What Method to Choose for Quantification of Cerebral Blood Flow in Experimental Research

QUANTIFICATION of perfusion is an important question in research in experimental cerebral ischemia, and numerous techniques of measurements based on various physical principles have been developed. The ideal method should permit continuous total, regional, and local flow determinations. It should be applicable in unanesthetized intact animals without causing any structural or functional impairment. No presently available method can meet all of these requirements. Each technique has certain advantages and limitations that must be considered with respect to the problem under investigation.

In the history of cerebrovascular research direct observation of the pial circulation was employed first. This method permits continuous monitoring of fluid and particle movement within the vessels under normal and pathological conditions. It made possible the detection of pial vascular responses to various physiological, pathological or pharmacological stimuli, e.g., the "sausage string phenomenon" at the point of break-through of autoregulation in hypertension. This method necessitates a large craniectomy and the flow rate per unit weight of brain tissue cannot be determined. In contrast to the direct observation of the pial circulation, which is still being used in several laboratories, another classical method, the measurement of blood flow in vessels by flowmeters has largely been abandoned because this method only yields an estimate of the volume of blood flowing through a vessel whose territory of supplied tissue is indeterminate. Flowmeters are difficult to calibrate and the positioning of the probe itself often impedes flow. Venous outflow methods can be calibrated more easily, but the tissue volume from which the blood is drained is not exactly known, and when a sinus or the jugular vein is used for sampling, extracerebral contamination may occur.

The heat clearance method may be used for estimation of flow in large vessels where the same limitations apply as for other flowmeter methods, or in the brain tissue. The latter application has been used extensively since the introduction of the heated thermocouple yielding reproducible flow estimates in terms of thermal conductivity. With this technique tissue perfusion can be monitored continuously in open skull preparations. The relationship of thermal conductivity and tissue perfusion is non-linear and may vary considerably from experiment to experiment.

More recently, interest has focused on tracer techniques that permit a quantitative assessment of cerebral blood flow. Various methods have been developed using diffusible tracers and calculation of flow per unit weight and time from arteriovenous differences of the tracer, from the tissue desaturation curve of the tracer, or from the tissue concentration of the tracer during saturation. The first method requiring direct determination of concentration curves in large cerebral veins or sinuses during saturation or desaturation with an inhaled tracer — N₂O, ¹³³Xenon, Kᵣ or molecular hydrogen — suffers from the same limitations as the venous outflow methods mentioned above: contamination by blood originating from extracerebral tissue cannot be eliminated and results represent an average flow in a poorly defined tissue volume. With these methods flow measurements may be repeated and venous blood may be drawn for determination of metabolic substrates. When the tissue clearance of gamma-emitting radioactive tracers — ¹³³Xe or H₂¹⁸O — is followed by external counting, no craniectomy is needed. Due to the high energy of gamma-rays the recorded count rates originate from large and not clearly defined volumes including extracerebral tissue when strictly intracarotid injections cannot be performed as in most experimental animals. This disadvantage can be overcome by local injection of ¹³³Xe — a technique that may seriously damage the tissue of interest.

When the clearance of Kᵣ is recorded the emitted beta-radiation originates from the cortex to the depth of 1 mm only. There is no extracerebral contamination and measurements can be taken over well localized areas, but the skull and even the dura mater must be removed.

When appearance and clearance of injected or inhaled molecular hydrogen is recorded polographic electrodes have to be advanced into the tissue or placed on the surface of the cortex. The volume of tissue where changes in H₂ concentration are recorded is quite small. Electrodes may be implanted for chronic experiments in freely moving animals. This approach traumatizes brain tissue, but with small and sharp electrodes the damage is minute as can be concluded from the normal activity of cortical cells in the vicinity, which may be recorded with the same electrode.

In contrast to the clearance techniques permitting repeated measurement, methods based on the determination of the concentration of tracer material...
during tissue saturation yield a single flow measurement only, because the animal has to be sacrificed during the procedure. Autoradiographic flow determinations are particularly localized and multiregional and, therefore, values obtained by these techniques generally are regarded as a reference representing "true flow" (133I-trifluo-iodomethan, 14C-antipyrine, 13C-iodoantipyrine). With methods of tissue sampling flow determinations are less regional, separation of different tissue compartments is difficult and may be a source of error (141I-antipyrine, 14C-antipyrine, 14C-ethanol and 1H-water).

When tissue sampling techniques are used for the determination of non-diffusible tracers, e.g., carbonized microspheres, up to 5 measurements may be performed using different nuclides for labeling. Surgery of the head is not necessary for this approach, but the animals must be anesthetized and immobilized for intracardiac injection and arterial blood sampling. Recently, it has been demonstrated that injections of microspheres have a short-lasting effect on the cerebral microcirculation. As with other methods based on tissue samples, pieces for examination have to be rather large (0.3 — 1g), and separation of tissue compartments may be inaccurate. Inhomogeneities in particle distribution and loss of trapped microspheres during subsequent flow alterations may cause additional errors. The sampled tissue may be used for a number of biochemical assays.

Flow values obtained by the various methods are rather discrepant, even in the same animal under comparable experimental conditions, both normal and pathological. The highest values are found by autoradiographic methods, but some of the reported flow data may be strongly influenced by anxiety in unanesthetized animals. The lowest values in normal animals usually are reported with the microsphere technique, and the results obtained by the clearance methods with radioactive tracers or H2 range between these 2 extremes. However, due to the heterogeneity of flow alterations concomitant with experimental focal ischemia, e.g., during and after middle cerebral artery occlusion, maximal changes of flow often cannot be resolved by the microsphere technique, because differences between adjacent small regions in the relatively large tissue samples needed for the counting of microspheres may cancel each other.

Keeping in mind the limitations of each technique the method to be employed should be chosen according to the individual experimental problem.

Great caution must be used in comparing results obtained from different methods. When exact absolute flow values with high regionality are of interest, autoradiography should be used, but repeated measurement cannot be performed. Several measurements in distinct, but relative large volumes can be achieved by microspheres without surgery to the head and brain. An unlimited number of flow determinations can be made by the clearance methods, but regional determinations may require craniectomy or even implantation of electrodes. If simultaneous determinations of other experimental variables are intended such procedures call for expensive double tracer techniques with autoradiography, but may easily be obtained in tissue sampling techniques or by applying the polarographic electrodes for electrophysiological recordings or determination of tissue oxygen tension.

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