roots (*7*). The powder was dried from solution on a 50-μm beryllium foil to a thickness of 30 to 50 μm. The nerve fibers were removed from freshly killed albino rats and air-dried under slight tension. Twenty fibers were mounted in parallel to cover the full aperture of the x-ray beam. Fiber thicknesses ranged from 30 to 100 μm.

The samples were placed in vacuum approximately 5 cm from the scintillator plane. A stop was placed in front of the samples to limit scattered x-rays in the camera system (*5*). Although this was adequate to permit observation of the diffraction in this case, a smaller stop will be used in the future to more fully eliminate the bright feature in the center of the patterns.

The sharpness of the first-order diffraction ring from cholesterol (34.9 Å) indicates that our camera was in good focus. The diffraction from the spinal roots is due to the 170-Å repeat period in the myelin membranes. The more diffuse second- and fourth-order diffraction spots from the nerve are probably due to structural imperfections produced during drying and mounting. We believe that the use of wet samples with improved ordering, along with straightforward improvements in our x-ray diffraction camera, will enable us to obtain patterns with much improved detail on a single laser shot.

Many kinetic experiments in organic and inorganic systems are possible. In particular, photoresponsive membranes such as the rod outer segment (*8*) and the purple membrane of *Halobacterium halobium* (*9*) seem ideal for study by low-angle x-ray diffraction techniques with the laser plasma x-ray source. In a typical experiment a laser-generated optical pulse would stimulate the sample. Then, with delays ranging from nanoseconds to milliseconds, the laser plasma-generated x-rays would interrogate the sample. X-ray diffraction patterns of the evolving structure would thus be obtained.

Other studies are possible and clearly important. Membrane systems with predominantly one protein, such as the postsynaptic membrane of the electric organ of the *Torpedo californica* electroplax (*10*) or the sarcoplasmic reticulum of muscle (*11*), should be suitable for study. Examination of contracting muscle (*12*) would be possible. The prospect of low-angle x-ray scattering by macromolecules in solution (*13*) with the added dimension of time resolution opens many new experimental paths. The temporal evolution of the binding of an enzyme to its substrate, the assembly of macromolecular structures such as ribosomes and nucleosomes, and the folding of proteins might be studied.

The power and energy of large laser systems continues to increase. At the same time the mechanisms of the laser light-matter interaction are becoming better understood. We are confident that in the near future the laser plasma will produce a sufficient flux of x-rays to give single-shot subnanosecond x-ray diffraction patterns of high quality for structural kinetic studies.

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Hydroxylase Cofactor Activity in Cerebrospinal Fluid of Normal Subjects and Patients with Parkinson's Disease

Abstract. *A method for measuring hydroxylase cofactor activity in human cerebrospinal fluid is described. The hydroxylase cofactor content of cerebrospinal fluid from Parkinsonian patients is approximately 50 percent that of normal subjects. A significant correlation between hydroxylase cofactor and the concentration of homovanillic acid in the cerebrospinal fluid was observed.*

The initial step in the biosynthesis of dopamine is the enzymic hydroxylation of tyrosine. This reaction is catalyzed by tyrosine hydroxylase, which utilizes tyrosine, tetrahydrobiopterin (BH_4), and oxygen as cosubstrates. Recent studies have suggested that the hydroxylase cofactor, BH_4, plays a regulatory role in the synthesis of catecholamine neurotransmitters (*1*, *2*). Since the presence of hydroxylase cofactor in cerebrospinal fluid might reflect the in situ functional capability of central catecholamine neurons in man, we attempted to devise a sufficiently sensitive assay for this compound in cerebrospinal fluid (CSF). The

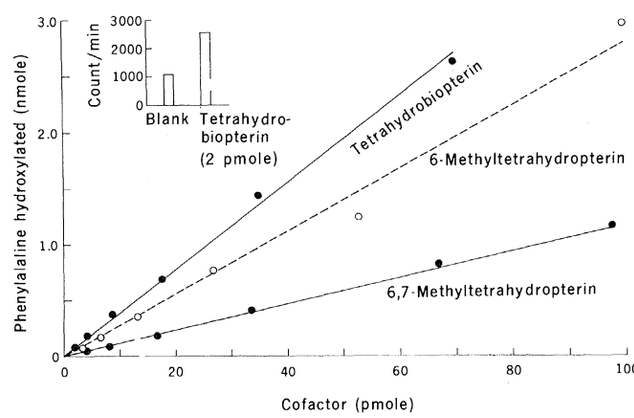

Fig. 1. Response of the cofactor assay system to various amounts of hydroxylase cofactors. In the absence of any cofactor the system gave a blank value of about 1000 count/min (upper left), whereas the addition of as little as 2 pmole of BH_4 generates more than twice this number of counts.

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system described by Guroff *et al.* (*3*) for measuring hydroxylase cofactor in tissue has been modified for this purpose, and we have observed significant hydroxylase cofactor activity in human CSF.

The assay consists of a purified rat liver phenylalanine hydroxylation system with the components adjusted so activity is linearly dependent upon the amount of BH_4. For analysis, CSF samples of approximately 2 ml were collected in a polypropylene tube and frozen immediately on Dry Ice. Samples were transferred as quickly as possible to a liquid nitrogen tank for storage of up to 1 week. Prior to analysis, the sample was thawed, and samples (500 μl) were placed in each of three 1.5-ml Eppendorf tubes. Standard tubes containing between 0 and 80 pmole of BH_4 in 500 μl of a bovine serum albumin solution (400 μg/ml) were also prepared. The standard solution of BH_4 was prepared by catalytic reduction (*4*). These CSF and authentic BH_4 samples were lyophilized to dryness and redissolved in 70 μl of a mixture containing 36 units of highly purified phenylalanine hydroxylase (*5*), 13.0 units of partially purified quinoid dihydropterin reductase from sheep liver (*6*), 10 μmole of potassium phosphate (*p*H 6.8), 0.85 μmole of nicotinamide adenine dinucleotide (NADH), 400 units of catalase, and 0.01 μmole of 4-L-[^{3}H]phenylalanine (30 μCi/μmole). After 45 minutes of incubation at 30°C, the reaction was terminated by the addition of 50 μl of 1.2*M* sodium acetate (*p*H 5.5). After all tubes were cooled to 0°C, 25 μl of a solution of *N*-iodosuccinimide (50 mg/ml in dimethyl sulfoxide) was added to each. The alkylation was terminated after 5 minutes by the addition of 50 μl of 30 percent trichloroacetic acid. The tritiated water was separated from other components of the reaction mixture by a small column of Dowex-50 (6 by 30 mm) under a 1-mm layer of charcoal and a 5-mm layer of Dowex-1 acetate. Since it is presumed that the natural cofactor in neuronal tissue is BH_4, the values of hydroxylase cofactor activity in the CSF are expressed as BH_4 equivalents.

In initial studies, the amount of reaction components and incubation time were optimized. Under the conditions described for this assay system, the amount of tritiated water released is proportional to the amount of BH_4 added to the system, up to 60 pmole (Fig. 1). The synthetic analogs, 6-methyl tetrahydropterin ($6MPH_4$) and 6,7-dimethyltetrahydropterin ($DMPH_4$) also give linear responses, with the activity of $6MPH_4$ being nearly equivalent to that of BH_4 and the activity of $DMPH_4$ being somewhat less. Control experiments demonstrated that the assay system did not respond to dihydrofolic acid, a potential interfering substance.

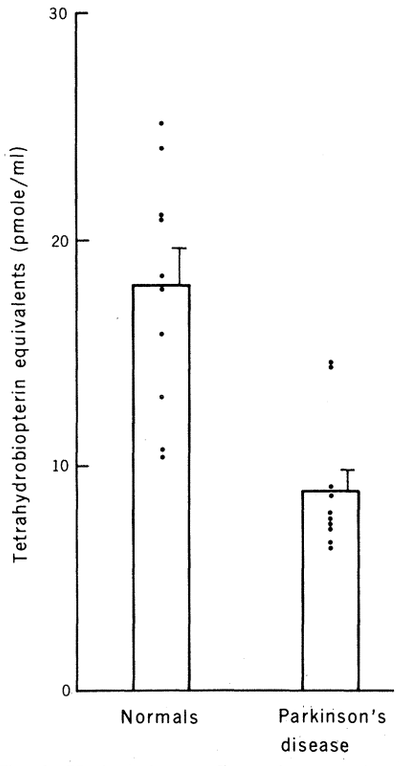

Fig. 2. Hydroxylase cofactor in normal and parkinsonian patients. The values are expressed as BH_4 equivalents. Each data point represents the mean of samples assayed in triplicate. All patients gave their informed consent to this study. Lumbar punctures were done at 9 a.m. after all patients had been at bed rest for 8 hours and had only a standard low monoamine diet for 48 hours. A series of 2-ml portions of CSF were collected, frozen by the bedside in Dry Ice, and then quickly transferred for storage in liquid nitrogen.

With this assay system, human CSF was found to contain significant amounts of hydroxylase cofactor activity. In any one sample, the activity was proportional to the volume analyzed. The concentration of hydroxylase cofactor in all normal subjects sampled extended over a threefold range, with a mean value of 20 pmole/ml. A comparison of this value with earlier work (*7*) in the Crithidia protozoological assay system for total biopterin indicates somewhat higher values in our work with the radioenzymic assay system. The reason for this difference is not known.

It was of interest to examine the content of hydroxylase cofactor in patients with untreated Parkinson's disease. These individuals have a marked reduction in the amount of hydroxylase cofactor in their CSF (Fig. 2). Since examination of a larger number of normal subjects indicated a significant inverse correlation of cofactor activity and age, the data (Fig. 2) consist only of patients with Parkinson's disease and age-matched controls. The mean value for the normal control group was 17.7 $\pm$ 1.69 pmole/ml, while the parkinsonian patients had a mean value of 8.9 $\pm$ 0.95 pmole/ml ($P < .001$). Another potential variable could be a gradient in cofactor concentration in the spinal column. For this reason, all samples used for analysis were from the 13th to 15th milliliter of CSF collected. The question of patient mobility and cofactor content has not been addressed, although all lumbar punctures were taken after 8 hours of bed rest. Because of the overlap of cofactor content in the CSF of normal subjects and those with Parkinson's disease, it would appear that the cofactor measurement would be of limited diagnostic value. Another question to be resolved is whether the decrease in cofactor activity is restricted to the central nervous system, or whether decrements would also be observed in serum, in urine, or in various tissues.

Since the level of cofactor in the CSF may reflect that in brain tissue and this in turn is important in controlling dopamine

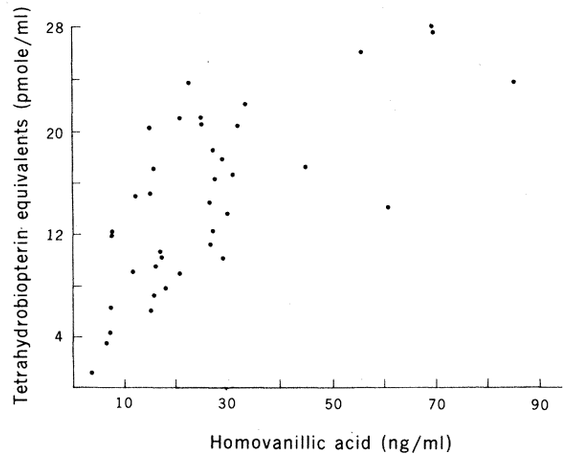

Fig. 3. Correlation of hydroxylase cofactor and homovanillic acid content of the CSF. The 35 samples analyzed in triplicate were from both normal and parkinsonian patients.

synthesis, we examined the correlation of the major dopamine metabolite, homovanillic acid (HVA), with hydroxylase cofactor. As is seen in Fig. 3, there is a significant correlation ($r = .73$; $P < .001$) between these two parameters of dopamine metabolism. However, there was no correlation between CSF cofactor levels and CSF 5-hydroxyindoleacetic acid (*8*).

The significance of the above observations is unclear. One explanation is that individuals with Parkinson's disease have a severe loss of dopamine cells and that these dopamine cells are the major contributors of CSF hydroxylase cofactor. An alternative hypothesis could be that the synthesis of the hydroxylase cofactor is limited in parkinsonian patients and that the inability of dopamine neurons to produce sufficient neurotransmitter results in an atrophy of the dopaminergic system. In any case, an interesting approach to therapy would be the pharmacological elevation of cofactor in brain by either administering the compound or stimulating its endogenous synthesis. Considerably more must be known about the regulation of BH_4 synthesis before the latter approach is taken.

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Local Cerebral Glucose Metabolism During Controlled Hypoxemia in Rats

Abstract. *2-Deoxy-[^{14}C]glucose metabolism was examined in brains of hypoxic, normotensive rats by autoradiography, which revealed alternating cortical columns of high and low metabolism. Activity in white matter was increased severalfold over that in adjacent gray matter. The columns were anatomically related to penetrating cortical arteries with areas between arteries demonstrating higher rates of metabolism. The results suggest the presence of interarterial tissue oxygen gradients that influence regional glucose metabolism. The relatively greater sensitivity of white matter metabolism to hypoxia may lead to an understanding of white matter damage in postanoxic leukoencephalopathy.*

The morphological and functional heterogeneity of the brain is reflected by marked regional differences in its rate of glucose metabolism (*1*, *2*) and blood flow (*3*). Consistent with this heterogeneity, the neuropathological picture that usually results from an apparently uniform hypoxic-ischemic insult to the brain is one of selective damage to vulnerable areas (*4*). Whether such differences in regional vulnerability reflect mainly variation in the severity of the hypoxic-ischemic insult or in the respective tissues' intrinsic metabolic responses to the same insult is unresolved. Measurements of whole brain metabolism during hypoxemia have yielded divergent results with reports of unchanged (*5*), increased (*6*), and decreased (*7*) metabolic rates. Such global measurements may vary according to the nature and severity of the hypoxic insult but are unlikely to accurately reflect, and may even mask, the metabolic responses of individual structural or functional components of the brain to hypoxia.

The 2-deoxy-[^{14}C]glucose ([^{14}C]DG) method (*2*) allows simultaneous measurement of in vivo cerebral glucose metabolism in discrete structural and functional components of the brain. In the present study, the [^{14}C]DG technique was used to assess qualitative differences in regional cerebral glucose metabolism under conditions of moderate hypoxia in physiologically controlled "Levine" rats (*8*). Male Wistar rats, weighing 250 to 300 g and fasted overnight, were lightly anesthetized with ether, paralyzed with tubocurarine chloride (2 mg/kg, intramuscularly), tracheotomized, and mechanically ventilated with a gas mixture of 70 percent N_2O and 30 percent O_2. In all animals, the right common carotid artery was ligated, and cannulas were inserted into the tail artery for monitoring arterial blood pressure and blood gases, and into a tail vein for the injection of [^{14}C]DG.

When the rats had achieved a respiratory steady state (PaO_2, > 90 mm-Hg; $PaCO_2$, 35 to 40 mm-Hg; pH_a, 7.42 to 7.44), were normotensive (mean arterial blood pressure, 120 to 140 mm-Hg), and normothermic (rectal temperature, 37.5° ± 0.5°C), the partial pressure of oxygen (FIO_2) was either continued unchanged (controls) or reduced by replacement with nitrogen in order to achieve an arterial PO_2 of 28 to 32 mm-Hg (experimentals). After a 10-minute equilibration period, the animals were injected intravenously with 50 μCi of 2-deoxy-D-[1-^{14}C]glucose (specific activity, 44 to 55 μCi/μmole; New England Nuclear). Arterial blood was sampled for gas measurements at 5, 15, and 25 minutes after the administration of [^{14}C]DG, and the FIO_2 was appropriately adjusted to maintain a PaO_2 in the experimental animals of approximately 30 mm-Hg. At 30 minutes after the injection of [^{14}C]DG, the rats were decapitated; the brains were quickly removed and frozen in Freon-12 chilled to −70°C in Dry Ice. Coronal sections (20 μm in thickness) were cut serially from the frozen brains in a −20°C cryostat, mounted on glass cover slips, and dried on a hot plate at 60°C. Autoradiographs were prepared in commercial x-ray cassettes by exposing the dried sections to Kodak SB-5 medical x-ray film for 3 days.

Representative autoradiographs of brain sections obtained from normoxic and hypoxic rats are shown in Fig. 1. The ^{14}C activity profiles of control animals (Fig. 1A) showed no right-left asymmetries despite occlusion of the right common carotid artery; gray matter structures consistently appeared darker than those of white matter. This qualitative pattern of higher [^{14}C]DG metabolism in gray versus white matter is in keeping with quantitative measurements showing that the rate of glucose metabolism in gray matter is normally two to three times that in white matter (*2*).

Autoradiographs prepared from hypoxic, normotensive (blood pressure, > 120 mm-Hg) rats revealed alternating light and dark bands of radioactivity in the right cerebral cortex which were not discernible in the left cortex (Fig. 1B).

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