EXTRACORPOREAL SHUNTING of canine blood through glass-wool prior to induction of global central nervous system (CNS) ischemia has been demonstrated to enhance post-ischemic brain blood flow and to eliminate focal zones of impaired reperfusion. The experimental model employed in these studies produces neurrayxias ischemia by cerebrospinal fluid (CSF) compression and serves as an assay system in which factors influencing the rate and distribution of post-ischemic CNS blood flow can be investigated. However, the dependent variable of concern in these experiments was blood flow and no index of neuronal function was measured so that conclusions regarding the effect of glass-wool filtration on nerve cell recovery could not be drawn from the data. If agents or measures that prevent impaired post-ischemic reperfusion should consistently fail to enhance return of some index of neuronal function after ischemic insults, the inference would seem warranted that such flow disruption was an epiphenomenon of irreversible nerve cell damage and, therefore, irrelevant to the ultimate level of functional return. If, however, these measures were to promote restoration of post-ischemic neuronal function, a relationship between the microcirculatory reflow impairment and the reversibility of ischemic nerve cell damage would appear likely.

The probability that measures counteracting impairment of microvascular reperfusion would contribute to functional recovery of neurons was judged greater for focal than for global ischemia. Accordingly, reversible focal ischemia was produced by selective injection of small volumes of air into the right internal carotid artery of dogs while monitoring the cortical sensory evoked response (CSER) as a quantifiable electrophysiologic index of neuronal function.

**Methods**

Twenty conditioned, male mongrel dogs were splenectomized 2–3 weeks prior to being entered into the experimental protocol. Each dog was administered xylazine 1.1 mg/kg and atropine 0.05 mg/kg subcutaneously followed by sodium pentobarbital 15 mg/kg i.v. initially with incremental doses as needed to suppress the corneal reflex. The animals were intubated and ventilated with a respirator. End-tidal PCO₂ and PO₂ were continuously monitored by Beckman LB-2 and OM-11 analyzers. Rectal temperature was maintained at 37–38°C with heating pads and infrared light except during recording of the CSER when hot water bottles were substituted to prevent generation of 60-cycle artifact. One or 2 catheters were placed in the right femoral artery. One was directed proximally into the aorta and, in 3 dogs, the other was directed into the distal femoral artery.
When these 2 catheters were later joined through a Y-connector in the 3 dogs, the arterial flow to the leg was externalized, permitting rapid sampling of arterial blood for the \(^{14}\)C-antipyrine autoradiographic blood flow assay. The aortic catheter permitted monitoring of systemic arterial blood pressure. A right ventricular catheter was placed via the right femoral vein. Catheters were threaded into the aorta from the left femoral artery and into the inferior vena cava from the left femoral vein. The arterial catheter permitted monitoring of arterial blood pressure. The venous catheter permitted intravenous infusion of various solutions and sampling of venous blood. In addition, the 2 catheters could be joined to form an arteriovenous shunt in which a glass-wool filter could be inserted. Glass-wool filters were assembled as previously described. The right internal carotid artery was carefully exposed and catheterized with PE 50 tubing. Glass-wool filters were assembled as previously described. The right internal carotid artery was carefully exposed and catheterized with PE 50 tubing. ECG leads were connected for monitoring heart rate and rhythm.

The dogs were placed in a Kopf® stereotaxic apparatus. During the series of experiments, 3 types of electrodes were used. The responses recorded with the 3 electrodes did not differ with regard to shape or latency and since each animal served as its own control, the percentage change in the amplitude of each response was directly comparable among electrodes. In early experiments, rigid stainless steel electrodes that were insulated except at the tips were impacted against the skull and held in place by stereotaxic electrode carriers. In later studies, holes were drilled in the skull after reflecting the scalp and nylon screws housing stainless steel electrodes were inserted so that the blunt electrode rested on the dura. In the most recent experiments, steel eyelet screws soldered to pin connectors were inserted into the skull. These latest electrodes were virtually noise-free and preserved an intact skull. The recording electrode was positioned over the right sensorimotor cortex and the reference electrode was abutted against the floor of the right frontal sinus in all experiments. Potentials from the recording electrodes were initially led to a preamplifier with a 0.1-100 Hz bandwidth and 4 x 10** gain. The output signal was led to another amplifier for a final gain of 8 x 10** and displayed on a Tektronix® oscilloscope while it was summed in parallel by a TMC-400C® computer of average transients (CAT). The CAT output was recorded on a Hewlet-Packard 7044A® XY-recorder. At least 60 evoked responses (epochs) were averaged to obtain each recorded response. The stimulating needle electrode was inserted percutaneously next to the closely apposed median and musculocutaneous nerves in the left upper foreleg, and an indifferent needle electrode was placed subcutaneously in the left lower foreleg. A square wave stimulus of 300 µ sec duration at a rate of one/sec was led through a Grass SIU5® stimulus isolation unit to the left foreleg. The strength of the stimulus was adjusted to cause minimal twitching of the left leg and paw. In this manner, a near-maximal evoked response was produced that was free of distortion from spread of the stimulus.

Animals were divided into 2 groups according to whether or not they underwent glass-wool filtration. The 9 animals in the first group were not exposed to glass-wool filtration. They were anticoagulated with 300 units/kg i.v. heparin and observed while the anesthetic level was maintained and blood gases, blood pH, blood pressure and rectal temperature were monitored. The 10 animals in the second group were anticoagulated with heparin and monitored as above but, in addition, were subjected to glass-wool filtration by extracorporeal shunting of blood from left femoral artery to left femoral vein through loosely packed glass-wool. The blood of each animal was filtered for 20 min through each of 3 glass-wool filters at an estimated flow rate of 50-100 ml/min for a total filtration period of one hour.

The subsequent operations were identical for both groups. Baseline CSERs were recorded and 50-100 µl of room air were injected into the right internal carotid artery catheter and flushed in with 500 µl of saline. After 2-3 minutes, another CSER was recorded. If the response was suppressed, no more air was infused. If the CSER was only partially suppressed, another 50-100 µl of air was delivered. Subsequently, the periodic re-emergence of an incipient CSER was suppressed by periodic infusion of 50-100 µl of air to restore the state of electrophysiologic neuronal silence. This cycle of alternating emergence and ischemic suppression of the evoked response was continued for 1 hour. After this, the CSER was repeatedly measured for a further 60 min to assess the degree of recovery.

Blood gases and pH were measured periodically throughout the experiments and appropriate adjustments of the respirator were made to maintain the values in the normal range. Hematocrit, white blood count and platelet count were measured initially and just prior to the recording of the final evoked response.

In 3 dogs, local brain blood flow was measured by the \(^{14}\)C-antipyrine autoradiographic blood flow technique. The blood flow study was performed at the conclusion of the 60 min CSER suppression in one animal and subsequent to the final CSER recording in 2 animals, one from each of the 2 groups. Essentially, this required intravenous infusion of 100 µc/kg of \(^{14}\)C-antipyrine for one min with serial sampling of arterial blood every 4 to 6 sec. Cardiac arrest terminated the procedure and was produced by injecting a bolus of 20 cc of saturated potassium chloride through the right femoral vein catheter into the right ventricle. The brain and heart were removed, frozen in −50°C to −60°C liquid freon suspended over liquid nitrogen, and cut into 20 micrometer sections. The tissue concentration of the isotope was determined autoradiographically. Local blood flow was calculated from the following formula: \(B_{x}(T) = \frac{\lambda k_r C_{x}(T)}{C_{x}(T) - C_{x}(0)}\) where \(C_{x}(T)\) = the concentration of tracer substance in the tissue at time \(T\); \(\lambda\) = the tissue-blood partition coefficient for the tracer material; \(k_r\) = the rate of blood flow per unit weight of tissue multiplied by the reciprocal of the partition coefficient for that tissue;
and \( C_a \) = the concentration of tracer substance in the arterial blood.

**Results**

A typical CSER is depicted schematically in figure 1. The wave designation is adapted from Ciganek\(^6\) and waves I-III are regarded as constituting the "primary response" of sensorimotor cortex to afferent stimulation via specific pathways. Latency A was measured from the stimulus to the peak of wave I and latency B from the stimulus to the peak of wave II. Latency A averaged 15 ms and latency B averaged 27 ms. There were no significant differences between control and final values within a group for either latency A or latency B. Also, latency values in one group did not differ significantly from the corresponding latencies in the other group.

By virtue of its relative reproducibility and stability, the peak to peak amplitude between waves I and II served as the quantifiable electrophysiologic index of neuronal function in this study. This amplitude was found to be less variable during serial recording than those measured between longer latency waves both in the control period and after ischemia. It was also the most resistant to obliteration by ischemia. Table 1 displays the mean ± standard error of the mean (SEM) and the range of amplitudes in the 2 groups at the 2 sampling points. There is considerable dispersion of the data at each sampling point and this is partially attributable to the use of several different electrode types during the study. Because of this large variance, recovery of the CSER at the final sampling point was expressed as a percentage of its respective control value in each animal. Table 2 shows for the 2 groups the mean ± SEM of the percentage recovery of the final amplitudes relative to their respective control amplitudes. The difference in degree of recovery between the 2 groups is highly significant.

Figure 2 condenses and compares the progression of CSER changes noted in the 2 experimental groups. The control CSERs in the 2 groups were sufficiently similar that tracing A may adequately represent either. In like manner, tracing B represents the ischemically suppressed CSER in both experimental groups. The degree of CSER recovery in unfiltered animals is shown in tracing C and reflects the 14 percent mean recovery in the unfiltered group. The CSER recovery in filtered animals is shown in tracing D.

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Control Unfiltered</th>
<th>Control Filtered</th>
<th>Final Unfiltered</th>
<th>Final Filtered</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean ± SEM</strong></td>
<td>45.8 ± 10.0</td>
<td>43.4 ± 17.7</td>
<td>5.6 ± 1.9</td>
<td>24.9 ± 8.0</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>5.7-197.7</td>
<td>9.1-197.0</td>
<td>0.0-14.9</td>
<td>3.1-72.2</td>
</tr>
</tbody>
</table>
which also corresponds closely to the 69 percent mean recovery in the filtered group. In the unfiltered group, 3 of the 9 animals manifested a declining peak to peak amplitude after an initial partial recovery. This secondary diminution in amplitude began about 45 min after the conclusion of the 60 min recovery period. Autoradiograms B and C illustrate the extent of blood flow return in an animal from each of the 2 groups at the conclusion of the 60 min recovery period. Autoradiogram B is from an unfiltered animal that received 100 μl air and recovered only 13% of its control CSER amplitude. It shows heterogeneous cortical blood flows with regions of relatively high blood flow adjacent to zones of residual perfusion impairment in which the rate of flow remains below levels critical for sustaining the CSER. Autoradiogram C is from an animal exposed to glass-wool filtration and shows post-embolic heterogeneity of flow. This animal received 350 μl air and fully recovered its evoked response. The trend for extremes of flow rate to occur contiguously is again apparent. The major difference between this autoradiogram and its counterpart, B, is that the foci of lowest cortical blood flow in this section are well above the threshold for maintenance of the CSER.

Figure 4 is an enlargement of autoradiogram C from Figure 3 with an insert from the anterior cerebral-middle cerebral watershed cortex. It shows an area of relatively low blood flow completely encircled by a zone of relative hyperemia. The well perfused zone may be diverting some fraction of its blood flow into the zone of relatively low flow as a source of collateral supply. The net result of this process is that flow in all cortical regions exceeds threshold levels for suppression or abolition of the CSER.

Discussion

There are a number of diverse clinical and experimental conditions involving acute CNS tissue damage which share in common, focal impairment of perfusion. This suggests that it is the presence of acute CNS tissue damage, rather than its specific cause, that is the important variable in the production of focal flow disruption. Findings in other studies suggest that blood has a positive role in the focal flow

Table 2: Percentage Recovery of Final Amplitudes Relative to their Respective Control Amplitudes

<table>
<thead>
<tr>
<th>Percentage recovery</th>
<th>Unfiltered</th>
<th>Filtered</th>
<th>Significance of differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>69</td>
<td>14 ± 4*</td>
<td>69 ± 12</td>
<td>p &lt; 0.0004*</td>
</tr>
</tbody>
</table>

*By Student t-test with the Welch approximation for unequal variance

Table 3: Blood Gases and pH at 2 Points during Experiment (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Final</th>
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<tbody>
<tr>
<td>Unfiltered</td>
<td>Filtered</td>
<td>Unfiltered</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>31.2 ± 1.9</td>
<td>33.9 ± 1.7</td>
</tr>
<tr>
<td>PaO₂</td>
<td>98.1 ± 3.4</td>
<td>88.3 ± 3.9</td>
</tr>
<tr>
<td>pH</td>
<td>7.40 ± 0.02</td>
<td>7.39 ± 0.01</td>
</tr>
</tbody>
</table>

Table 4: Mean Arterial Blood Pressure and Hematologic Indices at 2 Points during Experiment (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfiltered</td>
<td>Filtered</td>
<td>Unfiltered</td>
</tr>
<tr>
<td>Mean arterial blood pressure</td>
<td>101 ± 2</td>
<td>103 ± 5</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>43 ± 2</td>
<td>40 ± 2</td>
</tr>
<tr>
<td>WBC × 10⁶</td>
<td>13.4 ± 1.6</td>
<td>15.9 ± 2.6</td>
</tr>
<tr>
<td>Platelets × 10⁶</td>
<td>387.5 ± 48.7</td>
<td>305.5 ± 33.9</td>
</tr>
</tbody>
</table>
A B c

FIGURE 3. Autoradiograms developed from coronal sections of brain cut from similar neuroanatomic levels in each of 3 animals. Autoradiogram (A) is from an animal subjected to 60 min of ischemic suppression of the CSER by incremental air embolism. The flow measurement was performed at the conclusion of the 60 min suppression period. Autoradiograms (B) and (C) are from flow measurements performed at the end of the 60 min recovery period in an unfiltered and a filtered animal, respectively. Gray-scale densities are proportional to the rate of blood flow within each autoradiogram but are not directly comparable between autoradiograms. (See text for discussion).

The relationship, if any, between the presence or absence of post-ischemic impairment of microvascular reperfusion in brain and the level of functional recovery achieved by that brain remains to be clarified. A direct approach is to examine the influence of measures that prevent impaired reperfusion on the degree of neuronal recovery after ischemic insults. Should such measures prove beneficial, an integral role for impaired reperfusion in the evolution of cell damage would be suggested. Failure of these measures to promote return of neuronal function would be consistent with an incidental role for impaired reperfusion as perhaps an epiphenomenon of irreversible nerve cell damage.

The probability that impairment of microvascular perfusion could act as an integral force in the process leading to irreversible neuronal damage seems greater for focal than for global ischemia. The persistence of flow in circulatory beds adjacent to those supplied by the obstructed vessels affords an opportunity for collateral circulation. Focal ischemic lesions in monkeys secondary to middle cerebral artery clipping are largely reversible for 2 to 3 hours. Since this exceeds anyone's claim for neuronal ability to withstand glucose and oxygen deprivation, and since collateral circulation has been demonstrated angiographically after middle cerebral artery occlusion, the final infarct may depend on failure of collateral circulation over time. Progressive failure of collateral circulation has been observed in middle cerebral artery occlusion. The blood-damaged tissue interaction hypothesis would predict that slow flows in zones of acute tissue damage should tend to shut themselves off. Blood entering ischemic zones through circuitous collateral routes should theoretically be vulnerable to just such a progressive flow interference. Measured that eliminate or attenuate development of impaired microvascular perfusion could tend to protect and sustain nutrient flow in the injury zone that is supported by collateral circulation under these circumstances and facilitate neuronal survival.

Incremental air embolism, as used in this study, produces a resolving form of focal ischemia which is better standardized by monitoring its effect on a neuronal pool in the zone of flow disruption than by regulating the dose of air on a volume per kilogram basis. Small volumes of air delivered into the cerebral circulation obstruct the flow temporarily and its restoration, as viewed on the pial surface, is uneven with stasis and brisk flow juxtaposed in adjacent vessels (personal observation). Widely varying volumes of air are required to produce the same physiologic effect in different animals as demonstrated in this study and in others. For these reasons, periodic suppression of a returning electrophysiologic response generated by a neuronal pool on the lateral surface of the affected hemisphere, such that the response fluctuates around threshold, constitutes an appropriate end-point for regulating the volume of air delivered. Selective internal carotid artery catheterization was performed on the strength...
of studies demonstrating that small volumes of air injected from this site at close to the normal flow rate have a high probability of entering the brain without straying into extracranial vessels.

The results indicate that recovery of the CSER was enhanced in the group of heparinized dogs exposed to glass-wool filtration relative to the group of dogs receiving heparin alone. The CSER is a quantifiable electrophysiologic index of neuronal function although the cellular mechanisms of the neuronal pool "generator" have not been completely defined.

Recovery of the CSER has been shown to have prognostic significance in acute CNS injury but full return of an evoked response does not exclude the presence of a residual neurologic deficit. Persistent depression of the evoked response is associated with severe neurologic damage.

Electroencephalographic activity and evoked cortical potentials, as well as extracellularly recorded single-unit activity, bear an interesting threshold relationship to the rate of cerebral blood flow. The amplitude and wave-form of such electrophysiologic activity is preserved at flow rates greater than 16 to 20 ml/100gm/min. Between 12 and 16 ml/100gm/min, the CSER amplitude varies in direct proportion to flow rate and below 12 ml/100gm/min, the response is abolished. Furthermore, single-unit activity, which disappeared as flow fell below 20 ml/100gm/min, was observed to return when flow resumed near-normal rates. Conversely, cerebral cortical energy metabolism and electrophysiologic indices of neuronal function are not tightly coupled after a period of arrested circulation in brain. Despite post-ischemic restoration of cortical energy reserves to normal or near-normal levels, the recorded cortical potentials can remain distinctly abnormal. In addition, the normally high correlation between regional cerebral metabolic activity as manifested by the regional cerebral metabolic rate for oxygen and regional cerebral flow breaks down during acute brain disease.

We attribute the protection afforded by prophylactic glass-wool filtration in this study to the preservation of nutrient flow in the injury zone that is supported by collateral circulation. The observed contiguity of clearly demarcated extremes of flow in a region of heterogeneous reperfusion is probably not fortuitous. An interpretation of this phenomenon is that a fraction of the relatively high flow in a well perfused zone is superfluous to the metabolic needs of that tissue and can be diverted into the adjacent zone of impaired perfusion as collateral blood supply. Collateral circulation has been described on the pial surface and "thoroughfare channels" have been observed within the brain parenchyma. Collateral circulation has been measured within the middle cerebral artery distribution after middle cerebral artery clipping and its rate has been found directly related to the capacity for neuronal recovery. The occurrence of zones of relatively high flow at the periphery of ischemic zones has been well documented and has been referred to variously as luxury perfusion and cerebral vasomotor paralysis. Also, red venous blood has been observed issuing from such zones and angiograms have demonstrated early filling of veins. The stimulus for such luxury perfusion is probably the accumulation of acid metabolites. Residual air may underlie the increased cerebrovascular resistance in the regions of relatively low flow in this study and the combination of reduced perfusion rate and tissue damage would be predicted by the blood-damaged tissue interaction hypothesis to progressively staunch injury zone nutrient blood flow that is being supported by collateral circulation. The progressive shutdown of nutrient flow is viewed as continuing during the recovery period in unfiltered animals. Modification of animals' blood by exposure to glass-wool prior to induction of CNS ischemia has been demonstrated to eliminate focal zones of impaired reperfusion after global ischemia and presumably has attenuated or aborted progressive shutdown of nutrient flow in zones of residually high vascular resistance in the filtered animals of this series. Thus, with the aid of collateral circulation, the rate of blood flow in these zones was maintained at levels compatible with CSER recovery (fig. 4).

The results of this study offer some support for the possibility that measures preventing impaired microvascular reperfusion may find therapeutic
application in focal ischemic disorders affecting the central nervous system.

Acknowledgment

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