SUMMARY The elastic properties of the basilar artery were studied in control and treated dogs in which 3 ml of blood was injected intracisternally. Vascular specimens were resected transclivally as cylindrical segments and their external diameters were measured in vitro in the pressure range from 0 mm Hg to 250 mm Hg in the active condition of smooth muscle in Krebs-Ringer solution and in the passive condition in saline solution. The development of cerebral vasospasm was confirmed comparing the diameter difference between these two conditions. The experimental data indicated that vasospasm was most prominent on the 7th day after the treatment of blood injection. In the passive condition no significant dimensional change (i.e. radius and wall thickness) was observed between the control and the treated arteries at various pressure levels. These results imply that the luminal narrowing under vasospasm is not attributable to an irreversible organic change in the wall but to the constriction of vascular smooth muscle. The treated arteries are more distensible and have lower elastic moduli than the control arteries, possibly due to a change in the content of their connective tissues. These changes of the passive elastic properties of arterial walls after blood injection might be one of the factors affecting the development of cerebral vasospasm.

ALTHOUGH CEREBRAL VASOSPASM following the rupture of cerebral aneurysm has been studied intensively, its etiology and pathogenesis have not been fully established. There is no definite answer to such a simple question as whether the observable luminal narrowing is attributable to the contraction of vascular smooth muscle and hence reversible, or results from some reactive and organic change in the arterial wall associated with a decreased internal diameter. There have been several histological observations in the arterial walls of both human and experimental animals subjected to subarachnoid hemorrhage (SAH). In these studies, however, little information is available on change of dimensions of the wall (i.e. radius and thickness) as a function of time elapsed because insufficient data was provided on the wall dimensions nor were the arterial specimens fixed by a perfusion technique. Extrapolation of vascular dimensions measured at 0 mm Hg to those at the physiological pressure might result in a misunderstanding since the pressure-diameter relations of blood vessels are usually nonlinear. In addition, it is not fully clarified whether the luminal narrowing will be retained after the cessation of blood circulation and the preparation of specimens by fixation.

The arterial wall is composed of passive components (collagen and elastin) which determine its passive elastic properties, and an active component (smooth muscle). The force generated by the contraction of smooth muscle is transmitted to the passive components and is detected as a developed tension. The increase of collagen fibers in arterial walls which is observed histologically after SAH might change their passive elastic properties and eventually alter the degree of their constriction.

In this study, we used cylindrical segments of the basilar artery obtained from dogs in which blood was injected into the cisterna magna. Their wall dimensions and mechanical properties were measured at intervals after the blood injection to determine whether or not cerebral vasospasm is reversible. The connective tissue composition of the arterial walls was determined and compared with their passive elastic properties.

Materials and Methods

Experimental Procedures

A total of 35 mongrel dogs, weighing from 8 to 12 kg, were used in this study and divided into 6 groups: control group (10 dogs) with no treatment and treated groups (25 dogs) with the blood injection. The blood injection was performed in the following manner. Under sodium pentobarbital anesthesia (25 mg/kg, i.v.), a dog was placed in a prone position with the head fixed in a stereotaxic frame which was tilted downward 15°. A no. 22 spinal needle was inserted into the cisterna magna with the bevel directed rostrally. Three ml of autogenous fresh arterial blood was injected slowly with an exchange of the same amount of cerebrospinal fluid. The procedures employed here have been shown to produce a high incidence of vasospasm in dogs. After recovering from the anesthesia, the dog was fed in a conventional manner until the subsequent operation.

After a certain period of time following the blood injection, each dog was anesthetized again with sodium pentobarbital (25 mg/kg, i.v.) and intubated. A clivectomy was performed to resect the basilar artery, at about 20 mm from the junction of the vertebral arteries. The treated dogs were divided into five groups according to the period of time passed after the treatment: 2, 4, 7, 14 and 28 days. Each treated group was designated as 2-day, 4-day, 7-day, 14-day and 28-day
groups, respectively. The basilar artery was dissected from the surrounding arachnoid membranes and its branches were ligated (Crown 10-0 Nylon Monofilament Suture, Kono Seisaku-sho Co., Ltd, Japan) and severed. During these procedures great care was taken to keep the arterial wall wet with Krebs-Ringer Solution and to avoid even a tiny injury. The in situ length of the arterial segment was measured prior to its resection to determine its in vivo axial strain.

After washing the blood from the lumen of the vessel, the tubular segment was mounted horizontally in its in vivo axial length in a tissue bath containing Krebs-Ringer solution kept at 37°C and oxygenated with 95% O₂ - 5% CO₂. The composition of this solution, with a pH of 7.42 ± 0.02, was as follows in millimoles per litre: 115.3 NaCl, 22.1 NaHCO₃, 4.6 KCl, 2.3 CaCl₂, 1.1 MgSO₄, 1.1 KH₂PO₄ and 7.8 dextrose. Each segment was inflated with the solution from a reservoir under air pressure using the testing apparatus reported elsewhere. Its intraluminal pressure and external diameter were measured by a strain gauge manometer (MPU-0.5, Toyo Measuring Instruments Co., Ltd., Tokyo, Japan) and a displacement transducer, respectively.

After incubating the segment in the Krebs-Ringer solution for 30 minutes under an intraluminal pressure of 100 mm Hg, the pressure was elevated to 250 mm Hg and then lowered to 0 mm Hg at the rate of 1.0 mm Hg/sec. A reproducible pressure-diameter curve obtained after repeating this inflation and deflation procedure several times was considered to represent the active elastic properties of the arterial wall. In our preliminary study, the arterial specimens resected and incubated by these procedures responded well to some vasoconstrictors such as serotonin and KCl. After the intraluminal pressure was returned to 100 mm Hg, the bath was drained and rinsed with a saline solution and then incubated in the solution for at least 30 minutes. The pressure-diameter curve was obtained by the same procedure as carried out in the Krebs-Ringer solution and represents the passive elastic properties of the segment. Our preliminary study also showed that no difference was observed between the curves obtained in the saline solution mixed with a metabolic inhibitor, i.e. KCN 1 mM, suggesting that a blood vessel has little smooth muscle tone in the pure saline solution. Only the inflation curve was recorded and used in the subsequent analysis. At the end of the experiment the segment was removed from the bath, lightly blotted on filter paper, and weighed.

The composition of connective tissues in each arterial wall were determined by a modified method of the procedure employed by Neuman and Logan. The collagen and elastin contents were measured by a colorimetric assay of hydroxyproline, and expressed as percentages of the dry defatted weight.

Data Analysis
For the evaluation of the mechanical properties of blood vessels from their pressure-diameter curves, we calculated the incremental elastic modulus, which represents the elastic properties inherent in their materials and defined by the following equation:

\[ E_{inc} = \frac{\Delta P_t}{2(1-\nu^2)R_i^2 R_e}, \]

where \( P_t \) is the intraluminal pressure, \( R_e \) the external radius and \( R_i \) the internal radius. Poisson’s ratio, \( \nu \), is estimated to be 0.5. The internal radius was calculated from the external radius, the in vivo axial strain and the volume of the segment. The volume of the segment was calculated from its wet weight assuming the wall density to be 1.06 g/cm³.

Tangential wall stress, \( \sigma \), and tangential mid-wall strain, \( \varepsilon_m \), were calculated to study the stress-strain relations of walls with different cross-sectional areas. These stress and strain are defined by the following equations:

\[ \sigma = P R_i/(R_o - R_i), \]

\[ \varepsilon_m = [(R_o + R_i)/2]/((r_o + r_i)/2) - 1, \]

where \( r_o \) and \( r_i \) are the external and internal radii at 0 mm Hg, respectively, under the passive condition.

Diameter response, \( \Delta D/D_m \), was calculated to express the ability of smooth muscle to constrict a blood vessel at a given pressure level and is defined as:

\[ \Delta D/D_m = [(D_m)_{SS} - (D_m)_{KRS}]/(D_m)_{SS}, \]

where \( D_m \) is the mid-wall diameter. \( (D_m)_{SS} \) and \( (D_m)_{KRS} \) are the mid-wall diameter under the active condition in the Krebs-Ringer solution and the passive condition in the saline solution, respectively (fig. 1). The details of the experimental procedures and data analysis employed in this study have been described previously.

Results
Production of Cerebral Vasospasm
Clotted blood was always found on the ventral surface of the brain stem when the operation was performed on the 2nd and 4th day after the blood injection. However, the quantity of hematoma decreased gradually with time, and no clot was detectable in the 28-day group. Some thickening in the arachnoid membrane around the basilar artery was observed in the 14-day and 28-day groups. No thrombus was found in the lumen of any of these arteries.

Change in the diameter response at the intraluminal pressure of 100 mm Hg is shown in figure 2. The value of the diameter response in the control group is 0.02. The diameter response increases with time and reaches the maximum value of 0.19 on the 7th day after the treatment. The value decreases rapidly thereafter, falling to the control value in the 28-day group.

Dimensional Changes of Arterial Wall
Figure 3 summarizes the internal radius (\( R_i \)) and the ratio of wall thickness (\( T \)) to internal radius (designated as the wall thickness ratio; \( T/R_i \)) under the passive condition of smooth muscle component in the saline solution. There is no significant difference in each
dimension among the control group and the treated groups. The segments of each group have almost the same values at the intraluminal pressure of either 100 mm Hg or 200 mm Hg while some scatterings are observed at 0 mm Hg.

### Passive Elastic Properties

Averaged stress-strain curves of the basilar arteries are depicted in figure 4. The control artery yields a smaller strain change at any stress level than the other treated arteries except for that in the 7-day group. Although the curves in the control and 7-day groups appear similar in shape, the slope of the former is steeper than that of the latter in the higher stress range greater than 10^6 dynes/cm².

Change in the incremental elastic modulus, E_{inc}, at the intraluminal pressure of 100 mm Hg is shown in the table, where the incremental elastic modulus virtually represents the tangential slope of the stress-strain curve shown in figure 4. The elastic modulus decreases markedly to a value of 6.4 × 10^6 dynes/cm² on the 2nd day after the treatment. The value recovers to some extent on the 4th day, being in the range between 10.0 × 10^6 and 12.0 × 10^6 dynes/cm² afterwards.

### Connective Tissue Compositions

Changes of dry defatted weight, collagen and elastin contents of the basilar arteries after the blood injection are summarized in the table. There is no significant difference in the dry defatted weight among the control group and the treated groups. Although both the collagen and elastin increase continuously after the treatment, the increase of the elastin content is more marked in the initial period than that of collagen. These changes result in a remarkable decrease of the collagen to elastin ratio, (C/E), on the 2nd day. This decreased ratio increases gradually thereafter and keeps a constant value of about two-thirds of the control value on the 14th and 28th day.

### Discussion

Kuwayama et al.\textsuperscript{12} and White et al.\textsuperscript{13} have reported successful production of cerebral vasospasm in the dog by the same method as used in this study. Kuwayama et al. traced the change in the internal diameter of the canine basilar artery by means of angiography and observed a diameter change ranging over 37% in 2 days, 49% in 4 days, 20% (average of 46, 33, 0 and 0%) in 7 days and 5% (average of and 0%) in 14 days after the treatment. White et al. documented the persistence of cerebral vasospasm produced in this manner for at least one week. In the present study we measured the external diameter of the vessel in vitro and observed the difference of mid-wall diameter between the active and passive conditions of the smooth muscle component. We obtained diameter responses of 2% in the control group, 5% in the 2-day, 6% in the 4-day, 19% in the 7-day, 6% in the 14-day and 1% in the 28-day groups as shown in figure 2, where the diameter response of 2% in the control group is considered to correspond to the physiologically exhibited smooth muscle tone. The discrepancies in the magnitude and time course of the vasospasm between Kuwayama’s and our observations might be due to: 1) the use of the
Krebs-Ringer solution in our experiments, which is free from possible spasmogenic substances and therefore can decrease the smooth muscle tone, 2) the larger amount of blood (3 ml) injected into the cisterna magna than in the experiments conducted by Kuwayama (2 ml), which can prolong the vasospasm, and 3) the in vitro study employed here. In spite of these differences, the vasospasm produced by the intracisternal injection of blood could be detected in vitro to some extent.

When the smooth muscle was fully relaxed in the saline solution, no significant dimensional changes of the basilar arteries were observed between the control group and the treated groups at each intraluminal pressure of 0, 100 and 200 mm Hg as shown in figure 3. No thrombus was found in the lumen of these arteries by macroscopic examinations. These results imply that the luminal narrowing of the artery under vasospasm can not be ascribed to the irreversible organic changes of arterial wall but to a contraction of the smooth muscle. These findings appear to correspond to the fact that the intracisternal injection of saline solution reverses the experimentally induced vasospasm.13, 24

After the subarachnoid injection of blood, the arterial wall yields fairly complicated changes in passive elastic properties as shown by the table and figure 4. All of the treated arterial walls have lower elastic moduli than the controls with the minimum value in the 2-day group. Cox has extensively investigated the contractility and passive elastic properties of blood vessels in rats of different ages,25 different species of animals22 and in hypertensive rats.26 He hypothesized that the stiffer the vascular wall, the more efficient is the constriction produced by the activation of smooth muscle. Studies on the arterial wall mechanics11, 14, 22, 25, 26 suggest that the change in passive elastic properties of arterial walls observed in the present study might be one of the factors affecting the development of vasospasm. At this moment, however, we can not decide which is the main factor between the production of remarkably softened wall observed 2 days after the blood injection and the stiffening observed thereafter. We are investigating the change of cerebrovascular reactivity after the blood injection to solve this problem.

There have been few quantitative data with regard to the connective tissue contents in arterial walls subjected to SAH. Histological observations have indicated that collagen and elastic fibers proliferate in the stroma of the media within 1 to 2 days after SAH2 and that intercellular collagen in the media increases later than one week after SAH.6 We have observed a marked increase of elastin in the 2-day group and of collagen in

<table>
<thead>
<tr>
<th>Days after treatment</th>
<th>Einc (10^6 dynes/cm²)</th>
<th>Weight (µg/mm)</th>
<th>Collagen (%)</th>
<th>Elastin (%)</th>
<th>C/E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.6 ± 1.5</td>
<td>75 ± 10</td>
<td>25 ± 3</td>
<td>7 ± 2</td>
<td>5.1 ± 0.9</td>
</tr>
<tr>
<td>2</td>
<td>6.4 ± 0.5*</td>
<td>58 ± 10</td>
<td>28 ± 2</td>
<td>17 ± 4</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>11.6 ± 0.5</td>
<td>69 ± 5</td>
<td>34 ± 5</td>
<td>17 ± 3</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>7</td>
<td>11.2 ± 1.9</td>
<td>62 ± 7</td>
<td>40 ± 10</td>
<td>17 ± 5</td>
<td>2.7 ± 0.4</td>
</tr>
<tr>
<td>14</td>
<td>10.2 ± 1.4</td>
<td>59 ± 4</td>
<td>63 ± 9*</td>
<td>21 ± 6</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>28</td>
<td>12.0 ± 1.3</td>
<td>65 ± 4</td>
<td>69 ± 7*</td>
<td>17 ± 4</td>
<td>4.3 ± 0.6</td>
</tr>
</tbody>
</table>

Values of connective tissue contents are expressed as % of dry defatted weight.
C/E: collagen to elastin ratio.
*Significantly different from the control values (p < 0.05).
Values are expressed as means ± se.
EXPERIMENTAL CEREBRAL VASOSPASM/Nagasawa et al.

FIGURE 5. Correlation of incremental elastic modulus, $E_{inc}$, at the intraluminal pressure of 100 mm Hg and collagen to elastin ratio, $C/E$.

The 14-day group (table). The ability of arterial smooth muscle to synthesize and secrete the connective tissue proteins within 4 hours might explain such an early change in the contents as was observed 2 days after SAH. However, it remains open to further study what is responsible for the increase of the connective tissue content and for the different rates of increase between collagen and elastin.

Generally the more the intraluminal pressure, and hence the diameter increase, the more strongly blood vessels react against stretch (fig. 1). This nonlinear pressure-diameter relation has been ascribed to the heterogeneous structure of the wall, that is the composite structure consisting of easily stretchable elastin fibers and inextensible collagen fibers. The relative content of the connective tissues, which is represented by the collagen to elastin ratio ($C/E$), has been considered to have some relation to the passive elastic properties of blood vessels.

To understand the mechanism of the change in elastic properties due to the blood injection documented in the present study, we plotted the incremental elastic modulus versus the $C/E$ ratio in each specimen and obtained figure 5. A good correlation between these two values indicates that the change in the passive properties of the canine basilar artery due to the blood injection is ascribed to the change in the connective tissue contents.

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