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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Serial brain sections revealed that these alternating bands of high and low radioactivity were actually cortical columns. The columnar pattern appeared to be confined to the middle cerebral artery distribution of the right cerebral hemisphere (for example, no columnar arrangement was ever detected in the paramedian cortex, which receives its blood supply from the anterior cerebral artery) and extended from the frontal to the occipital poles. In addition, the hippocampal formation and caudate nucleus of the right hemisphere appeared darker than the corresponding structures of the left. Subcortical white matter in both hemispheres appeared as dark or darker than adjacent gray matter. In a group of hypoxic animals whose blood pressure was maintained at 90 to 100 mm-Hg, the alternating light-dark cortical columns in the right hemisphere (Fig. 1C) were even more prominent. The greater incorporation of radioactivity into white matter structures, as compared to gray, is further emphasized in an autoradiograph prepared from more anterior sections of such brains (Fig. 1D); the dark corpus callosum and anterior commissure contrast with the paler left cerebral cortex and other gray structures.

Because these experiments differed substantially from the normoxic, equilibrium conditions used to assess cerebral glucose metabolism in normal rats by the [^{14}C]DG technique (*2*), it seemed necessary to establish chemically that the radioactivity accumulated in the brains of the hypoxic animals represented mainly phosphorylated [^{14}C]DG. This was accomplished in parallel experiments with similarly prepared hypoxic, normotensive rats. The frozen brains were divided and the posterior one-third was subjected to autoradiography (to confirm the local metabolic patterns) and the anterior two-thirds to chromatographic analysis of metabolized and unmetabolized [^{14}C]DG. For the chromatographic procedures, cerebral cortex (75 to 100 mg) and subcortical white matter (10 to 13 mg) were dissected from right and left cerebral hemispheres in a room at −20°C, weighed, and homogenized in 1.3 ml of ice-cold 1.0*M* $HClO_4$ containing 4 m*M* EDTA. The homogenates were centrifuged (5000*g*) for 30 minutes at 0°C, and the supernatant fluids (1.2 ml) were neutralized with 3.0*M* KOH and 60 μl of 0.5*M* imidazole-HCl (*p*H 7.0). Portions of these supernatants were added to Dowex 1-X8 columns (formate form; 0.5 by 5 cm) together with 10 μmole of unlabeled 2-deoxy-D-glucose (DG) and 2-deoxy-D-glucose-6-phosphate (DGP) as carriers. The DG was eluted (> 97 percent) from the columns in the application volume (0.8 ml) plus the first 4 ml of a 9-ml water wash; the DGP was eluted (99 percent) in the 3rd to 8th ml of a 12-ml wash with 23*M* formic acid. Portions of these eluates were added to 10 ml of an Omnifluor-dioxane scintillation mixture (New England Nuclear) and counted in a Searle Mark III liquid scintillation spectrometer. Also, portions (50 μl) of the neutralized sample extracts were applied to silica gel thin-layer plates. The chromatograms were developed in a mixture of *n*-butanol, ethanol, and water (50:32:18) for 4 hours; the R_F values for authentic DG and DGP in this system were 0.55 and 0.20, respectively. Areas corresponding to these R_F values were scraped from the thin-layer plates into scintillation vials containing 0.5 ml of water plus 10 ml of Omnifluor-dioxane scintillation mixture and were counted for ^{14}C activity.

Column chromatographic analysis of 12 extracts of cerebral cortex and of subcortical white matter from four animals revealed that, of the total activity present, 88 to 93 percent was recovered in the formic acid fraction, presumably as 2-deoxy-[^{14}C]glucose-6-phosphate ([^{14}C]DGP). Thin-layer chromatography yielded identical values for percentage conversion to [^{14}C]DGP; therefore, the radioactivity that was detected autoradiographically in the cortex and subcortical white matter reflected cerebral metabolism and not simply the accumulation of substrate. The specific radioactivity (disintegrations per minute per milligram, wet weight) for white and gray matter obtained from two control and two experimental rats is shown in Table 1. Brains from hypoxic, normotensive rats demonstrated a two- to threefold increase in specific radioactivity of subcortical and corpus callosum white matter compared to that of normoxic rats. There was no difference in the specific radioactivity of cerebral cortex obtained from normoxic and hypoxic animals. Moreover, the ratio of the specific activity of [^{14}C]DGP in white matter to that in gray matter (Table 1) was higher in both cerebral hemispheres of hypoxic animals than in controls and was highest in the right hemisphere, which presumably sustained the more severe hypoxic insult.

We determined the spatial relationship of the alternating light-dark cortical columns (Fig. 1, B to D) to the cortical vasculature. Accordingly, animals made hypoxic (mean arterial blood pressure, 110 mm-Hg) and injected with [^{14}C]DG were perfused, just prior to being killed, with

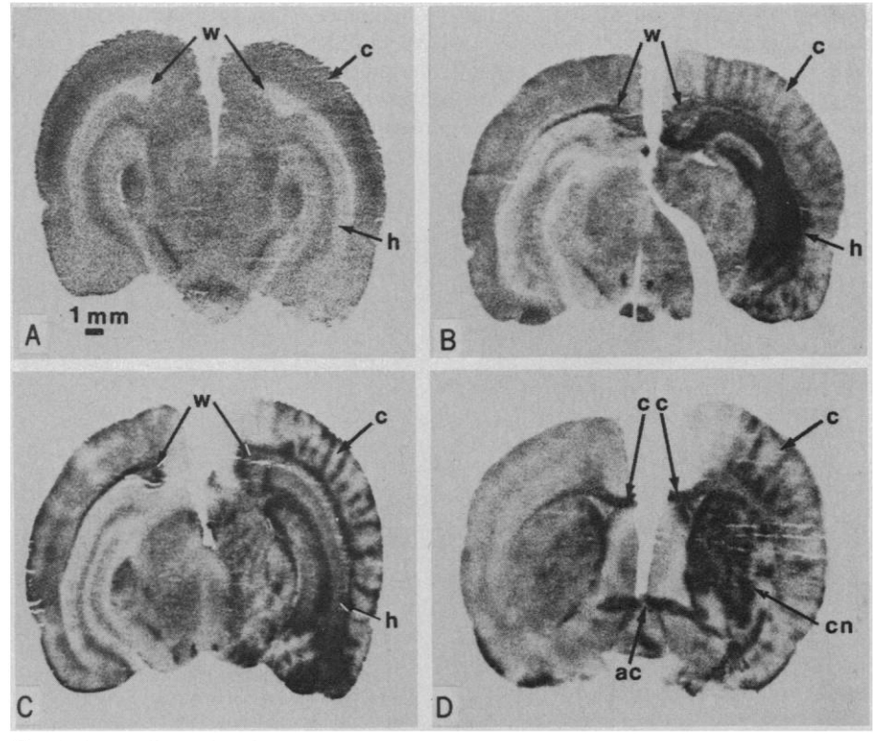

Fig. 1. Autoradiographs of coronal brain sections from rats subjected to right common carotid artery ligation followed by either normoxia or hypoxia. The right cerebral hemisphere is shown on the right-hand side of each photograph. The respective autoradiographs were obtained from animals that were (A) normoxic and normotensive, (B) hypoxic and normotensive, and (C and D) hypoxic and mildly hypotensive (mean arterial blood pressure, 95 mm-Hg). Labeled anatomical structures include cerebral cortex (*c*), hippocampus (*h*), subcortical white matter (*w*), corpus callosum (*cc*), anterior commissure (*ac*), and caudate nucleus (*cn*).

Table 1. Specific radioactivity of 2-deoxy-[^{14}C]glucose-6-phosphate in the cerebral cortex and subcortical white matter from normoxic and hypoxic rats. The autoradiographs of normoxic rat brain revealed no left-right asymmetries, and therefore tissue samples from both hemispheres were pooled.

Condition	Animal No.	Disintegrations per minute per milligram, wet weight				Ratio of white to gray matter	
		White matter		Gray matter			
		Left hemisphere	Right hemisphere	Left hemisphere	Right hemisphere	Left hemisphere	Right hemisphere
Normoxic	1	647		1321		0.49	
	2	552		1185		0.47	
Hypoxic	1	1125	1731	1217	1313	0.92	1.32
	2	871	1189	955	967	0.91	1.23

black plastic microspheres (15 ± 5 μm; 3M Company) via the ascending aorta. These microspheres are too large to pass through the capillary lumen and become trapped in the small arteries and arterioles, thus providing an outline of the cerebral arterial system. As seen in coronal sections, the penetrating cortical arteries and arterioles appeared as black lines perpendicular to the cortical surface (Fig. 2B). When the autoradiographs (Fig. 2A) were superimposed upon the brain sections from which they were derived, the microsphere-laden arteries lined up with the pale cortical columns, and the darker columns lay between penetrating arteries (Fig. 2C). If one assumes that cortical areas lying between penetrating arteries constitute arterial boundary zones, then it seems reasonable to conclude that the dark cortical columns, which represent regions of higher [^{14}C]DG phosphorylation (glucose metabolism), correlated with areas of lowest oxygen tension in the tissue. In this connection, Welsh *et al.* (*9*) demonstrated alternating columns of high and low fluorescence from the reduced form of nicotinamide adenine dinucleotide in the cerebral cortices of hypotensive cats. These authors proposed that the areas of high fluorescence and, presumably, inadequate tissue oxygenation were indicative of "microwatershed" zones between penetrating cortical arteries.

The physiological significance of the cortical columns of high and low glucose metabolism (Fig. 1, B to D) remains to be determined. Their anatomical relation to the cortical blood supply (Fig. 2) and prominence during hypoxia suggest the existence of tissue oxygen gradients between penetrating arterioles analogous to the Krogh model proposed to account for gaseous diffusion at the capillary level (*10*). Nevertheless, it is conceivable that the columns represent functionally heterogeneous areas of cortex that respond in different ways to cerebral hypoxia. Functional columns have been reported in the striate (*11*), sensory (*12*), and motor (*13*) cortices, but metabolic measurements of such columns during altered physiological states have been limited (*14*). Whether these metabolic cortical columns, detected under hypoxic conditions, have any counterpart during normoxia or are related to the eventual development of anoxic-ischemic brain damage are questions for future research.

The increase in [^{14}C]DG metabolism by white matter and the dark cortical columns clearly reflect an increased glycolytic flux in these tissues. However, the neurochemical interpretation of this increase in glucose metabolism in terms of local energy demands is complicated by the fact that the degree of hypoxemia achieved in this animal preparation leads to increased tissue lactate in both cerebral hemispheres and decreased concentrations of adenosine triphosphate in the hemisphere ipsilateral to the occluded artery (*8*). Such changes imply some uncoupling of the normal link between cerebral glycolysis and oxidative phosphorylation. Although the contribution of anaerobic glycolysis in supplying tissue energy demands undoubtedly increases in response to hypoxia, the greater phosphorylation of [^{14}C]DG in white matter compared to that in gray matter raises the question: Is the relatively greater change (increase) in the rate of glucose metabolism in white matter simply a reflection of anaerobic glycolysis supplying the normal energy requirements or does it also reflect increased energy utilization? If one assumes that hypoxia does not alter the proportional energy requirements of cerebral gray and white matter structures, then anaerobic glycolysis, activated to maintain normal energy needs, would be expected to produce the same incremental rise in glucose metabolism in both tissues. As a result, gray matter structures would appear darker than white matter when viewed autoradiographically, as is the case in control animals (Fig. 1A). However, Fig. 1, B to D, demonstrates that this was not true for hypoxic animals and suggests that energy requirements in at least some white matter structures are increased relative to gray matter.

An alternative explanation for the disproportionately greater change in glucose metabolism in white matter is that

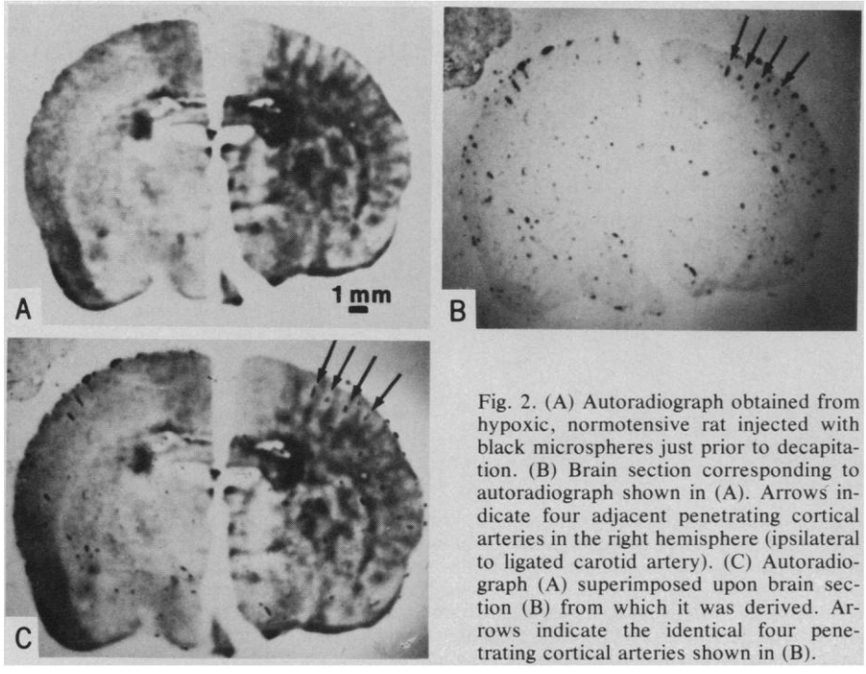

Fig. 2. (A) Autoradiograph obtained from hypoxic, normotensive rat injected with black microspheres just prior to decapitation. (B) Brain section corresponding to autoradiograph shown in (A). Arrows indicate four adjacent penetrating cortical arteries in the right hemisphere (ipsilateral to ligated carotid artery). (C) Autoradiograph (A) superimposed upon brain section (B) from which it was derived. Arrows indicate the identical four penetrating cortical arteries shown in (B).

white matter became more hypoxic than gray matter. In a model of cerebral oligemia Welsh *et al.* (*15*) observed a disproportionately greater accumulation of lactate in white matter compared to gray matter. These authors postulated that this "failure" of white matter metabolism resulted from a relatively greater reduction in blood flow to white matter. During severe hypoxia, cerebral blood flow may increase up to fourfold (*16*), thereby promoting the delivery of oxygen to the tissues. However, Ginsberg *et al.* (*17*) demonstrated in moderately hypoxic, normotensive "Levine" rats that the rise in blood flow to several gray matter structures was variable, ranging from 40 to 100 percent. Although blood flow data for white matter are presently unavailable, it is conceivable that regional cerebral blood flow to white and gray matter during hypoxia is altered disproportionately to their metabolic needs and that white matter may sustain the more severe insult.

The present findings of regional differences in cerebral glucose metabolism during hypoxia may be relevant to human stroke and other clinical conditions of cerebral hypoxia-ischemia. Postanoxic leukoencephalopathy in man is a condition that is characterized by edema and necrosis of white matter with relative sparing of gray matter structures (*18*). The precise hypoxic insult or combination of insults necessary to reproduce this clinical condition experimentally are unknown. Further elucidation of the biochemical and physiological factors responsible for the greater sensitivity of white matter metabolism to hypoxia may lead to the understanding of the susceptibility of white matter to hypoxic injury.

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Clomid Administration to Pregnant Rats Causes Abnormalities of the Reproductive Tract in Offspring and Mothers

Abstract. *In rats, a single injection of clomiphene citrate (Clomid) during pregnancy causes multiple abnormalities of the reproductive tract in the offspring and mothers. These abnormalities probably result from the ability of Clomid to cause long-term estrogenic stimulation.*

Clomiphene citrate (Clomid) is used extensively for the induction of ovulation in women with secondary amenorrhea (*1*). Injection of Clomid to neonatal rats causes extensive abnormalities of the reproductive tract in adult rats (*2*). In this report we demonstrate that, in rats, a single injection of Clomid during pregnancy results in the development of abnormalities of the reproductive tract in the pups when they become adults, as well as in the mothers. These results indicate the potential danger which may be inherent in the use of this drug in women.

Rats of the Sprague-Dawley strain were mated, and the morning that copulatory plugs were found was designated as day zero of pregnancy. Clomid (2.0 mg/per kilogram of body weight) was injected on days 0, 5, or 12 of pregnancy (*3*). At birth, the number of pups was adjusted to eight per mother rat. The pups remained with the mother rat until the day of weaning (day 21) without further handling except for weekly determinations of body weight. At weaning, males and females were separated and caged in groups of four and checked daily for preputial separation or vaginal opening. Once this was determined, rats were kept undisturbed until they were killed at

Table 1. Incidence of epithelial abnormalities in the organs of rats treated with Clomid during pregnancy. The rats were injected with Clomid (2.0 mg/kg body weight) on day 1, 5, or 12 of pregnancy. Rat mothers (N = 12) and offspring (N = 28) were autopsied 100 days postpartum. Control females received either no treatment or 0.1 ml of oil. Results are expressed as percentages.

Group	Highly disorganized epithelium	Extensive hyperplastic vacuolated atypical epithelium	Extensive metaplastic epithelium	Degenerating epithelium	Cysts	Sloughing of noncornified epithelium
			Vagina			
Control	0	0	0	0	0	0
Mothers	0	10	0	10	24	31
Offspring	0	0	0	0	8	21
			Cervix			
Control	0	0	0	0	4	8
Mothers	12	35	35	12	0	12
Offspring	14	21	18	0	0	14
			Uterus			
Control	0	0	0	0	8	0
Mothers	12	47	47	0	12	12
Offspring	14	46	68	0	25	14
			Oviduct			
Control	0	0	0	0	0	0
Mothers	0	6	0	47	18	18
Offspring	0	11	0	14	0	0

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