Autoradiographic Comparison of Thallium-201 Diethyldithiocarbamate, Isopropyliodoamphetamine and Iodoantipyrine as Cerebral Blood Flow Tracers

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We investigated [^{201}Tl]diethyldithiocarbamate (DDC) as a tracer for local cerebral blood flow (LCBF). Awake male rats were given intravenous infusions of a mixture of DDC and reference tracer(s): [^{123}I]isopropyliodoamphetamine (IMP) and/or [^{14}C]iodoantipyrine (IAP). LCBF values for DDC, IMP, and IAP were measured using simultaneous multiple radionuclide autoradiography and quantitative digital image analysis. Patterns of local cerebral blood flow (LCBF) obtained with DDC were intermediate compared to IMP and IAP, although they were more similar to those of IMP. DDC and IMP underestimated LCBF in some white matter and adjacent structures, while IAP underestimated LCBF values in high flow regions. We conclude that DDC uptake generally reflects local cerebral blood flow and that it can therefore be used as a cerebral perfusion tracer in humans using SPECT imaging.

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Diethyldithiocarbamate has been used as a chelating agent for the treatment of poisoning with heavy metals such as thallium. Clinical reports indicated that increased neurological symptoms developed in some patients (*1*), which suggested that a thallium-diethyldithiocarbamate complex might be crossing the blood-brain barrier. This observation lead to preliminary studies which showed that total brain uptake of thallium-201 diethyldithiocarbamate ([^{201}Tl]DDC) was similar to that of iodine-123 isopropyliodoamphetamine ([^{123}I]-IMP) following i.v. injection in rats and rabbits (*2*). Initial clinical studies in normal humans were then performed with single photon emission computed tomography and the DDC cerebral uptake pattern was thought to be similar to that of IMP (*3*). Quantitative local cerebral blood flow (LCBF) measurements were not performed in these animal and human studies.

Studies have indicated that carbon-14 iodoantipyrine ([^{14}C]IAP) and [^{123}I]isopropyliodoamphetamine can be used to measure LCBF in rats using autoradiography (*4,5*). We therefore decided to measure LCBF values obtained in rats with DDC, and to compare the results with those of IAP and IMP using simultaneous quantitative multiple radionuclide autoradiography (*6*) and digital autoradiographic image analysis (*7*).

METHODS

DDC solutions containing ~1 mCi of DDC per ml saline were prepared as previously described (*2*) by mixing [^{201}Tl]-chloride and diethyldithiocarbamate. IMP and IAP were obtained commercially.

Male Sprague-Dawley rats were anesthetized with a mixture of 1–2% halothane and room air. Catheters were inserted in a femoral vein and artery and the animals were then allowed to recover for 3–4 hr before each study.

The brain-blood partition coefficient of DDC was first measured. Approximately 500 μCi of DDC was administered as a 30-sec infusion to each of four rats. Two hours after the DDC administration, arterial blood samples were obtained, the rats were killed and their brains were removed and frozen in powered dry ice. Small tissue specimens were obtained from the frontal cortex, weighed, and counted for ^{201}Tl activity. Twenty-micron-thick transverse sections of the brain were cut using a cryomicrotome, placed on cover slides, and dried in preparation for autoradiography. The sections were placed

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against sheets of Kodak NMB film adjacent to calibrated ^{14}C standards for 5 days to produce autoradiographic exposures and the films were then developed. The amount of darkening produced by the tissue sections was compared to the darkening produced by the ^{14}C standards, the activity measured in the tissue specimens, and the activity in the arterial blood samples. Results were used to determine the relative exposure efficiency of the ^{201}Tl compared with the ^{14}C and the brain-blood partition coefficient.

Local cerebral blood flow measurements were made with DDC and IAP, DDC and IMP, as well as DDC, IAP, and IMP in a series of rats using double or triple label autoradiography. In the double tracer studies, 30–40-sec i.v. infusions of 500–700 μCi of DDC and 50–70 μCi of IAP or 1.5–2 mCi of IMP were administered. In the triple label studies, the infusions consisted of 600–800 μCi of DDC, 75 μCi of IAP and 2.5 mCi of IMP. Arterial blood samples were obtained continuously during the infusion and the rats were killed by KCl administration at the end of the infusion. The brains were rapidly removed and frozen in powdered dry ice. Twenty- and forty-micron thick sections were prepared for autoradiography.

In the DDC-IAP studies, an autoradiographic exposure was first made for 3 days. Next, the sections were removed from the film for 21 days to allow the ^{201}Tl to decay, and a second exposure of 7–10 days was then made. In the DDC-IMP studies a first exposure of 13 hr was made, the sections were removed from the film for 3 days to allow most of the ^{123}I to decay, and a second exposure of 5 days was made. In the DDC-IMP-IAP studies, a first exposure of 13 hr duration was made, a first decay period of 3 days was allowed to elapse, a second exposure of 3 days duration was made, a second decay period of 21 days was allowed to elapse, and finally a third exposure lasting 7–10 days was made. These exposure durations and waiting periods were chosen to produce images which represented ~80% or higher of a particular radionuclide (*6*).

The autoradiographic images were digitized into 512 × 512 matrices with 256 gray levels per pixel using a high performance digital autoradiographic analyzer (*7*). Values for the ^{14}C standards and exposure durations were entered into the system's computer and the density images were transformed into exposure images. The cross contamination between exposures was subtracted on a pixel by pixel basis, and images representing local cerebral concentration of DDC, IMP, and IAP were created.

Cerebral tracer concentration was related to LCBF using a standard diffusible tracer model (*8*).

$$C_b = F\int_0^T C_a\, e(-F/\lambda)\,(T - t)\,dt,$$

where C_b = brain concentration of tracer; C_a = arterial concentration of tracer; F = local cerebral blood flow; λ = brain-

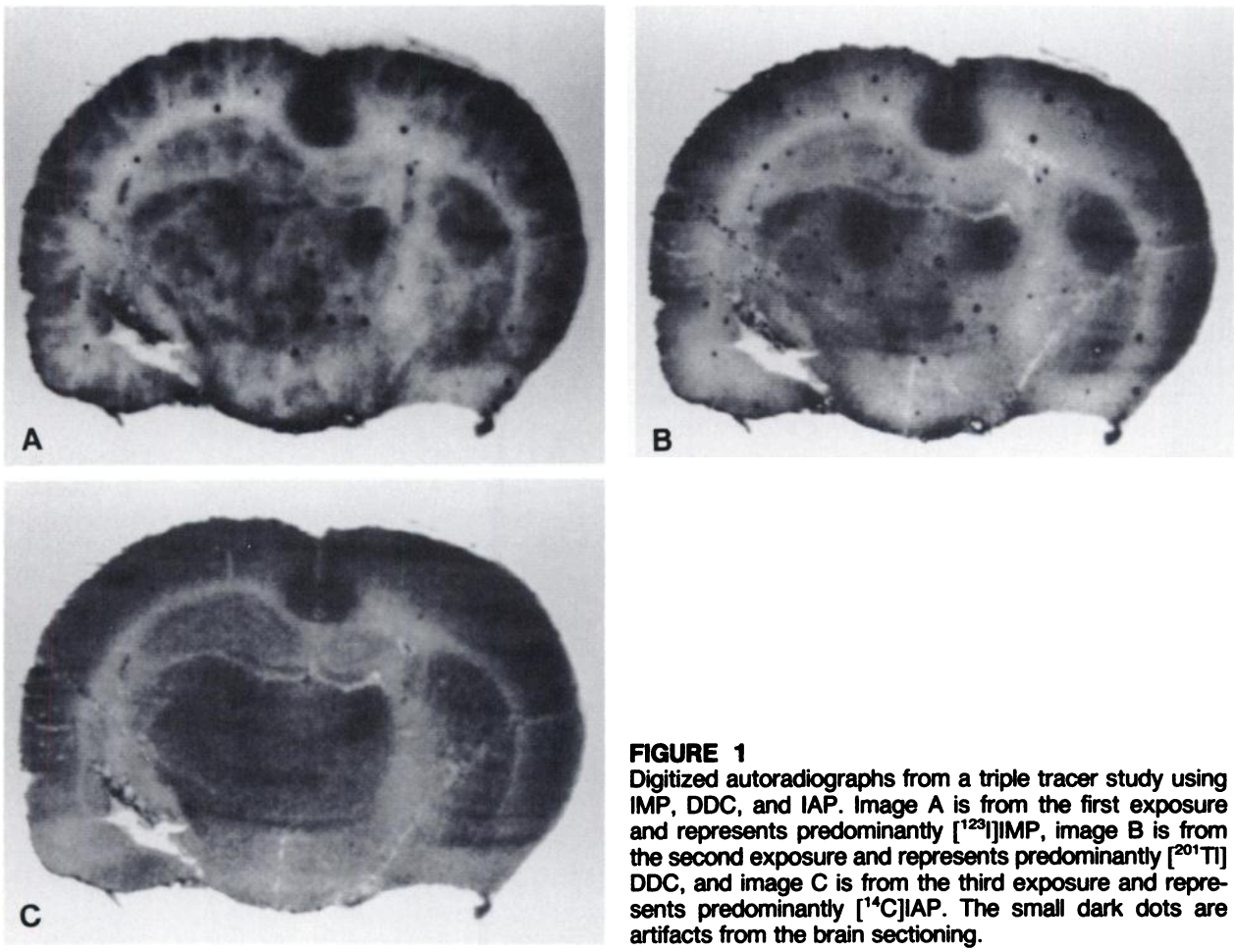

FIGURE 1
Digitized autoradiographs from a triple tracer study using IMP, DDC, and IAP. Image A is from the first exposure and represents predominantly [^{123}I]IMP, image B is from the second exposure and represents predominantly [^{201}Tl] DDC, and image C is from the third exposure and represents predominantly [^{14}C]IAP. The small dark dots are artifacts from the brain sectioning.

blood partition coefficient; T = time of death. Arterial blood concentration data and time of death were entered into the computer and the tracer concentration images were converted into LCBF images. Images representing ratios of LCBF values of the different tracers were created to help explore subtle differences in uptake patterns (see Results).

RESULTS

The brain-blood partition coefficient of DDC was found to be ~18/1. This is similar to that of IMP (25/1) and quite different from that of iodoantipyrine (0.8/1).

Autoradiograms differed in appearance for the three different exposures (Fig. 1). Similarly, LCBF images produced from the original autoradiograms differed in appearance for the three tracers (Fig. 2). IMP had a less homogeneous appearance in cortical and deep gray matter regions than both DDC or IAP because of curvilinear areas of high values. Areas of high LCBF showed more structural variation with DDC and IMP than IAP. Cortex to white matter LCBF ratios were higher with DDC and IMP than IAP.

LCBF values obtained from corresponding regions of interest in the LCBF images were therefore not identical. In the midrange of LCBF values expected in normal awake rats (75–150 ml/100 g/min), all three tracers had similar measured LCBF values (Fig. 4). Values with DDC and IAP averaged slightly, though not significantly, lower than those of IMP. Significantly higher values, however, occurred in some regions with high blood flow with DDC compared to IAP, but not to IMP. Both DDC and IMP had significantly lower LCBF values than those of IAP in some white matter structures.

These differences were clearly illustrated in the LCBF ratio images (Fig. 3). Some areas of high LCBF have small regions within them with DDC/IAP and IMP/IAP LCBF ratios >1. White matter structures such as the corpus callosum and adjacent gray matter regions had IAP/DDC and IAP/IMP LCBF ratio values >1. The DDC/IMP LCBF ratio images had the least structure. With the exception of variations caused by the curvilinear pattern of IMP activity and a slightly greater than 1 value for the corpus callosum, DDC/IMP LCBF ratio images are uniform.

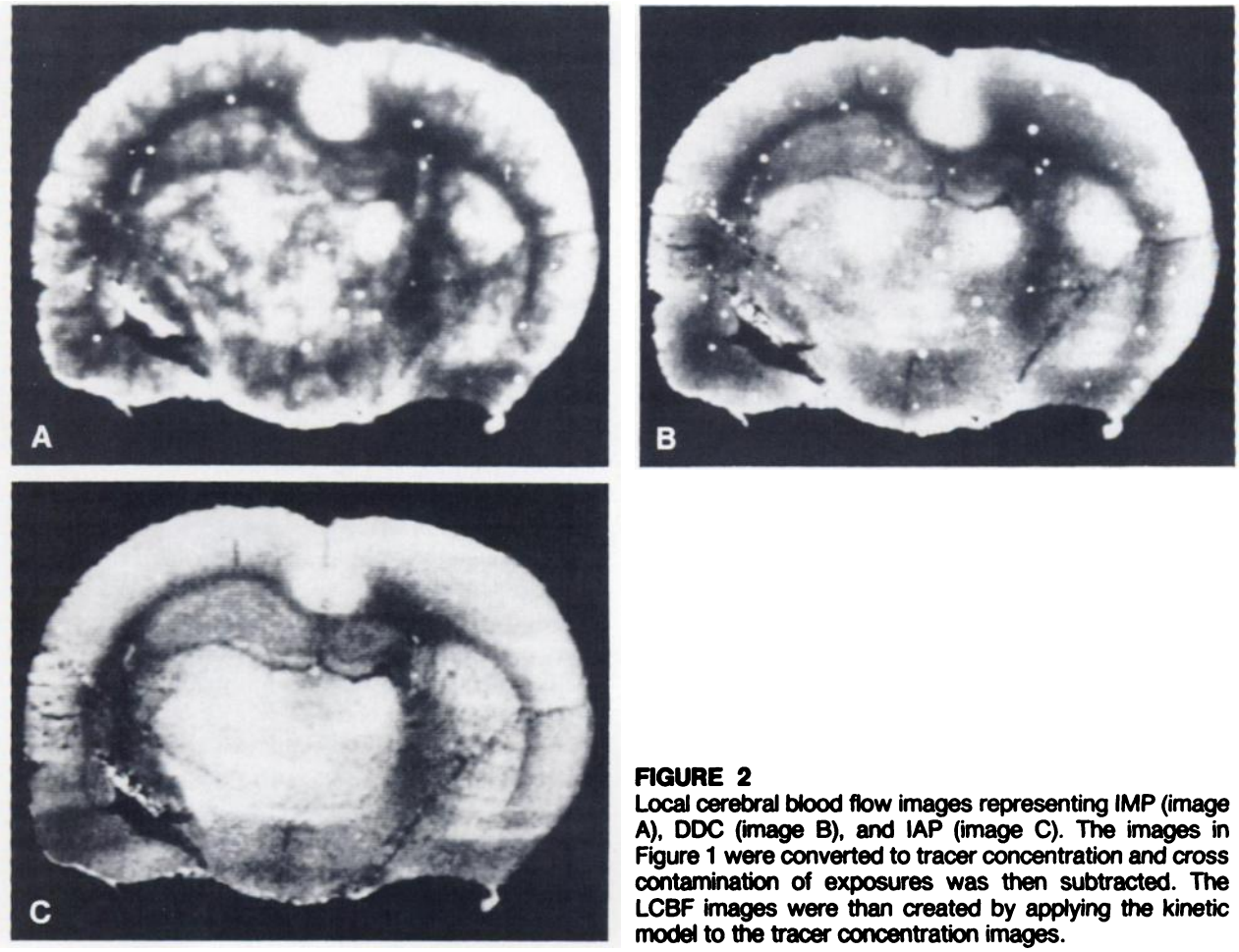

FIGURE 2
Local cerebral blood flow images representing IMP (image A), DDC (image B), and IAP (image C). The images in Figure 1 were converted to tracer concentration and cross contamination of exposures was then subtracted. The LCBF images were than created by applying the kinetic model to the tracer concentration images.

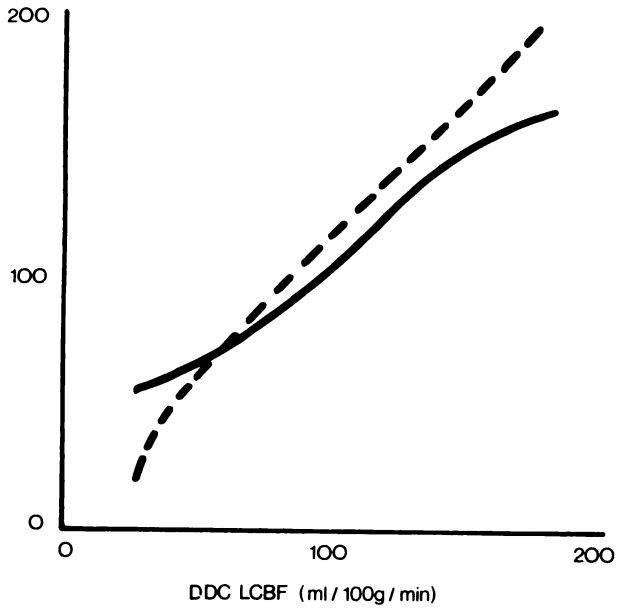

FIGURE 4
Comparison of values of LCBF obtained with IAP and IMP compared to DDC. Lines represent the mean from five rats for each comparison. Because the LCBF relationships are functions of both LCBF and brain location, this correlation is an incomplete representation of the causes of the relationships. (—) IAP LCBF; (- - -) IMP LCBF.

DISCUSSION

The partition coefficient of DDC (18/1) is much closer to that of IMP (25/1) than that of IAP (0.8/1). This is not unexpected because IAP is a freely diffusible tracer (*4*), while IMP and DDC have been previously shown to have prolonged cerebral retention (*2,3,9,10*). Although the mechanisms causing the high retention of DDC and IMP have not yet been fully illucidated, their distributions are more similar to each other than to IAP.

This study was performed using techniques designed to optimize the ability to compare values of cerebral uptake of several tracers with high spatial and concentration resolution. The activities of the various tracers used in the experiments were chosen so that the images produced represented primarily a single tracer (*6*). This minimized the amount of cross contamination which had to be subtracted and thus minimized the amplification of error associated with the subtraction. Autoradiographs were produced so that their darknesses were optimized for having wide ranges of transmittance values (*11*). This maximized the precision of measurement of tracer concentration values so that small differences could be measured. Finally, a high performance digital autoradiographic image analyzer was used for density measurements (*7*). This allowed very precise and accurate tracer concentration and LCBF maps to be made having intrinsic resolutions of ~50 μ.

Because of these optimizations, this study has provided important insights into differences in cerebral uptake and LCBF values obtained with DDC, IMP, and IAP. In a previous double tracer study, it was shown that LCBF values measured with IMP were similar to IAP for structures larger than 500 μ in diameter (*5*). Although the images from IMP and IAP did not appear identical, the use of a microdensitometer with an aperture diameter of 250 μ precluded detailed assessment of the differences. These differences, which were also seen in this study, as well as differences in uptake patterns of DDC compared to both IMP and IAP, could now be more closely examined.

The data in Figure 4 shows that LCBF values obtained with IMP and DDC are generally consistent throughout physiological ranges of LCBF. On the other hand, some low flow regions have higher LCBF values with IAP than either with IMP and DDC, while some high flow regions have higher LCBF values with IMP and DDC in comparison to IAP. The LCBF ratio images show that differences are related to both LCBF and brain location. Thus, the correlation shown in Figure 4, which is a common type of correlation, is an approximation which does not include the spatial parameters. It is therefore important to consider possible reasons relating to both LCBF and brain structure as to why the LCBF values are different for the three tracers.

The kinetic model used in this study is based upon the widely used, two compartment model originally proposed by Kety (*8*). The model represents the brain as a system of identical parallel units, each consisting of a capillary and associated tissue region. Tracer exchange is assumed to occur only between a capillary and its tissue region. Diffusion of the tracer is assumed to be rapid enough so that complete equilibrium occurs between the brain and blood by the time the blood reaches the venous end of the capillary. Each tissue region is assumed to be well mixed, i.e., tracer concentrations are identical throughout a region.

This model is clearly an oversimplification. Diffusion of a tracer may not be rapid enough to assure complete capillary-brain tissue region equilibrium in structures with very rapid blood flow. Some tracer exchange may occur through the walls of arterioles as well as capillaries. Capillaries arise from different places along an arteriole and downstream arterioles may have lower tracer input concentrations than upstream ones. Also, a tissue region consists of a number of cells and cells near the arterial end of the capillary may be exposed to higher tracer concentration than cells near the venous end. The significance of the inaccuracies in the model with respect to LCBF measurement will vary depending upon parameters such as LCBF, permeability of vessel walls to the tracer, and the diffusion pressure of the tracer in blood.

The most likely reasons for the relatively low LCBF

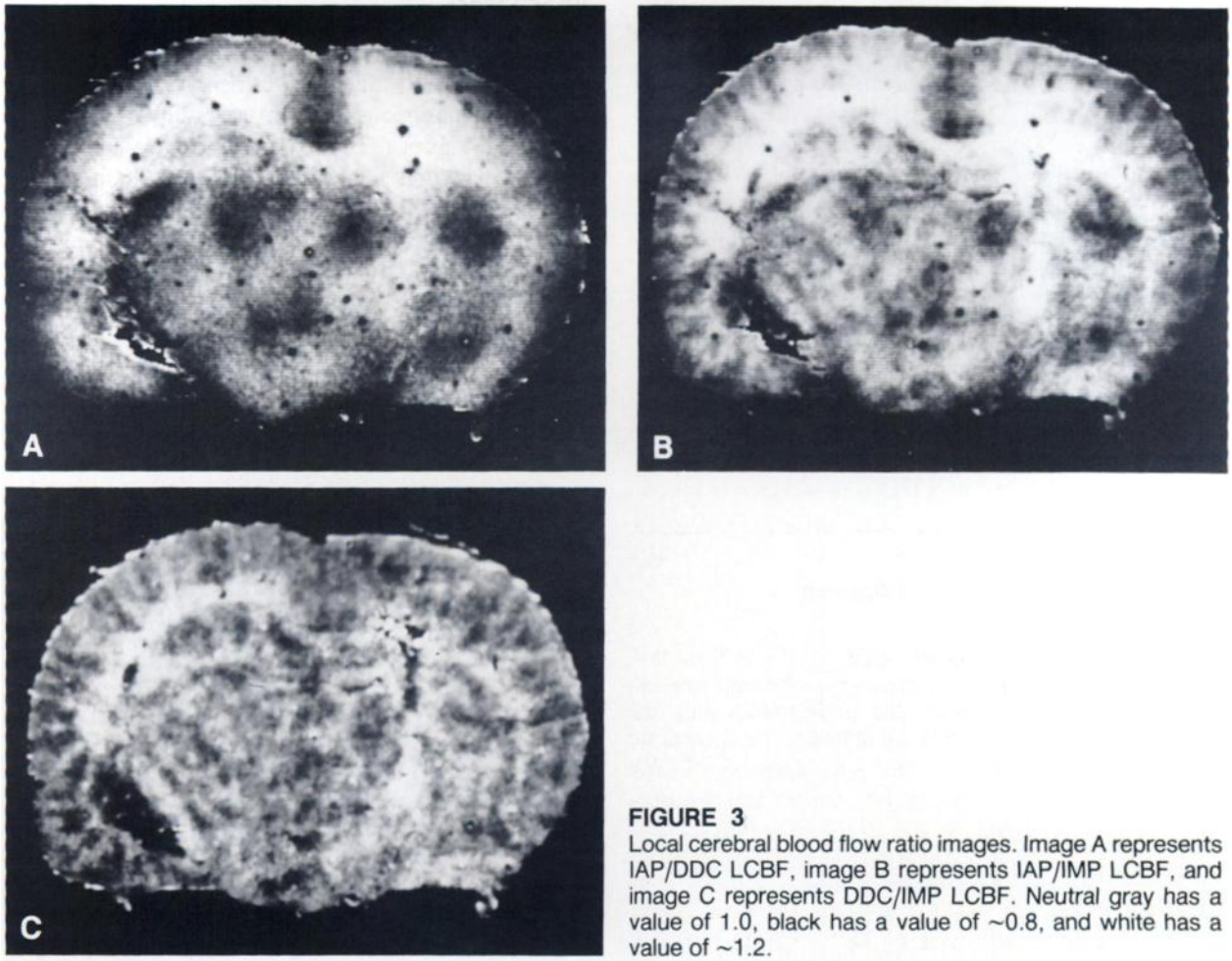

FIGURE 3
Local cerebral blood flow ratio images. Image A represents IAP/DDC LCBF, image B represents IAP/IMP LCBF, and image C represents DDC/IMP LCBF. Neutral gray has a value of 1.0, black has a value of ~0.8, and white has a value of ~1.2.

values in high flow structures with IAP relate to its diffusion properties. The assumption of complete capillary-brain tracer equilibrium has been challenged with respect to IAP (*12*). While the conclusions of that study have been shown to be at least partially incorrect (*5*), the actual experimental data tend to support the contention that full equilibrium does not occur. This would cause underestimation of LCBF in areas with very high blood flow. The second possibility for underestimation of LCBF in high flow structures is that diffusion within the brain after death can affect tracer distribution. A few minutes pass before the brain can be removed and frozen. During this time, a highly diffusible tracer can diffuse from areas of high concentration to areas of lower concentration. This phenomenon would also cause underestimation of LCBF in high flow regions. Because IMP and DDC are more tightly localized, they would not be as affected in this manner.

The underestimation of LCBF by DDC and IMP in some white matter and adjacent structures probably results from errors in the assumptions that all capillary-brain tissue regions are identical and that tracer distribution is uniform within a region.

IMP has a very great affinity for the brain in comparison to blood, as evidenced by its partition coefficient. This causes very rapid extraction and localization, so that tissue adjacent to the arterial side of the capillaries may have higher IMP concentration than tissue adjacent to the venous end. IMP may also be partially extracted from arterioles themselves. Capillary beds which are distal in the cerebral circulation therefore have a lower input concentration than proximal regions. Because cerebral circulation flows from the cortex inward, the inner cortex and white matter regions would be most affected (*13*). Evidence of this occurrence can be seen in Figures 1 and 2. The curvilinear areas of high IMP activity correspond to arterioles which can be seen on stained sections (*5*). The IAP/IMP ratio increases inwardly from the outer cortex. Thus the underestimation of LCBF by IMP in comparison to IAP in white matter results from the distal location of the white matter rather than its LCBF. DDC has a partition coefficient, and thus cerebral avidity, which is only slightly less than that of IMP. It therefore probably behaves similarly with respect to proximal extraction and this is supported by the IAP/DDC LCBF ratio image in Figure 3. The DDC/IMP LCBF ratio image in Figure 3 shows that this effect is slightly more

pronounced with IMP as evidenced by the slightly higher than average ratio in the corpus callosum.

Because of the short infusion times and type of kinetic model used, LCBF values will tend to be underestimated rather than overestimated if equilibrium assumptions are not entirely valid. Thus, we can conclude that IAP underestimates absolute LCBF in some areas of very high LCBF, and that IMP and DDC underestimate absolute LCBF in some distal structures.

This study indicates that, in general, cerebral uptake of DDC occurs in a manner which is intermediate to IAP and IMP, although closer to IMP. While none of the tracers was found to be a "gold standard" for LCBF measurement, all three can be used in animal studies with autoradiography as long as their limitations are appreciated. Only IMP and DDC, however, are practically suitable for *in vivo* studies in humans because of times needed for imaging.

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