IT HAS BEEN KNOWN that hematocrit (HCT) is a major determinant of blood viscosity and plays an important role in regulating cerebral blood flow (CBF). In either humans or animals with anemia, CBF is increased, while in those with polycythemia or high HCT, CBF is significantly decreased. HCT is also one of the risk factors in cerebral infarction. There have been many studies describing the relationship between HCT and either cerebral hemodynamics or metabolism in an intact brain, but it is little known how HCT affects the metabolism of the ischemic brain. In this communication, we measured brain tissue metabolites in experimentally induced cerebral ischemia in hypertensive rats, in which HCT was varied by the following way; withdrawing 3-5 ml of blood and simultaneously substituting it by exchanging with isovolemic homologous red cells, plasma or whole blood.

Materials and Methods

Forty-four female SHR, weighing 190-260g, aged 5 to 9 months, were anesthetized with intraperitoneal amobarbital (10mg/100g of body weight). Animals were allowed to breathe room air spontaneously. Both femoral arteries were cannulated, one for continuous blood pressure recording with an electromanometer, and the other for blood withdrawing. A femoral vein was also cannulated for infusion. The rectal temperature was kept near 37°C with aid of a heating lamp. HCT was varied by the following way; withdrawing 3-5 ml of blood and simultaneously substituting it by intravenous infusion of an equal amount of homologous plasma, red cells or whole blood, which were previously prepared. The animals were divided into 4 subgroups by HCT values before carotid ligation; high (\( \geq 50\% \)), normal (40-49\%), low (30-39\%) and very low HCT subgroups (< 30\%). In these animals, in which blood pressure was altered by more than 20 mmHg during 30 min interval after blood exchange, data were excluded from the present results.

Thirty minutes after blood exchange in the ischemic groups of animals both common carotid arteries, previously exposed through ventral midline cervical incisions, were doubly ligated at the same time. One hour after carotid ligation the head was frozen in situ by pouring liquid nitrogen into a plastic funnel placed on the skull bone. Then, the whole brain was chiselled out and separated grossly into the supra- and infratentorial portions in the frozen state. Each part of the brain was weighed and ground in a rapid sequence and homogenized after the addition of ice cold perchloric acid. The tissue homogenate, maintained at 0°C to 4°C, was centrifuged and neutralized with potassium hydroxide at a pH of between 4.5 and 5.0. Lactate, pyruvate and adenosine triphosphate (ATP) concentrations were determined by the standard enzymatic methods. Arterial blood samples were taken for a blood gas analysis and HCT measurements at three occasions, such as resting state, 30 min after blood exchange, and 1 hr after carotid ligation. Arterial acid-base parameters were determined with an IL meter (Model 113) and duplicate HCT were measured with microhematocrit method by centrifuging at 11,000 rpm for 5 min.

Non-ischemic control groups of animals were also prepared in similar manner, namely both carotid arteries were exposed but not ligated (sham-operation). Arterial gas analysis and HCT determinations were made at resting state, 30 min after blood exchange and...
60 min after second sampling of arterial blood. Brain tissue metabolites were measured in these animals without carotid ligation, of which the brains were frozen 90 min after exchanging blood.

In comparing parameters among HCT subgroups, one way (ANOVA-I) or two way analysis of variance (ANOVA-II) was applied. In case of inhomogeneity of within-sample variances, mean values for each parameter in experimental and control HCT subgroups were compared separately by ANOVA-I. Simultaneous multiple comparisons among HCT subgroups were applied by using Bonferroni's t-test at α = 0.05 level, and also, for comparison between two groups, e.g. ischemic and corresponding control groups, Welch's t-test was used. The best fit curvilinear relations between supratentorial metabolites and HCT in ischemic and corresponding control groups, Welch's t-test was used. The best fit curvilinear relations between supratentorial metabolites and HCT in ischemic and corresponding control groups were fitted more appropriately than any others. The constants (a₀ to a₄) available by the computer were given in figures. For control rats, simple regressions were used to analyze relations between supratentorial metabolites and HCT.

Results

Before exchanging blood, there were no differences in average values for HCT, mean arterial pressure (MAP) and acid-base parameters among HCT subgroups (table 1). After blood exchange, as shown in table 2, HCT was varied ranging from 24% to 57%, although there were no differences in mean values for each parameter between ischemic and non-ischemic control animals. Also, there were neither differences in MAP and acid-base parameters among HCT groups nor between ischemic and control animals.

In table 3 are shown average values for differences in the parameters from before to 1 hr after carotid ligation or sham operation in HCT subgroups. Neither carotid ligation nor sham operation affected HCT values and MAP. But, in ischemic animals, arterial pCO₂ was decreased by more than 13 mmHg after 1 hr carotid ligation regardless of HCT values, whereas in non-ischemic animals, the reduction in arterial pCO₂ after sham operation was slight. Arterial pH was increased markedly after carotid ligation in ischemic animals, reflecting the decreased pCO₂. Likewise, in ischemic animals arterial pO₂ tended to increase during ischemia, but did not reach statistical significance compared with that of non-ischemic ones.

In table 4 are summarized metabolic data of supratentorial brain tissue in ischemic and control animals with various HCT. Among ischemic subgroups, an

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Table 1: Average Values for Hematocrit (HCT), Mean Arterial Pressure (MAP) and Arterial Acid-Base Parameters before Alteration of HCT in Spontaneously Hypertensive Rats (SHR)

<table>
<thead>
<tr>
<th>HCT groups</th>
<th>Very low (&lt; 30%)</th>
<th>Low (30–39%)</th>
<th>Normal (40–49%)</th>
<th>High (≥ 50%)</th>
<th>Pooled s² ANOVA-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>7</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>44.9</td>
<td>45.6</td>
<td>45.6</td>
<td>44.3</td>
<td>46.0</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>171</td>
<td>166</td>
<td>170</td>
<td>168</td>
<td>171</td>
</tr>
<tr>
<td>pCO₂ (mm Hg)</td>
<td>42.2</td>
<td>43.7</td>
<td>44.7</td>
<td>48.7</td>
<td>41.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.404</td>
<td>7.383</td>
<td>7.385</td>
<td>7.355</td>
<td>7.365</td>
</tr>
<tr>
<td>pO₂ (mm Hg)</td>
<td>87.8</td>
<td>80.7</td>
<td>98.6</td>
<td>98.1</td>
<td>81.2</td>
</tr>
</tbody>
</table>

I: ischemia, C: control. Pooled s²: pooled estimate variance of within-sample variances. Standard errors of parameters in each HCT subgroup are calculated by equation: s.e. = √(pooled s²/No. of rats in each HCT subgroup). ANOVA-I: one way analysis of variance.

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Table 2: Average Values for Changes in HCT, MAP and Arterial Acid-Base Parameters from before to after Alteration of HCT in SHR Prior to Induced Cerebral Ischemia

<table>
<thead>
<tr>
<th>HCT groups</th>
<th>Very low (&lt; 30%)</th>
<th>Low (30–39%)</th>
<th>Normal (40–49%)</th>
<th>High (≥ 50%)</th>
<th>Pooled s² ANOVA-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>7</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>-18.7</td>
<td>-18.4</td>
<td>-12.6</td>
<td>-9.5</td>
<td>-0.7</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>-10</td>
<td>-12</td>
<td>-8</td>
<td>-8</td>
<td>-4</td>
</tr>
<tr>
<td>pCO₂ (mm Hg)</td>
<td>-4.2</td>
<td>-3.4</td>
<td>-8.6</td>
<td>-3.6</td>
<td>-4.0</td>
</tr>
<tr>
<td>pH</td>
<td>0.016</td>
<td>0.058</td>
<td>0.026</td>
<td>0.050</td>
<td>0.023</td>
</tr>
<tr>
<td>pO₂ (mm Hg)</td>
<td>4.2</td>
<td>0.7</td>
<td>9.5</td>
<td>9.1</td>
<td>12.8</td>
</tr>
</tbody>
</table>

I, C and Pooled s² are same as in table 1. ANOVA-II: two way analysis of variance; HCT, difference among HCT groups, regardless of classification by presence or absence of cerebral ischemia; I/C, difference between SHR with and without cerebral ischemia, regardless of classification according to HCT levels.

*significant by design.
increase in lactate was related with HCT values (HCT ≥ 30%), namely lactate level was greater by 29% in high HCT and lower by 46% in low HCT than in normal HCT subgroup. In these animals with very low HCT (< 30%), however, lactate increased more than it did in those with either high or normal HCT (ANOVA-I p < 0.0001). In non-ischemic animals, there was also significant difference in lactate among the HCT groups, namely a slight but significant increase in lactate in very low HCT compared with normal (Bonferroni method).

Relations of cerebral lactate levels to preligating HCT values in individual animals with or without cerebral ischemia are demonstrated in figure 1. In ischemic animals, there was a U-shaped relation between lactate and HCT (R² = 0.423). The minimal point of the line was about 37% of HCT. On the other hand, although there was a U-shaped correlation between lactate and HCT in non-ischemic animals, the slope of this line was negligible (y = -0.05x + 4.69).

Cerebral pyruvate levels tended to be lower in either ischemic or non-ischemic animals with very low and higher HCT compared with normal or low HCT. Changes in L/P ratio were substantially the same as those in lactate, namely a U-shaped correlation to be present between L/P ratio and HCT values in ischemic animals (R² = 0.523) but no relation in non-ischemic ones (fig. 2). In contrast, cerebral ATP levels in ischemic animals were significantly lower than those in corresponding non-ischemic ones with high, normal and very low HCT (table 4). In low HCT group, however, cerebral ATP averaged 2.76 mM/kg in ischemic animals and 3.12 mM/kg in non-ischemic ones. The difference not being significant. Figure 3 reveals an inverse U-shaped correlation between cerebral ATP and HCT in ischemic animals (R² = 0.383), of which the maximal point was at 37% of HCT. On the other hand, ATP levels remained unchanged among non-ischemic animals with high to very low HCT.

Infratentorial brain metabolites in ischemic as well as non-ischemic animals are summarized in table 5. Lactate, pyruvate and ATP were normal in both groups of animals with various HCT, indicating that neither HCT changes nor carotid ligation caused impaired metabolism of the infratentorial tissue in this animal model.

**Discussion**

In comparison with normotensive rats, SHR have a greater increase in cerebral lactate and L/P ratio with a concomitant decrease in ATP following bilateral carotid ligation, suggesting that SHR are more susceptible
to cerebral ischemia. In addition, the ischemic changes of the brain have been confirmed histologically and electron microscopically in these hypertensive rats, in which the increased cerebrovascular resistance and an upward shift of CBF autoregulation probably due to persistent hypertension seem to be responsible for such susceptibility to cerebral ischemia. By using this animal model of cerebral ischemia in the present study, we investigated how HCT affects the glucose and energy metabolism of the ischemic brain. It has been well documented in the intact brain that HCT has an inverse effect on CBF due to alterations of blood viscosity and arterial oxygen content. Name-ly, an increase in HCT such as polycythemia reduces CBF, whereas a decrease in HCT such as various types of anemia increases CBF. Hyperviscosity at high HCT, which reduces CBF, overrides a benefit of increased arterial oxygen content, and consequently, oxygen supply to the brain is diminished. The present study showed that both supratentorial lactate and L/P ratio 1 hr after carotid ligation increased and ATP decreased with an elevation of HCT without alteration of MAP or arterial acid-base balance, suggesting adverse effects of high HCT on ischemic brain. The higher viscosity in the presence of high HCT leads to a greater reduction of regional CBF to the ischemic lesion and also to its surrounding tissue. Since blood is a non-Newtonian fluid and its viscosity is dependent on shear stress applied to the fluid, the viscosity markedly increases with a greater rise in flow resistance, when blood flow and shear stress decline to the critical low level. Therefore, high HCT generates a greater reduction of blood flow in the ischemic brain, leading to rather severe derangement of brain metabolism. In addition to viscosity, red blood cells have physical and chemical effects on the interaction between platelets and blood vessel surfaces, and high HCT enhances thrombus formation and causes a more pronounced decline of blood flow in the occluded vessels.

Under normal flow condition, the HCT value in microcirculation is lower than in large vessels, and HCT affecting viscosity is less important for regulating the flow in small vessels or negligible in microcirculation. This is not the case when normal flow is disturbed by occlusion of the supplying arteries. In such situations, the perfusion pressure is lowered with diminished blood flow, although small vessels are rather dilated due to hypoxia-induced vasoparalysis. Physiological hemodilution, regulated by resistance vessels, is no longer possible in microcirculation, resulting in a steep increase in HCT to the level of large vessels. Also, by lowering the perfusion pressure a passive adaptation of red cells as fluid droplets to narrow vessels is disturbed due to a lack of forces necessary for their deformation. These factors lead to aggregation of red cells and stasis of microcirculation. Such stasis phenomenon, however, is extremely dependent on HCT value in large vessels. At HCT values ranged 30% to 35%, fluidity of capillary blood is maintained, even when shear stress is deeply reduced. Further-
more, yield shear stress required for forcing stationary blood to start moving, is negligible independent of concentration of fibrinogen, which is considered the major determinant of viscosity and red cells aggregation in microcirculation, in other words, stasis seldom occurs and never persists. On the other hand, when HCT is elevated to 55% fluidity of capillary blood drops to zero at low shear stress and its yield shear stress is increased with elevation of HCT as well as concentration of fibrinogen.

Our findings of adverse effects of high HCT on ischemic brain are compatible with those on the other experimental and clinical observations. Pollock et al have shown in the ischemic model with gerbils that at high HCT a larger non-perfusion area with a carbon suspension was more commonly encountered, and their survival rate was adversely affected. In cats with acute cerebral ischemia, the administration of packed erythrocytes leads to a larger infarct volume than in those without treatment, while hemodilution significantly reduces the infarct volume.

In patients with completed stroke, who have carotid stenosis or occlusion, a positive correlation between HCT value and the size of infarcts on brain CT scan is found, suggesting that reduced blood flow due to an increase in blood viscosity associated with high HCT adversely affects collateral flow, thereby increasing the size of infarct.

At low HCT, in general, the increased preload due to increment of venous return and reduction of viscous resistance permits an increase in stroke volume of the heart, and a marked increase in viscous blood flow, the latter of which counteracts decreased arterial oxygen content and maintains oxygen transport to organs. However, Borgström et al have shown that acute normovolemic anemia can cause a linear increase in CBF at hemoglobin content lowering to 9g/100ml, but at lower hemoglobin such flow increase is much greater than may be accounted for by changes in viscosity alone. They also found a moderate increase in brain tissue lactate, but no alteration of energy metabolism with reduction in hemoglobin content to 3g/100ml, suggesting that such severe anemia leads to tissue hypoxia. According to our unpublished data concerning hemoglobin content and HCT in SHR (y = 0.28x + 0.5, r = 0.98, p < 0.001), 30% of HCT is equivalent to 9g/100ml of hemoglobin content. Our present results together with other studies indicate that 9g/100ml of hemoglobin or 30% of HCT is a critical value, below which CBF increases without affecting metabolism in non-ischemic brain, but both glucose and energy metabolism are severely impaired in the ischemic brain.

U-shaped correlations of lactate or L/P ratio to HCT were demonstrated among ischemic animals in this study. Either the minimal point of such regression for lactate and L/P ratio or the maximal point for ATP was about 37% of HCT, around which lactate as well as L/P ratio was little increased and ATP remained unchanged even 1 hr after bilateral carotid ligation, suggesting that a moderate anemia is less vulnerable to cerebral ischemia. Gottstein described interestingly that hemodilution therapy, keeping HCT value about 35%, would be more beneficial in patients with cerebral infarction who have HCT above 40%. Likewise, Wood et al have reported that the lowering of HCT from 42% to 34% by isovolemic hemodilution caused a significant increase in CBF concomitant with clinical and electroencephalographical improvements in patients with cerebral infarction at least 2 weeks after the onset. Additionally, our results suggest that HCT should not be reduced to below 30%, when stroke patients are submitted to hemodilution therapy.

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