INTRACRANIAL HEMORRHAGE due to rupture of subarachnoid aneurysm is usually associated with an immediate cerebral vasospasm of variable duration. Several approaches to the prevention and treatment of cerebral vasospasm have been proposed including expansion of intravascular fluid volume, reserpine and other anti-serotonin drugs, α-adrenoceptor antagonists and β-adrenoceptor agonists, and various vaso­ dilators such as nifedipine and sodium nitroprusside.

While investigating the cardiovascular actions of the potent vasoconstrictor angiotensin II, we observed that the inhibition of the conversion of angiotensin I to angiotensin II resulted in fall of peripheral vascular resistance and increase in cardiac output, with wide redistribution of regional blood flow to the kidneys, adrenals, brain, and heart, at the expense of blood flow to the musculocutaneous tissues, without substantial change in systemic blood pressure. This suggested that the arterial trees of the "favored" organs were particularly sensitive to the vasoconstrictor effect of angiotensin II.

The present study was designed to investigate the possibility that angiotensin II might play a role in acute cerebral vasospasm following introduction of blood into the subarachnoid space. Accordingly, we studied the effect of the angiotensin converting enzyme inhibitor, teprotide (SQ20881) on the acute vasospasm as determined angiographically in the dog.

**Animals and Procedures**

Ten male beagle conditioned dogs weighing approximately 10kg were used for these experiments. They were maintained on Purina dog chow and tap water ad lib. The animals were anesthetized with sodium pentobarbital 30mg/kg I.V., intubated, and placed on a Harvard respirator. The left femoral artery was cannulated for blood sampling and blood pressure recording. Through an incision in the right femoral artery a no. 5 french Newton Cerebral catheter was inserted and a vertebral artery was selectively catheterized. A baseline cine-arteriogram was obtained at this point. After surgical exposure of the atlanto-occipital membrane, a 22 gauge spinal needle was inserted into the cisterna magna and 2 ml of cerebrospinal fluid was removed. Two ml of autologous blood was then placed in the subarachnoid space through the same needle. Thirty minutes after this a repeat cine-arteriogram was obtained and a 7 ml blood sample was drawn for determination of blood PCO₂, pO₂, and plasma renin activity. Arterial pressure was measured directly via a Statham P23Db pressure transducer. Thereafter, on seven dogs teprotide 1 mg/kg was injected intravene­ nously. Repeat cine-arteriograms were obtained again 30, 60, and 90 minutes later. At 60 minutes and 90 minutes arterial blood pressure was measured again and 7 ml blood sample was again drawn for blood gases and plasma renin activity. Three control dogs were subjected to the same procedures under identical conditions, except that they received an injection of 2 ml 0.9% saline instead of teprotide.

Blood pH, pO₂ and pCO₂ were determined by a Radiometer pHm71 Acid Base Analyzer (Rainin Instrument Co., Inc., Brighton, MA) and plasma renin activity was measured by radioimmunooassay of generated angiotensin I after three hours incubation.

Angiography was obtained with Iothalamate Meglumine (Conray) 60% as contrast medium, injected at the rate of 1.5 ml per second for a total of 3 ml. The arteriogram was recorded on a 35 mm Cine Fluorography system with the image intensifier at a fixed 45 cm from table top. Films were taken at the rate of 60 frames per second. After processing, the films were evaluated dynamically and the frame demonstrating the widest diameter of the basilar artery on each angiogram was selected for magnification. The selected frames were magnified ten times with the use of a standard enlarger onto Kodak NMB film. This film was then processed through a usual X-ray processing...
unit and the enlarged angiograms were evaluated by microdensitometry (Joyce Loeb and Co., MK III C Double Beam Recording Microdensitometer) according to the following procedure: The paper tray speed to scanning speed ratio (peak width on paper/basilar artery width on film) was set at 5:1. The scanning beam slit width was set at 40 μ. For each experiment, the appropriate optical density gradient wedge was selected to produce peaks of sufficient height as to yield the most accurate width measurements.

The artery width of each angiogram was determined by averaging approximately 15–20 scans across the vessel. Scans were performed perpendicular to the basilar artery and were spaced approximately 2 mm apart, starting at the junction to the Circle of Willis. The integration length (beam length) was approximately 3 mm. The position of each scan was recorded on the film for correlation of photo and resulting plot, if necessary.

The width of the peak plotted on a given scan was measured by first fitting straight lines to the slopes of the peak, and then determining average background values on either side of the peak. The slope lines were graphical averages of the noise fluctuations on the sides of the peak. When necessary, the photo was examined at the point of the scan to determine whether a particular glitch was background or basilar artery.

The average background values were determined by using the points of the scan trace before and after the peak. The intersection of the two slope lines and the intersections of the slope lines with their respective average background lines defined the two slope line segments. The midpoints of the two slope line segments were measured, and the distance between them, projected along the direction of the paper tray motion, was called the width.

For each angiogram, the mean and standard error of these widths was calculated. Difference in changes of basilar artery width after teprotide or saline infusion in experimental and control animals respectively, were evaluated by Student's t test for non-paired data.

### Results

Table 1 indicates the percent decrease in the width of the basilar artery produced in all dogs 30 min after the introduction of blood intracisternally, and the increase in width observed at 30 and 90 minutes after the injection of teprotide. It is apparent that two of the dogs (#4 and 6) had either complete restoration of arterial diameter or vasodilation beyond the baseline and the remaining four had very substantial improvement, with reversal by 78% to 40% of arterial spasm at 90 minutes post-teprotide. In the three control animals (which received 2 ml of saline instead of teprotide) 90 minutes later the diameter of the basilar artery was either unchanged or showed a further decrease. Despite the small numbers in each group, the difference in changes is highly significant (t = 3.674, p < 0.005).

### TABLE 1 Change in Width of Basilar Artery (% of Controls)

<table>
<thead>
<tr>
<th>Dog</th>
<th>30 minutes post hemorrhage</th>
<th>30 minutes post teprotide</th>
<th>90 minutes post teprotide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-26%</td>
<td>-6%</td>
<td>-11%</td>
</tr>
<tr>
<td>2</td>
<td>-24%</td>
<td>-9%</td>
<td>-10%</td>
</tr>
<tr>
<td>3</td>
<td>-15%</td>
<td>-4%</td>
<td>-9%</td>
</tr>
<tr>
<td>4</td>
<td>-8%</td>
<td>+2%</td>
<td>+5%</td>
</tr>
<tr>
<td>5</td>
<td>-19%</td>
<td>-15%</td>
<td>-5%</td>
</tr>
<tr>
<td>6</td>
<td>-21%</td>
<td>-1%</td>
<td>-1%</td>
</tr>
<tr>
<td>7</td>
<td>-30%</td>
<td>-7%</td>
<td>-10%</td>
</tr>
<tr>
<td>Controls</td>
<td>30 minutes post saline</td>
<td>90 minutes post saline</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>-17%</td>
<td>-13%</td>
<td>-15%</td>
</tr>
<tr>
<td>9</td>
<td>-1%</td>
<td>-12%</td>
<td>-12%</td>
</tr>
<tr>
<td>10</td>
<td>-15%</td>
<td>-21%</td>
<td>-24%</td>
</tr>
</tbody>
</table>

did the basilar artery (fig. 1a and b). Unfortunately this could not be quantified. However, 90 minutes post teprotide, the branches and collaterals were again clearly visible (fig. 1c). These pictures suggest that restoration of collateral circulation may be an equally or more important indicator of release of spasm than the recovery of the width of a major artery.

The blood pressure and biochemical parameters measured during the experiments are shown in table 2.

It is apparent that baseline values were similar for both the experimental and the control dogs. After infusion, animals that received teprotide had as expected, elevation of plasma renin, whereas all other parameters were similar in both groups.

### Discussion

The possibility that angiotensin may participate in cerebral vasospasm has been dismissed by most investigators. It was reasoned that the angiotensin II contained in extravasated blood would be rapidly destroyed by angiotensinases. However, there is evidence that angiotensin II can be generated locally at the peripheral vascular wall level if substrate is available. Renin-like enzymes and angiotensin converting enzyme have both been detected in cerebral microvessels. Our results demonstrate angiographically that inhibition of angiotensin converting enzyme with teprotide administered thirty minutes after blood was placed in contact with the adventitial surface of cerebral arteries could partially or totally reverse the established acute arterial spasm within thirty to ninety minutes in all the animals tested. Thus, at 30 min after bleeding, animals had a decrease in basilar artery width ranging between 8 and 30% (table 1); at 90 min after teprotide, the width of the basilar artery had been completely or partially restored, and, in fact, dog #4 ended up with a basilar artery larger by 5% over its original width. On the contrary, the spasm in control dogs either remained unchanged over this period or actually intensified further at 90 min after saline infusion as compared to the degree of spasm at 30 min post bleeding. The basilar...
artery was chosen for objective measurement of changes in arterial width according to the method of Zervas.\textsuperscript{10} However, the disappearance of the branches around the Circle of Willis at thirty minutes and their reappearance after injection of teprotide is also very striking (fig. 1) and probably is more representative of changes in cerebral blood flow, though this is difficult to quantify.

Acute cerebral vasospasm is often followed by delayed cerebral vasospasm which is an important determinant in the outcome of subarachnoid hemorrhage. A variety of pharmacologic agents have been used in an effort to release the spasm.\textsuperscript{1,4, 10, 14, 18, 19} In particular the use of vasodilators such as sodium nitroprusside has not met with success, possibly because it is associated with fall of systemic blood pressure and therefore reduced cerebral blood flow. A similar drawback might be expected from agents which decrease cardiac output, such as reserpine.

Angiotensin inhibition does not produce a hypertensive effect in normotensive subjects, probably because the fall in systemic vascular resistance is compensated by an increase in cardiac output.\textsuperscript{6, 13} This is associated with a change in the fractional distribution of cardiac output, with the most angiotensin-sensitive vascular trees such as renal, adrenal, coronary and cerebral vasculature receiving preferentially larger fractions of blood flow at the expense of the least sensitive musculocutaneous tissues, after administration of teprotide.\textsuperscript{6} Although the percent fraction to the cerebral circulation did not change in those experiments, the absolute cerebral blood flow increased significantly in proportion to the augmented cardiac output.

Cerebral hypoxia and hypercapnia are potent non-specific stimuli known to cause arteriolar dilatation. The $pO_2$ and $pCO_2$ values determined after induction of anesthesia and 90 minutes after teprotide or saline injections in our animals indicate that the observed vasodilatation was not due to alteration in blood gasses. Moreover, in a parallel series of experiments testing the effect of converting enzyme inhibition on the delayed established cerebral arteriospasm, we found that teprotide could reverse that spasm as well.\textsuperscript{8} Our preliminary experience with the injectable form of the converting enzyme inhibitor enalapril, (the diacid MK-422) in four dogs with established delayed cerebral arteriospasm, indicates that this agent is at least as effective as teprotide in restoring arterial width in these animals. (Unpublished data).

From our clinical experience with the use of angiotensin converting enzyme inhibitors in the treatment of human hypertension and congestive heart failure, we know that an intravenous injection of teprotide produces effects lasting up to sixteen hours\textsuperscript{5, 7} whereas the orally active converting enzyme inhibitors captopril and enalapril (MK-421) can be administered once or twice daily for chronic maintenance.\textsuperscript{3, 9} It remains to be determined whether administration of a converting enzyme inhibitor in humans soon after the diagnosis of a subarachnoid hemorrhage can prevent or reverse cerebral arterial spasm, thus duplicating clinically the ef-

\begin{table}[h]
\centering
\caption{Blood Pressure and Biochemical Parameters Before and After Injection of Teprotide or Saline (Mean ± SEM)}
\begin{tabular}{lcccc}
\hline
 & \textbf{Experimental dogs} & & \textbf{Control dogs} & \\
 & \textbf{Before} & \textbf{90' after} & \textbf{Before} & \textbf{90' after} \\
\hline
\textbf{Blood} & & & & \\
pressure mm Hg & 121 ± 2 & 113 ± 1 & 115 ± 3 & 117 ± 2 \\
\textbf{PRA} ng/ml/h & 84 ± 2 & 83 ± 1 & 78 ± 2 & 80 ± 0 \\
\textbf{pH} & 6.5 ± 0.6 & 31.7 ± 12.8 & 9.1 ± 2.4 & 9.4 ± 2.5 \\
\textbf{pO}_2 mm Hg & 7.41 ± 0.0 & 7.41 ± 0.0 & 7.46 ± 0.03 & 7.45 ± 0.0 \\
\textbf{pCO}_2 mm Hg & 88 ± 1.8 & 86.5 ± 3.0 & 84.2 ± 2 & 87.3 ± 2.9 \\
\textbf{PRA = plasma renin activity.} & & & & \\
\end{tabular}
\end{table}
fects observed in the experimental animals with these compounds.

References

Measurement of Regional Blood Flow Using Hydrogen Gas Generated by Electrolysis

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SUMMARY Electrochemically generated hydrogen gas was used to measure local blood flow by Stosseck et al. The data obtained by their method, however, did not correlate well with those obtained by hydrogen inhalation.

We have modified the equation proposed by Stosseck, prolonging the stimulus duration in order to increase the amount of hydrogen generated. In dog white matter the resulting clearance curves were formed to be monoexponential both in the living animal as well as after circulatory arrest when all the clearance is by diffusion away from the electrode. The values calculated by our equation correlated well with those obtained by hydrogen inhalation.

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ELECTROCHEMICALLY GENERATED H₂ was utilized for the measurement of cerebral blood flow (CBF) by Stosseck et al. The values obtained using their method differed from those obtained by hydrogen inhalation and were thought to reflect the blood flow in a smaller region of tissue, which Stosseck et al. called "microflow." Their values were greater in general than those found in the H₂ inhalation method and variations in the values obtained were frequent indicating that there would be still problems remaining in its practical application.

We have attempted to measure regional CBF (rCBF) in the dog brain following generation of H₂ gas by electrolysis as Stosseck et al. did, and found that if much more H₂ gas was produced, the changes in H₂ concentration were approximately monoexponential for some minutes both when the dogs were alive and dead.

A simple equation to calculate rCBF has been developed, modified from that of Stosseck equation. This

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