Experimental Studies of Intracranial Arterial Spasm Using Aortic Strip Assays*

ROBERT H. WILKINS, M.D., GLORIA K. WILKINS, B.A., J. CAULIE GUNNELLS, M.D., AND GUY L. ODOM, M.D.

Division of Neurosurgery, and Department of Medicine, Duke University Medical Center, and the Durham Veterans Administration Hospital, Durham, North Carolina

SUBARACHNOID hemorrhage from intracranial arterial aneurysms is complicated frequently by intracranial arterial spasm that can lead to cerebral ischemia, infarction, and death.²,¹⁴,³⁷,⁵¹,⁵² The exact mechanisms responsible for this spasm are unknown, although catecholamines, serotonin, and other vasoactive biochemical agents have been implicated.¹⁶,²⁷

Usually after the rupture of an intracranial aneurysm, the escaping blood forms a clot around the aneurysm and the adjacent arteries. In experimental animals, blood, serum, serotonin, and angiotensin have each been shown to cause cerebral arterial constriction when they are applied topically to these vessels.¹⁶,²⁷,⁴³ A similar mechanism for human intracranial arterial spasm has been postulated, and in three cases reported by Buckell, increased amounts of serotonin were demonstrated in the hematoma fluid surrounding intracranial aneurysms.¹²

In order to investigate this idea further, we have analyzed the effects of fresh and altered blood, plasma, serum, and cerebrospinal fluid on arterial tone, using spirally cut strips of rabbit aorta. The rabbit aorta

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Fig. 1. Diagram of aortic strip, suspended in muscle chamber containing oxygenated Krebs solution at 37.5°C.
method has been used as a model of vascular reactivity in a number of previous studies, and it has been shown to be sensitive to some of the agents known to affect the tone of cerebral arteries.

**Materials and Methods**

*Muscle Bath.* (Fig. 1). Spirally cut strips of thoracic aorta from female, white, New Zealand rabbits (4 to 6 lb) were prepared as described by Furchgott and Bhatrakom.44 For each experiment, a strip measuring 4 cm in length was mounted in a 5 ml muscle chamber in a muscle bath assembly† maintained at 37.5°C. The strip was bathed with freshly prepared Krebs bicarbonate solution55 containing 0.01 M glucose, and test solutions were added in varying volumes as described below. After each test, replacement of the solution in the muscle chamber was accomplished by thorough overflow flushing with fresh Krebs solution from a reservoir kept at 37.5°C. A mixture of 95%O2-5%CO2 was bubbled through the Krebs solution in the muscle chamber and in the reservoir to keep the pH at 7.4. The responses of the aortic strip were recorded on kymograph paper, moving at 1.5 cm/min, by an ink-writing lever counterweighted to exert a 4 gm tension on the strip.

† Isolated Organ/Tissue Bath (§70-534), Phipps & Bird, Inc., Richmond, Va.

**Standard Solutions.** (Fig. 2, Table 1). Because of an initial elongation and increase in sensitivity to stimulating drugs,23,54 each strip was suspended in the muscle chamber for at least an hour before any assays were performed. At the beginning and end of each day’s experiment, two standard strengths of angiotensin II* (0.1 ml aliquots of 0.1 and 1.0 μg/ml solutions, giving in the 5 ml muscle chamber concentrations of approximately 0.002–0.02 μg/ml) were tested to assess the reactivity of the aortic strip. By a series of separate tests at the beginning of the present investigation, it was established that similar concentrations of epinephrine, norepinephrine, and serotonin† could also be detected, in keeping with the results of others. Vasopressin‡ in dosages up to 50 I.U. stimulated no significant contractions of the aortic strip, and the smallest dose of oxytocin§ to give a positive response was 10 U.S.P. units. Dilutions of all drugs were made in a 0.9% NaCl solution.

* Hypertensin-CIBA, CIBA Pharmaceutical Co., Summit, N. J.
‡ Pitressin, 20 pressor units/ml, Parke, Davis & Co., Detroit, Mich., and L-S-Vasopressin, 50 I.U./ml, San-
doz, Inc., Hanover, N. J.
§ Pitocin, Parke, Davis & Co., Detroit, Mich.
TABLE 1

Sensitivity of the rabbit aorta method

<table>
<thead>
<tr>
<th>Substance</th>
<th>Approximate Strip Sensitivity (µg)</th>
<th>Approximate Concentration in Body Fluids From Humans With No Intracranial Disease (µg/ml)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Blood, Plasma, or Serum</td>
<td>CSF</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>0.001–10</td>
<td>0.0001–0.001</td>
<td>—</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>0.001–10</td>
<td>0.0005–0.0005</td>
<td>—</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>0.001–0.1</td>
<td>&lt;0.0005</td>
<td>—</td>
</tr>
<tr>
<td>Serotonin</td>
<td>0.001–0.1</td>
<td>0.01–0.03</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Histamine</td>
<td>0.1–100</td>
<td>0.01–0.1</td>
<td>0.01–0.1</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>1–1000</td>
<td>—</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The standard solutions of angiotensin II were prepared each month and kept refrigerated during and between experiments. The occasional aortic strip that initially was poorly reactive was not used. Rarely a strip lost its reactivity during an experiment, and in that case all of the day’s results were discarded. In general, different aortic strips responded similarly to equal concentrations of angiotensin II.

Patient Distribution. (Table 2). During a period of 3 years, samples of the various biological fluids to be tested were obtained from 296 patients with various diagnoses. All of the patients' charts were reviewed and all of the arteriograms were examined. In each case with spontaneous subarachnoid hemorrhage the diagnosis was confirmed by lumbar puncture and at least one arteriogram was performed. Of the 121 patients with aneurysms, angiomas or spontaneous subarachnoid hemorrhage, 39 were treated by carotid occlusion, 28 by craniotomy, and 17 by both procedures. Of the 93 patients with other intracranial diseases, 26 underwent craniotomy or craniectomy. For the purposes of this study, a patient was called hypertensive if he had a brachial cuff pressure exceeding 150/100 on at least 2 separate days, or a history of previous treatment for hypertension. The arteriographic pattern of segmental narrowing of intracranial arteries was identified as spasm provided that the narrowed areas were not: 1) irregular and ragged, suggesting atheromata, 2) in arteries known on occasion to be hypoplastic (proximal anterior cerebral artery or distal vertebral artery), or 3) radiographic artifacts due to laminar flow of the contrast medium.

Test Samples. Specimens of cerebrospinal fluid (CSF) were collected in iced containers during lumbar puncture, pneumoencephalography, or ventriculography. Erythrocytes were removed from bloody samples by refrigerated centrifugation. Blood was withdrawn without significant hemolysis by antecubital venipuncture into plain or heparinized* syringes using 19-gauge needles. In the former instance, the blood was added immediately to iced tubes containing CSF or was transferred to plain glass vacuum tubes where it was allowed to clot at room temperature. The samples of heparinized blood were placed in ice immediately. Within an hour of collection, the clotted and heparinized blood samples were centrifuged under refrigeration, and the serum and plasma were removed. Samples for the incubation experiments were collected and incubated using aseptic technique. To minimize any possible catecholamine response to fear and pain, lumbar punctures and ventriculopunctures were performed as rapidly and smoothly as possible, with no prior announcement to the patient.

* Liquasemin sodium, 1000 or 5000 U.S.P. units/ml, Organon, Inc., West Orange, N. J.
### TABLE 2

**Patient distribution**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Patients</th>
<th>No. of Bilateral Carotid, Unilateral Carotid or Brachial Arteriograms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous subarachnoid hemorrhage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spasm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Hypertension</td>
<td>22</td>
<td>40</td>
</tr>
<tr>
<td>2. No Hypertension</td>
<td>20</td>
<td>38</td>
</tr>
<tr>
<td>No Spasm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Hypertension</td>
<td>34</td>
<td>61</td>
</tr>
<tr>
<td>2. No Hypertension</td>
<td>30</td>
<td>48</td>
</tr>
<tr>
<td>Aneurysm or angioma; no spontaneous subarachnoid hemorrhage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spasm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Hypertension</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2. No Hypertension</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>No Spasm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Hypertension</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>2. No Hypertension</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Other intracranial diseases*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Hypertension</td>
<td>21</td>
<td>9</td>
</tr>
<tr>
<td>2. No Hypertension</td>
<td>72</td>
<td>27</td>
</tr>
<tr>
<td>Extracranial diseases and volunteers†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Hypertension</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2. No Hypertension</td>
<td>72</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>296</td>
<td>246</td>
</tr>
</tbody>
</table>

*Trauma—29, epilepsy—14, tumor—7, psychiatric illness—7, infarction—7, other—29. Cerebrospinal fluid specimens from 42 of these 93 patients were examined preoperatively. Bloody CSF was encountered in 9 cases, all with craniocerebral trauma. Arteriograms were performed in 30 cases, and spasm was present in 3 (trauma, tumor, infection).


Most samples were obtained between 7 and 9 a.m. They either were kept in ice and tested the same day or were kept frozen at $-18^\circ$C until testing. Each day's experiment usually involved specimens of from one to three patients with subarachnoid hemorrhage and from one to three control patients, resulting in 10 to 15 samples for assay. The sequence in which the samples were obtained and tested was varied from day to day and did not influence the results. The pH of each sample was measured with a Beckman pH meter.†

† Model GS, Beckman Instruments, Inc., Fullerton, Calif.

Samples were added through a polyethylene catheter to the bottom of the muscle chamber. Unless otherwise specified, 1 ml samples were used. When 1 ml of plasma was added, it forced 1 ml of Krebs solution into the overflow tube at the top of the chamber, resulting in a final dilution in the chamber of 1 ml of plasma to 4 ml of Krebs solution. Occasionally, 5 ml of the test solution was added to the chamber, resulting in essentially total replacement of the previous Krebs solution by the test solution. The strip was exposed to any one test solution for a minimum of 2 minutes. At the end of that time, the kymograph was stopped, the test solution was flushed from
the chamber by fresh Krebs solution, and the aortic strip was allowed to relax to its previous length before another test solution was added.

Positive Contractive Response. A response was called positive if the resulting contraction of the aortic strip was greater than that elicited by 0.01 μg of angiotensin II.

Control Experiments. (Fig. 2). The following fluids were tested to establish that contraction of the aortic strip caused by the test samples was not due to heparin or to simple changes in pH and temperature; heparin (10 units, 100 units, and 1000 units); isotonic saline at 37.5°C, containing various amounts of 1 N HCl or 0.75 N NaOH (1 ml pH 7.8, 5 ml pH 7.8, 1 ml pH 7.4, 5 ml pH 7.4, 1 ml pH 5.5, 1 ml pH 11.0); and isotonic saline at pH 7.8 (1 ml 37.5°C, 5 ml 37.5°C, 1 ml 21°C, 5 ml 21°C, 1 ml 10°C, 5 ml 10°C, 1 ml 0°C, 5 ml 0°C).

Experiments involving dialysis, boiling, and freezing of the standard solutions were also performed. Prior to the testing of 1 ml aliquots, 10 ml specimens of the 1.0 μg/ml solutions of angiotensin II, norepinephrine, epinephrine and serotonin, and 2 ml specimens of the 20 units/ml and 10 units/ml solutions of vasopressin and oxytocin either were dialyzed* with continu-


**TABLE 3**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Patients</th>
<th>No. of Samples</th>
<th>Average pH</th>
<th>Samples Giving Positive Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Iced Frozen</td>
<td></td>
<td>Iced Frozen</td>
</tr>
<tr>
<td>Spontaneous subarachnoid hemorrhage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spasm</td>
<td>29</td>
<td>25 10</td>
<td>7.8</td>
<td>56* 80</td>
</tr>
<tr>
<td>No Spasm</td>
<td>44</td>
<td>50 9</td>
<td>7.7</td>
<td>70 89</td>
</tr>
<tr>
<td>Aneurysm or angioma; no spontaneous</td>
<td>10</td>
<td>13 2</td>
<td>7.7</td>
<td>85* —</td>
</tr>
<tr>
<td>subarachnoid hemorrhage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other intracranial diseases</td>
<td>43</td>
<td>40 4</td>
<td>7.8</td>
<td>63 —</td>
</tr>
<tr>
<td>Extracranial diseases and volunteers</td>
<td>46</td>
<td>44 9</td>
<td>7.8</td>
<td>61 78</td>
</tr>
<tr>
<td>Total</td>
<td>172</td>
<td>172 34</td>
<td></td>
<td>65* 76*</td>
</tr>
</tbody>
</table>

* Not significantly different by the chi square test (p > 0.1).
sured values of serotonin and histamine in blood samples drawn at the same time or within 3 days.\textsuperscript{22}

Experiments with Plasma. 2. Dilution, pH changes, dialysis, denaturation, and precipitation. To define further the substances in plasma responsible for contraction of the aortic strip, plasma samples were altered in various ways before being tested. With a 0.9% NaCl solution, 105 samples were diluted 1:1, 1:5, or 1:10, and the pH values of 224 other specimens were changed to 2.0, 3.0, 5.5, 9.0, or 11.0 by the addition of appropriate amounts of 1 N HCl or 0.75 N NaOH. To remove relatively small molecules such as catecholamines, serotonin, and angiotensin, 68 10 ml samples were dialyzed for 24 or 48 hours with continuously running cold tap water. To remove large molecules such as proteins, 31 specimens were boiled for 10 to 30 minutes, and 21 other specimens were mixed with equal volumes of 6% perchloric acid. With both procedures the resulting insoluble substances were then removed by centrifugation. Following the addition of the perchloric acid to each of the latter 21 samples, the pH of the supernatant fluid was returned to the original pH value by the addition of appropriate amounts of 1 N KOH, and the precipitate of potassium perchlorate was subsequently removed by centrifugation.

Experiments with Hemolyzed Plasma. To assess the effects of hemolysis, two samples of heparinized blood were drawn from each of 16 patients under identical conditions. One sample from each patient was placed in a freezer at \(-18^\circ\text{C}\) until frozen, then thawed and centrifuged. The supernatant plasma in each case was discolored by hemolysis. The second (control) sample of heparinized blood from each patient was centrifuged, and only the clear plasma was frozen and thawed.

Experiments with Serum. (Table 4). The experiments performed with serum were similar to those performed with plasma, but were not as extensive. The effects of temperature were tested using 153 serum samples from 77 patients. Of the 153 samples, 107 were tested after being kept in ice for a few hours, 23 after being held at room temperature for a similar length of time, and 23 after being frozen and thawed. Other experiments used an additional 148 serum samples. With a 0.9% NaCl solution, 85 specimens were diluted 1:1, 1:5, or 1:10; the pH values of 26 specimens were changed to 5.5 by the addition of appropriate amounts of 1 N HCl; dialysis with cold running tap water was performed using 26 10 ml serum specimens; and 11 samples were boiled.

Experiments with Cerebrospinal Fluid. (Table 4). The CSF samples either were clear and colorless, clear and xanthochromatic, or were the clear xanthochromatic

\textbf{TABLE 4}

\textbf{Contrative effect of serum and cerebrospinal fluid}

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Patients</th>
<th>No. of Samples</th>
<th>Avg. pH</th>
<th>Samples Giving Positive Response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. SAH, spasm</td>
<td>13</td>
<td>14</td>
<td>7.9</td>
<td>98</td>
</tr>
<tr>
<td>2. SAH, no spasm</td>
<td>21</td>
<td>24</td>
<td>7.8</td>
<td>92</td>
</tr>
<tr>
<td>3. Controls</td>
<td>43</td>
<td>46</td>
<td>7.8</td>
<td>91</td>
</tr>
<tr>
<td>All CSF samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. SAH, spasm</td>
<td>10</td>
<td>11</td>
<td>8.0</td>
<td>36</td>
</tr>
<tr>
<td>2. SAH, no spasm</td>
<td>14</td>
<td>19</td>
<td>7.9</td>
<td>31*</td>
</tr>
<tr>
<td>3. Controls</td>
<td>23</td>
<td>22</td>
<td>8.2</td>
<td>3*</td>
</tr>
<tr>
<td>Xanthochromic CSF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Within 1 day of SAH</td>
<td></td>
<td>7</td>
<td>7.6</td>
<td>26</td>
</tr>
<tr>
<td>2. Days 2–6</td>
<td></td>
<td>9</td>
<td>7.8</td>
<td>33</td>
</tr>
<tr>
<td>3. Days 7–21</td>
<td></td>
<td>14</td>
<td>8.2</td>
<td>36</td>
</tr>
</tbody>
</table>

* Significantly different by the chi square test (p<0.01).
supernatant fluids from bloody specimens. Nineteen 1-ml samples were tested as were 32 colorless 5-ml samples from 23 control patients and 30 xanthochromic 5 ml samples from 24 patients with subarachnoid hemorrhage. Of the 5-ml specimens, 23 were stored in ice and tested on the day of collection. The remaining 39 were frozen at \(-18^\circ\text{C}\) until the day of assay. The 5-ml aliquots of 62 samples were at different temperatures when added to the muscle chamber; 33 were at 37.5°C, 20 at 21°C, and 9 at about 0°C.

Experiments with Blood-CSF Mixtures. (Fig. 3). Subarachnoid hemorrhage was simulated by the mixture of blood and cerebrospinal fluid specimens from each of seven control patients. In each case, the blood and CSF samples were drawn on the same day and the following fluids tested: 1 ml aliquots of plasma, serum, and CSF, 1 ml and 5 ml aliquots of the supernatant fluids from clotted 1:10 and 1:1 mixtures of blood and CSF, and a 5 ml aliquot of the 1:10 mixture. Additional aliquots of plasma, serum, and CSF were frozen and tested 3 days later. The 1:10 and 1:1 blood-CSF mixtures were incubated at 37.5°C for 3 days, and 1 and 5 ml aliquots of the supernatant fluids were tested, as well as 5 ml of the 1:10 mixture.

Results

Control Experiments. (Fig. 2). Heparin and saline under varied conditions of pH and temperature caused no contraction of the aortic strip. However, complete replacement of the balanced Krebs solution with saline caused slight transient relaxation of the strip that was more noticeable with the colder saline preparations.

No activity could be detected in the fluid remaining within the dialyzer tubing after dialysis of the standard solutions of angiotensin II, epinephrine, norepinephrine, serotonin, vasopressin, and oxytocin. Boiling of the standard solutions resulted in diminished activity, whereas freezing and thawing had no effect.

Experiments with Plasma. 1. Unaltered plasma, temperature changes. (Table 3). Positive responses were elicited by 65% of iced and 76% of frozen plasma samples. The three samples quickly frozen in dry ice and ethanol were markedly positive. In each of the six cases where similar plasma samples were kept at different temperatures for a few hours between collection and assay, the samples that had been frozen were the most reactive, the iced samples were less reactive, and the samples kept at room temperature were the least reactive. Only one of the 14 plasma samples incubated at 37.5°C gave a positive response.

Qualitatively and quantitatively, the responses of the samples from patients with subarachnoid hemorrhage and/or intracranial arterial spasm were not significantly different from the responses of the control samples. Even in the 10 instances in which plasma samples were obtained within 1 day of the demonstration of intracranial spasm, responses were similar (8 positive and 2 negative, with an average pH of 7.7).

Of the 29 patients who had two plasma samples withdrawn and tested on different days, 17 had positive responses on both occasions and three had negative responses both times. The other nine patients had either positive or negative responses initially and the opposite response subsequently.

In the 19 cases in which the results of
plasma testing were compared with measured values of blood serotonin and histamine, no correlations could be made.

Experiments with Plasma. 2. Dilution, pH changes, dialysis, denaturation and precipitation. Dilution of plasma with saline resulted in diminished activity that was roughly proportional to the dilution. None of the samples diluted 1:10 caused significant contraction of the aortic strip. Plasma samples also lost activity with acidification, but no changes were noted at basic pH values of 9 or 11. Dialysis with tap water was associated with a moderate decrease in sample activity. Only 35% of the 68 dialyzed plasma samples gave positive responses, and, in general, these were of reduced amplitude. Denaturation of plasma samples by boiling resulted in a minor diminution in activity, whereas precipitation with perchloric acid led to increased activity.

As with the unaltered plasma samples, the responses and the average pH values of the specimens from patients with subarachnoid hemorrhage and/or intracranial arterial spasm were not significantly different from those of the control specimens.

Experiments with Hemolyzed Plasma. Without exception, the 16 hemolyzed plasma samples (average pH 7.9) stimulated greater contraction of the aortic strip than did their nonhemolyzed controls (average pH 8.0).

Experiments with Serum (Table 4). Serum samples were uniformly more reactive than plasma samples. The variations in serum activity due to changes in temperature resembled those of plasma but were less pronounced. As with plasma, dilution and acidification of serum resulted in definite loss of activity, and denaturation was associated with a slight loss. However, in contrast to the results with plasma, dialysis of serum with tap water caused a marked reduction in activity. Only four of 26 specimens gave positive responses.

Experiments with Cerebrospinal Fluid. (Table 4). None of the 1 ml aliquots of CSF was reactive. Of the 5 ml samples, only one of 32 clear and colorless specimens gave a positive response, in contrast to 10 of 30 xanthochromic samples (a statistically significant difference). However, there was no correlation between the activity of the xanthochromic samples and the time after subarachnoid hemorrhage (up to 21 days) or the presence of intracranial arterial spasm. Likewise the results were not influenced by whether the samples were tested after freezing or refrigeration, or whether they were tested at 0°, 21°, or 37.5°C. When the 5 ml samples were tested, there frequently was an initial brief relaxation of the aortic strip similar to that caused by the 5 ml saline samples (Fig. 2).

Experiments with Blood-CSF Mixtures. (Fig. 3). In each of the seven cases the plasma was more reactive after being frozen for 3 days than it was initially. The serum and CSF responses were unchanged after freezing. As expected from previous observations, serum samples were more reactive than plasma, and the unaltered 1 ml CSF specimens were negative. The supernatant fluids produced contractions of the aortic strip that were related to the amount of serum in the mixture. In most of the 1:1 and 1:10 mixtures there was no hemolysis or clot lysis after 3 days of incubation at 37.5°C, and the test results of the supernatant fluids were approximately the same at that time as they were originally. The 5 ml 1:10 mixture samples elicited the same response as did the 5 ml 1:10 supernatant fluids.

Discussion

The smooth muscle fibers of the major cerebral arteries are thought to be responsive to neurogenic control, to mechanical stimuli, and to hormonal, chemical, and metabolic influences. The neurogenic and mechanical control of intracranial and extracranial vascular tone, as well as the effects of administered drugs and inhaled gases, have been studied extensively and are beyond the scope of the present study.

We have confined our interests to the role of possible humoral influences on arterial spasm, for which there has been previous experimental and clinical evidence. Intracranial arteries have been shown to constrict when exposed to intra-arterial noradrenaline, serotonin, or angiotensin, or to topical serotonin, angiotensin, blood, or serum. In addition, spasm of intracranial arteries has been demonstrated in a patient with elevated levels of circulating norepinephrine and epinephrine caused by a pheo-
chromocytoma. Finally, as mentioned in the introduction, in three cases increased amounts of serotonin have been demonstrated in the hematoma fluid surrounding intracranial arterial aneurysms.12

Stimulated by this information and the report by Barnes and Hunt,8 we have approached the problem using the rabbit aortic strip technique, a method that has been shown to be responsive to some of the agents known to affect the tone of cerebral arteries. In attempting to assess conditions affecting human cerebral arteries, this method introduces sources of error by employing a denervated, distorted artery of a different type9,9 and from a different species. However, the rabbit aorta has a large amount of circularly oriented smooth muscle (almost half of the contents of the vessel wall),12 and it has been used as a model of vascular reactivity in a number of previous studies.

In the present study there were several other factors that may have introduced errors. The rabbit aorta is sensitive to alterations in electrolytes,7,8,11,24 and in view of the electrolyte disturbances occasionally associated with subarachnoid hemorrhage33,32 and intracranial operations,34 the samples tested may have had abnormal concentrations of electrolytes. Also, the samples were exposed to the air, resulting in pH values above the normal range.42 However, in most instances the sample fluids were diluted 1:4 with the Krebs bicarbonate solution in the muscle chamber as they were tested, thus reducing the possible effects of electrolyte imbalance. In addition, though above the normal range, the pH values were similar for patients with or without subarachnoid hemorrhage.

As demonstrated in Table 1, normal blood levels of catecholamines, angiotensin, and serotonin are at or below the minimum sensitivity of the aortic strip method. It is interesting, then, that most of the plasma and serum specimens tested showed significant activity. This probably represents the effect of other vasoactive compounds. Our data do not allow precise chemical definition of these compounds, but we can speculate about their nature. Since the activity of plasma and serum was diminished by denaturation and precipitation, we estimate that the active substances may have a molecular weight of roughly 500–5000. They may be alkaline in nature and may be subject to enzymatic degradation which is enhanced by incubation and retarded by reduced temperature.

It is also possible that the observed effects were the result of various vasoactive compounds with opposite properties acting simultaneously. The loss or destruction of inhibitory compounds might explain why serum was more reactive than plasma, and why plasma activity was increased by freezing and by precipitation with perchloric acid.

The effects of erythrocytes, leukocytes, and platelets on vasoconstriction have not been investigated in the present study. However, the experiments involving hemolysis suggest that these solid elements may also play a role in intracranial arterial spasm. The specific influences of blood-clotting and fibrinolysis have also not been investigated, although these might be fertile areas for future experiments.15,17,41,53

Summary

More than 1000 samples of blood and cerebrospinal fluid were obtained from 121 patients with intracranial aneurysms, angio- mas, and/or spontaneous subarachnoid hemorrhage, of whom 43 had intracranial arterial spasm, and also from 175 control patients. The vasoactivity of these fresh and altered specimens of blood, plasma, serum, and CSF was tested using strips of rabbit aorta mounted in a muscle bath.

Despite the fact that normal blood levels of epinephrine, norepinephrine, angiotensin, and serotonin are at or below the minimum sensitivity of the aortic strip method, 92% of the serum samples and 67% of the plasma samples caused significant contractions of the aortic strip. The responses of the samples from patients with subarachnoid hemorrhage and/or intracranial arterial spasm were the same as the responses of the control samples. Only 3% of clear and colorless CSF samples showed significant activity, in contrast to 33% of xanthochromic specimens.

These and the other data from this study suggest that vasoconstrictors other than epinephrine, norepinephrine, angiotensin, and serotonin are normally present in human blood. We have reported preliminary experi-
ments to identify these substances and have speculated that these substances, when extravasated into the subarachnoid space from an intracranial arterial aneurysm, may play a role in intracranial arterial spasm.

Acknowledgments

The authors thank Drs. Oscar Helmer and Norman Kirshner for their advice, Drs. George Tindall and Eugene Day for the use of their equipment and facilities, and Mr. W. Guarrant for his care of our rabbits.

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