroxide (*7*). The brains of

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