Cerebral Blood Flow and Edema Following Carotid Occlusion in the Gerbil

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SUMMARY A technique for measuring focal cerebral blood flow (CBF) and brain specific gravity (SG) in gerbils is described; CO2 reactivity and autoregulation were tested. The mean CBF was 29.5 ± 4.5 ml/100 gm/min and brain SG 1.0500 ± 0.0004. Unilateral carotid occlusion resulted in a reduction of flow to 12.8 ± 5.8 ml/100 gm/min in the ipsilateral hemisphere; there was also a decrease in brain SG. One hour after occlusion, brain edema, as judged by decreased SG, developed at CBF less than 20 ml/100 gm/min and reached maximal levels at 7 ± 2 ml/100 gm/min. The amount of edema appeared to be related chiefly to the residual post-occlusion flow. With bilateral occlusion, CBF was close to zero and there was no change in SG, indicating that in the "no flow" situation, there is no edema.

BRAIN SWELLING, or edema, is associated with many of the major causes of intracranial catastrophe and its formation may result in the patient’s death by trans-tentorial herniation. It is frequently associated with subarachnoid hemorrhage and vasospasm. Up to a third of the patients with cerebral infarction may have edema sufficient to cause trans-tentorial herniation and most head injuries have a greater or lesser degree of brain swelling. Recent studies have shown that there are many components of brain swelling; Klatzo1 has emphasized vasogenic edema due to leakage of protein-rich fluid from damaged blood vessels and cytotoxic edema, characterized by the accumulation of intracellular fluid. Edema associated with hydrocephalus has been termed interstitial edema2 and, more recently, ischemic edema is thought to be sufficiently different to require a separate classification. The initiating factors in the development of brain swelling are still incompletely understood. Much of the early work has been histological and electronmicroscopic and, more recently, these qualitative results have been quantified in various ways. Previous work in this laboratory3–5 has shown the close relationship of flow, oxygen and electrical activity in the area around a stroke and has related the outflow of potassium to the residual blood flow in an area of infarction. This functional approach has recently been extended to the study of the development of edema and flow in experimental stroke in primates.6

Many species have been used in the study of cerebral edema. Early studies have been with rats.7 O’Brien et al.8 and Hossmann9 have investigated the problem in cats, and, more recently, the development of edema in sub-human primates has been described.10 The Mongolian gerbil preparation10 has been used extensively in clinical,11 pathological,12, 13 blood flow, and oxygen14 availability studies. Thus, it seemed an appropriate model to study for cerebral blood flow and edema formation.

Materials and Methods

Measurements

Local cerebral blood flow was determined using the hydrogen clearance technique developed in these laboratories.15 Electrodes of 1 mm were made from Teflon-coated 90% platinum, 10% iridium wire, 100 µ in diameter and the distal 0.5 mm tip of the electrode was bared. The electrodes were polarized 400 mv positive to a silver/silver chloride reference electrode placed subcutaneously in the back of the animal. Hydrogen of 2% in oxygen was delivered by an animal respirator through a funnel held over the animal’s face. After saturation, clearances were recorded and replotted on semilogarithmic paper. Flow was calculated using the “initial slope” technique.16 To ensure that this animal model fulfilled the indicator dilution requirements for the mathematical modelling of the flow curves, a platinum electrode was inserted in the aorta of several animals and simultaneous aortic and cerebral tissue wash-out curves were studied in several animals. The rate at which the arterial H2 concentration fell towards zero after discontinuing the H2 administration was extremely fast with 90% of systemic hydrogen cleared within 5 sec. The calculation of slow flow, i.e. less than 5 ml/100 gm/min, presented some problem; we considered zero flow to exist only if there was no clearance of hydrogen from the tissue and also no subsequent change on resaturation of the animal.

Specific gravity measurements were made by the technique of Nelson et al.17 using a variable density bromobenzene-kerosene column. After calibration of
the column with liquids of known specific gravity, small pieces of grey matter, 1 mm or less, were dropped into the fluid column and their position noted 3 min after insertion. Four to 6 specimens of grey matter were obtained from the tissue adjacent to each hydrogen electrode placement and the specific gravity of these were expressed as a mean of these results and related to the local flow. The brain was rapidly removed from the animal, stored immediately under bromobenzene-kerosene and the specimens for assay dissected, using the operating microscope. All measurements were performed immediately after the dissection; no specimens were stored or frozen. Using these rigorous techniques, there was an extremely small variation in specific gravity of samples taken from a particular area.

Preparation

Forty Mongolian gerbils (Meriones unguiculatus) weighing between 40 and 60 gm, were anesthetized using 60 mg/kg pentobarbital, injected intraperitoneally. Four small drill holes were made in the skull, 2 in front of the coronal suture and 2 behind, on each side; the prepared electrodes were inserted with micromanipulators through these into grey matter. Care was taken to avoid any obvious surface vessels. The electrodes were fixed into position with cool setting dental acrylic cement. A silver/silver chloride reference electrode was placed subcutaneously in the back. The abdominal aorta was exposed and a 21-gauge polyethylene catheter was inserted above the iliac bifurcation with the tip of the cannula fixed below the origin of the renal arteries. After placement of the catheter the abdomen was resutured. The cannula was connected to a system blood pressure transducer (Statham P. 23G transducer); during the experiment, up to 3 samples of blood, 0.15 ml in volume, were removed for blood gas analysis on a Radiometer ABL3 blood gas analyzer. Care was taken throughout the procedure to maintain the gerbils' extremely small blood volume. The right common carotid artery was exposed in the neck and, after the control observations, it was occluded with an Acklund vascular clamp. One hour after the carotid artery occlusion, the animal was sacrificed, the brain rapidly removed and immersed in a bromobenzene-kerosene solution for dissection and specific gravity measurements. If there was excessive bleeding during the preparation, a high CO₂ or a low blood pressure, the preparation was discarded.

Twenty-five of the 40 gerbils described in this study were treated as described. Two animals underwent sham operations, that is, blood flow estimations, blood pressure and blood gas analysis without carotid artery occlusion. Repeated estimations of local cerebral flow were performed over 3 hours to determine if there was any alteration with time in our preparations' cerebral blood flow. Five animals had specific gravity measurements of their brains, after anesthesia with 60 mg/kg pentobarbital, to determine if placement of the hydrogen electrodes in the brain was itself causing edema. Specific gravity measurements of these animals were identical to those having the sham operation. CO₂ activity and autoregulation were also assessed in 4 animals, and the CO₂ reactivity was assessed at 1.9% per torr. Autoregulatory response to hypotension induced by bleeding was intact to 45 mm Hg mean blood pressure. In 4 other animals, the preparation was identical except that both carotid arteries were exposed in the neck and, after controlled observations, both carotids were occluded simultaneously.

Procedure

After control observations of blood pressure, blood gases and local cerebral flow, the animal, which was spontaneously breathing, was again saturated with hydrogen and at the peak of saturation the carotid clamp was applied. Further clearances were performed at 30 min and at 50 min and the brain was removed exactly one hour after the ligation of the carotid artery. After medullary transection the brain was quickly removed, and the specific gravity estimations were performed.

Results

With the animal breathing spontaneously, the mean blood pressure was 80 ± 6.4 mm Hg and the arterial CO₂ was 34 ± 5.9 torr, arterial oxygen was 90 ± 10 torr.

The mean control cerebral blood flow for all observations was 29.2 ± 4.9 ml/100 gm/min and carotid occlusion resulted in a reduction of 12.8 ± 5.8 ml/100 gm/min flow on the ipsilateral side, that is, a reduction of 43% (p < 0.001). This initial reduction of flow was maintained and at 1 hour after the carotid occlusion the group flow had reduced by 0.9 ± 7.1 ml/100 gm/min (NS) as compared to the clearance immediately after the application of the carotid clip. In the contralateral hemisphere, however, there was a wide variety of results. In some there was a marked reduction in flow and in others an increase in flow (as compared to the ipsilateral hemisphere where only one animal showed no decrease in flow) but, in general, flow in the contralateral hemisphere was similar to control values (control 28.1 ± 5.3 ml/100 gm/min, post clip 25.6 ml/100 gm/min). Four animals had bilateral simultaneous carotid occlusions and, following this, the blood flow in both hemispheres was reduced to less than 4 ml/100 gm/min. In 2 there was no flow detected. The group mean result was 2.8 ± 1.3 ml/100 gm/min (p < 0.0001) initially and 1 hour after occlusion the final flow was 0.5 ± 0.5 ml/100 gm/min. The specific gravity of grey matter in the control animals and those with sham operations was 1.050 ± 0.0004 and the mean specific gravity in the ipsilateral hemisphere following 1 hour occlusion was 1.0470 ± 0.0017 while that in the contralateral hemisphere was 1.0490 ± 0.0013; both these latter figures were significantly different from control values (p < 0.001). Individual flow measurements were correlated with individual specific gravity estimations and
they are represented in figure 1. There was no change in brain specific gravity until the flow was reduced below 19 ml/100 gm/min; thereafter, reductions in flow down to 7 ± 2 ml/100 gm/min resulted in decreasing specific gravity. In those animals with no flow, confirmed by no penetration of hydrogen on resaturation, there was no difference in brain specific gravity from control measurements. However, those which had very low flows, less than 4 ml/100 gm/min, showed edema but not to the extent noted at flows between 10 and 5 ml/100 gm/min. In those with bilateral occlusion there was no change in brain specific gravity. Several points of interest emerged in that the flows and their corresponding specific gravity measurements have been plotted regardless of their site of origin. Thus, in the contralateral hemisphere, although the group mean showed only a 2.5 ± 7.0 ml/100 gm/min reduction in flow, there were individual animals who showed a reduction comparable to the mean of the reduction on the affected ipsilateral hemisphere, and in these there was a decrease in specific gravity corresponding to the decrease in flow. As there was no obvious pattern, it seemed to be reasonable to correlate all the flows and specific gravities on the one graph as shown. When the data are expressed as grouped flow and grouped specific gravity measurements (fig. 2), a smooth curve can be seen connecting the points from the control values up to or down to a reduction in flow of 15 ml/100 gm/min; thereafter, the curve is not so smooth.

Discussion

For over 15 years the gerbil has been intensively used to study stroke. Klatzo's group has used the model to investigate the histological changes associated with carotid occlusion. There is, however, a degree in selection of their material as only those with severe neurological signs were selected for further histology. Fugimoto et al. have assessed flow by autoradiography. Although their paper does not publish the actual results of the flow measurements, it does show marked changes in the ipsilateral hemisphere, particularly the subcortical grey matter, with a suggestion that the contralateral thalamus may also be affected. Halsey and co-workers have used the hydrogen clearance technique to assess blood flow before and after carotid ligation. In the ventilated, anesthetized gerbil, their controlled blood flow was 36 ± 10 ml/100 gm/min while in the awake gerbil, using an isotope dilution technique, van Uitert and Levy estimated the flow to be 102 ± 14 ml/100 gm/min. In none of these experiments were the blood flow results related to the blood pressure or to CO2 levels. To our knowledge, this study presents for the first time a correlation of blood pressure, CO2 and cerebral blood flow in the spontaneously breathing gerbil. Our blood flow results are lower than the previously published results. This difference may be methodological on our part, in that our analysis of the flow curves is by the "flow initial" technique. Many of the curves that we obtained were monoeponential as we took care to place the hydrogen electrodes in grey matter. The relatively high Pco2 (35 mm Hg) may have been the result of the degree of hypoventilation in the animal secondary to neck and abdominal surgery, but we were careful to exclude all animals in which there was a significant hypercapnia due to respiratory obstruction. The hypoventilation may also have been due to our barbiturate anesthesia, which, in turn, may have resulted in a slowing of the cerebral circulation as compared to the results published by Halsey's group using 70% nitrous oxide-30% oxygen mixture. Most of their experiments were conducted on curarized and ventilated animals. Initially, we attempted inhalational anesthetic agents but found
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Figure 2. The same data as figure 1 but condensed to show mean and standard error for data at 2 ml/100 gm/min intervals. Note the decrease in specific gravity around 20 mg/100 gm/min. Flows of 7 ± 2 ml/100 gm/min were associated with more edema than expected from the projected curve, suggesting additional factors in its production at these low flows.

wide variations in blood pressure and blood gases; thus we compromised with steady, if slightly metabolically depressing, anesthesia.

With the application of the carotid clip, there was slight rise in blood pressure and a change in pulse pressure as would be expected with a carotid manipulation. Although our animals were spontaneously breathing and lightly anesthetized, we were unable to confirm Brierley's et al.29 observations of a high incidence of epilepsy following carotid occlusion in gerbils. The sudden and permanent drop in blood flow in the most affected area is in keeping with previous work24 and does not agree with Osburne and Halsey21 who showed a progressive decline in flow in the ipsilateral hemisphere.

The brain's specific gravity changes, in our experiment, are in keeping with those of Fugimoto et al.38; for the gerbil, in particular, our results from cortex were almost identical. As much of the literature on brain water is expressed in terms of specific gravity, we did not consider it justified to convert our results into absolute values of brain water.9 To our knowledge, this is the first report on regional cerebral blood flow and regional brain specific gravity following ischemia in the gerbil. It is interesting in view of Waltz's22 comments on the importance of blood flows in the region of 20 ml/100 gm/min as the point at which electrical activity begins to fail in a wide variety of species and also as the point at which edema is noted in the baboon cortex. The plot of our results (fig. 1) very closely resembles that published for baboon brain following middle cerebral artery occlusion.6

Cerebral blood flow and regional specific gravity, when correlated, demonstrate that the factor which determines the presence or absence of edema is the local residual flow, at least during the first hour after occlusion. The decrease in specific gravity occurred at identical blood flow values to that already reported.8 The point of maximum edema was associated with flows in the region of 7 ± 2 ml/100 gm/min and this is at the level at which previous work showed that there was a massive efflux of potassium,19,20 also maximum changes in brain tissue impedance.28 Using the bilateral carotid ligation technique we were able to obtain a "no-flow" situation and after one hour of this, we found that there was no change in brain specific gravity. This is in keeping with Hossmann's work6 which showed only a small increase in water content in the cat after complete ischemia; the experiment was also repeated in the rhesus monkey.29 Thus, with this model, we have been able to study a spectrum of flows ranging from zero to normal.

We believe there are 3 stages in the relationship between flow and edema.

In the initial phase, at blood flows between 11 and 20, edema may be due to the efflux of potassium and the ionic redistribution and the metabolic impairment, caused by ischemia, resulting in an increase in tissue osmolality which will attract water from the blood to the extra and intracellular compartments. With further reduction in flow, around 7 ± 2 ml/100 gm/min the specific gravity decreases more than expected from the projection of the exponential line. This may be due to metabolism failure disrupting ionic homeostasis leading to the following sequence. The outflow of potassium is coupled with an intracellular flow of sodium and water. Sodium would then move down its concentration gradient from blood to ECS carrying water and further lowering the specific gravity. This is equivalent to a combination of the ischemic and "recirculation phase 1" in Hossmann's model of complete ischemia.8 Another possibility may be the leakage of protein-rich intravascular fluid into the ischemic area which would
increase the local water content more than we could explain by the small changes in total ionic concentration (both sodium and potassium). There may be a vasogenic component to the edema at these values of flow since the "no flow" state shows no change in specific gravity. This may result from the sudden drop in metabolism causing ionic redistribution between the intracellular and the extracellular compartment, but with no blood stream for the osmotic pull to act on, there can be no change in specific gravity.

In this animal model and for this length of ischemia, it is the absolute level of flow in the ischemic area, rather than the change or reduction in flow, which is most closely correlated to the amount of edema. If our model is correct, it demonstrates that there is no real variance between the work of Hossmann and Reulen et al., but rather that the development of edema increases with decreasing flow to a point about 7 ml/100 gm/min; thereafter, because of the very poor flow or absent flow, edema does not occur. These results do not agree with Osborne and Halsey's conclusion that a residual flow, no matter how low, during ischemia appears to minimize the likelihood of brain death. Our initial impression is that the worst of all the models is correct, it demonstrates that there is no real variance between the work of Hossmann and Reulen et al., but rather that the development of edema increases with decreasing flow to a point about 7 ml/100 gm/min; thereafter, because of the very poor flow or absent flow, edema does not occur. These results do not agree with Osborne and Halsey's conclusion that a residual flow, no matter how low, during ischemia appears to minimize the likelihood of brain death. Our initial impression is that the worst of all possible combinations would be a flow of about 7 ml/100 gm/min.

In a gerbil model, focal cerebral blood flow has been correlated to focal brain specific gravity as a measure of water content. The model can be rigorously tested in terms of autoregulation and CO2 reactivity, as described in large animals. Edema and flow were correlated and there was an increase in water content as judged by the decrease in brain specific gravity at around 20 ml/100 gm/min. Water content increased to a maximum at a blood flow of about 7 ml/100 gm/min. Thereafter, with further decrease in flow, there was a marked reduction in edema formation. There is no edema in the "no flow" situation.

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*Stroke.* 1980;11:494-498
doi: 10.1161/01.STR.11.5.494

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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