IT WAS PREVIOUSLY REPORTED that pentobarbital (PBT) and a novel imidazole derivative (Y-9179 or Nizofenone) reduced the size of infarction in the brain after permanent middle cerebral artery (MCA) occlusion in cats. The protective action of Y-9179 against hypoxia and ischemia had already been demonstrated with various models using rats and mice. In addition, Y-9179 has few side effects such as anesthetic or cardio-pulmonary depressant action. Although the mechanism of action of Y-9179 has not been fully elucidated, its moderate depressant action on the cerebral metabolic rate may be involved in its cerebral protective action. However, this effect is not so potent as with PBT, and the role of the decreased cerebral metabolic rate in the mechanism of cerebral protection is controversial.

In animal experiments it was shown that a barbiturate (methohexital) altered cerebral blood flow (CBF) so that local CBF (lCBF) in the severely ischemic area was increased and that in the unaffected area was decreased (inverse steal). Since such a hemodynamic effect might also be relevant to the action of Y-9179, we undertook the present experiments to evaluate i) effect of delayed administration of PBT or Y-9179 on lCBF as measured by the hydrogen clearance method and ii) overall effects of these drugs in terms of histological changes one week after the ischemic insult, using a permanent MCA occlusion model in cats.

Materials and Methods
Twenty-four adults cats of both sexes weighing 2.0–3.9 kg were used in the present study. One or two weeks prior to MCA occlusion, the implantation of glass-coated platinum electrodes with bared tips plated with platinum black was carried out under PBT anesthesia (35 mg/kg). The positions of the electrodes are shown in figure 1. The electrode tips of channels 1 and 6 were located in the right and left internal capsule and the remaining eight in predetermined positions in the cerebral cortex. The diameters of the electrodes in the internal capsule and the cerebral cortex were 200 and 100 micra, respectively.

The insertion of the electrodes into the cortex was carefully undertaken using an operating microscope, through small burr holes in the calvarium and tiny incisions in the dura mater. Those electrodes together with an indifferent reference electrode were placed in the subcutaneous tissue and were then connected to a socket which was fixed to the skull with Bio-resin.

A few days following electrode implantation, lCBF measurements were recorded and repeated daily until the MCA was occluded. The lCBF fluctuated little, and the means for obtained values were calculated for each electrode. This value of the lCBF was used as the control lCBF for each electrode during later statistical analyses.

All lCBF measurements were painlessly carried out without anesthesia. Hydrogen, at a flow rate of 1.5 liter per minute, was given to the animal through a mask inhalator held a slight distance from the nose. Having been trained prior to MCA occlusion, the animals exhibited little agitation during the procedure so that only moderate restraint was necessary. The lCBF values were calculated according to the two-minute initial index method.

Then, transorbital occlusion of the MCA was carried out under halothane anesthesia. The details of this procedure have been described in a previous report. In the present experiment, the right femoral artery was catheterized for the monitoring of blood pressure, heart rate and blood gases. Immediately after clipping the MCA, halothane was discontinued and the recov-
The values in the text are shown as means ± standard errors. Statistical analyses, except for mortality rates, were performed using Student’s t-test.

Results

Four out of 11 cats in the control group and one out of the seven in the PBT group died during the observation period. None died in Y-9179 group. Autopsy of the dead animals revealed that the cause of death was transtentorial or tonsillar herniation due to massive brain edema. Mortality rate was greater in the control group than in the other two groups, although the difference was not statistically significant ($\chi^2$-test). In the remaining 19 cats (control: 7; PBT: 6; Y-9179: 6), the following results were obtained.

1. Vital signs, blood gases and neurological scores

Except for slight diminution in the respiratory rate as well as an increase in the heart rate in the PBT group, no statistical differences were observed in the vital signs and blood gases among the three experimental groups. During the period of drug administration, the neurological scores were high in the PBT group, but these dropped rapidly upon discontinuation of the drug (fig. 2). The rectal temperature fell to about 34°C during surgery. This seemed to be due to halothane anesthesia. After several hours, the rectal temperature of each animal returned to normal. At the dose used in the present study, PBT did not cause any significant fall in the rectal temperature.

2. The courses of the $\ell$CBF following MCA occlusion

The electrode values from which stable data were included in the above category were classified as borderzone electrodes.

FIGURE 1. Position of electrodes.

![POSITION OF ELECTRODES](https://example.com/figure1.png)

Each Observation Item (Left). The Observation Items are Shown in the Upper Square. Drugs Administered for the First Three Days are Shown in the Lower Square.
not obtained prior to MCA occlusion and those which were accompanied by evidence of local hemorrhage were excluded from study. The total number of electrodes were, therefore, 63 in the control, 58 in the PBT and 56 in the Y-9179 groups, respectively. The average of the cortical \( \bar{\text{CBF}} \) prior to MCA occlusion in all the electrodes was 83 \( \pm \) 7 ml/100g/min and that of the white matter (the internal capsule) was 53 \( \pm \) 7 ml/100g/min.

In the control group, the \( \bar{\text{CBF}} \) dropped in all the channels in the affected hemisphere following MCA occlusion. This was particularly prominent in channels 2, 3, 8 and 9, which were in the territory of the MCA. The electrodes in channels 4, 5 and 10, which were in the peripheral zone of the MCA territory, generally showed less marked drops in the \( \bar{\text{CBF}} \) (fig. 3A). In the PBT and Y-9179 groups, a drop of the \( \bar{\text{CBF}} \) similar to that in the control group was observed following MCA occlusion. However, there were considerable differences in the later courses of the \( \bar{\text{CBF}} \) between the two groups. As shown in figure 3B, a striking increase in the \( \bar{\text{CBF}} \) of channels 2 and 3 in the PBT group was observed at 30 minutes after drug administration. This was significantly greater than that of the P-\( \bar{\text{CBF}} \) (\( p < 0.05 \)). This was maintained with some fluctuations throughout the observation period. In the Y-9179 group, the courses of the \( \bar{\text{CBF}} \) in each channel were almost the same as in the control group (fig. 3C).

To further analyze the \( \bar{\text{CBF}} \) changes in the early ischemic period, the difference between the P-\( \bar{\text{CBF}} \) and the mean of later \( \bar{\text{CBF}} \) values during the first three hours (\( \Delta \bar{\text{CBF}} \)) was calculated with each electrode in all experimental groups. The correlation between the \( \Delta \bar{\text{CBF}} \) and P-\( \bar{\text{CBF}} \) was analyzed in each group (fig. 4). A linear correlation was found in the control group and the regression line crossed the abscissa at about 40 ml/100g/min. Similar correlations were revealed in the Y-9179 and PBT groups. However, since the distribution of points was apparently different in the PBT group, the data were reassembled into four subgroups according to the values of P-\( \bar{\text{CBF}} \) (fig. 5).

In each subgroup, the \( \Delta \bar{\text{CBF}} \) was compared among the three experimental groups. No significant differences were found between the control and Y-9179 groups. However, in the subgroups with a P-\( \bar{\text{CBF}} \) less than 40 ml/100g/min, the \( \Delta \bar{\text{CBF}} \) in the PBT group was...
significantly greater than those in the control and Y-9179 groups. On the other hand, in the subgroup with an \( \Delta \)CBF greater than 40 ml/100g/min, no statistical significance was found among three groups. Thus, PBT caused a selective \( \Delta \)CBF increase in the area where the P-\( \Delta \)CBF was less than about 40 ml/100g/min. Most of the electrodes showing the above increase of the \( \Delta \)CBF belonged to channels 2 and 3. Once the redistribution of the \( \Delta \)CBF was established during the early ischemic period, \( \Delta \)CBF values in each experimental group shifted without major fluctuations throughout the observation period (fig. 3A, 3B and 3C). Figure 6 shows the \( \Delta \)CBF courses in the contralateral hemisphere in the control group. There was a statistically significant \( \Delta \)CBF reduction in both the gray (channel 7) and the white (channel 6) matters following MCA occlusion, indicating the occurrence of diachisis.\(^{15-18}\) This \( \Delta \)CBF reduction recovered to the control level 120–144 hours after MCA occlusion. The PBT and Y-9179 groups showed courses of contralateral \( \Delta \)CBF similar to that of the control group. Drug administration did not significantly influence the contralateral \( \Delta \)CBF.

3. Correlation between the infarction ratio and the mean P-\( \Delta \)CBF

Histological examination revealed zones of four distinct pathological changes as described by Garcia et al.,\(^{19}\) i.e., the normal, marginal, reactive and central zones. For the convenience of calculation of the infarction ratio, area A (severely affected area) was defined as including the central and reactive zones and area B (moderately affected area) as corresponding to the marginal zone.

As shown in the previous report,\(^{1}\) there was a wide variation in the infarction ratio (the ratio of area A or B to the entire hemispheric area, being averaged in the four coronal sections in each animal). On the assumption that the variability of the infarction ratio was due to the difference in the severity of the initial flow reduction following MCA occlusion, the relationship between the infarction ratio and the mean P-\( \Delta \)CBF in the affected hemisphere was examined (fig. 7). In the control group, there was a close correlation between the infarction ratio and the mean P-\( \Delta \)CBF (\( r = 0.98 \) for area A and \( r = 0.95 \) for area A + B). Thus, the variability in the infarction ratio almost exclusively depended on the severity of the initial flow reduction. The same procedure undertaken with the PBT and Y-9179 groups revealed that the points were mostly situated below and to the left of the regression line of the control group.

No significant differences in the infarction ratio were obtained among the three groups. This seemed to be due to the fact that the control group incidentally included a few animals with only slight initial flow reductions following MCA occlusion (fig. 7). Therefore, a statistical analysis was again carried out after excluding the animals with a mean P-\( \Delta \)CBF greater than 50 mg/100g/min (fig. 7 below) from all groups. Significant differences between the control and drug-administered groups were then revealed.

4. Effects of drugs on vasogenic edema

Histological examination was carried out using light-, fluoro- and electron microscopes. The results will be reported in detail elsewhere. In the present section, findings in reference to vasogenic edema will be briefly described. Vasogenic edema is characterized by the perivascular exudation of plasma, as evidenced by fluid accumulation and leakage of Evans blue.\(^{20}\) Such changes were most prominent in the periphery of the central zone as well as in the inner layer of the reactive zone (photo). To evaluate the severity of vasogenic edema in each experimental group, the extent of the perivascular fluid accumulation was graded into four classes, i.e., negligible, mild, moderate and severe changes, in each specimen.
The results are shown in table 2. From this, it seems clear that vasogenic edema was almost absent in the Y-9179 group, whereas it was more evident and close to the control in the PBT group.

5. The course of the $\Delta$CBF as related to each histological change

Although the number of electrodes in the affected areas was relatively small, there was a clear-cut difference in the courses of the $\Delta$CBF among the four histologically different cortical areas in the control group (fig. 8A). In the central zone, a $\Delta$-CBF of below 10 ml/100g/min was maintained. The reactive zone exhibited a little higher $\Delta$CBF, around 20 ml/100g/min. The border zone showed a wide variation of the $\Delta$CBF. In the Y-9179 group, no electrode was found in the central zone. In the other areas, the courses of the $\Delta$CBF were almost similar to those in the control group (Fig. 8C). In the PBT group, on the contrary, a remarkable difference in $\Delta$CBF courses was revealed in the border and reactive zones where a prominent increase in the $\Delta$CBF occurred following PBT administration (fig. 8B). This corresponded to the

![Figure 6. The time-course of the $\Delta$CBF in the contralateral hemisphere in the control group.](image1)

![Figure 7. The relationship between the infarction ratio of severely affected area and the mean P-$\Delta$CBF of all the seven electrodes implanted in the cortex of the affected hemisphere. Averaged values of the mean P-$\Delta$CBF and the infarction ratio in each group were shown in the table below. Those animals which showed the mean P-$\Delta$CBF greater than 50 ml/100g/min were excluded.](image2)

![Figure 5. Histograms comparing the mean of the $\Delta$CBF among the three groups according to the values of the P-$\Delta$CBF. The open, hatched and dotted columns indicate the control, PBT and Y-9179 groups, respectively.](image3)
TABLE 2  The Severity of the Perivascular Exudation of Plasma Fluid in Areas of Different Histological Changes is Shown With Each Animal. Only Those Animals Adopted for the Statistical Evaluation of the Infarction Ratio (fig. 7) Were Included.

<table>
<thead>
<tr>
<th>Infarcted lesions</th>
<th>Central zone</th>
<th>Reactive zone</th>
<th>Marginal zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inner areas</td>
<td>Outer areas</td>
<td>Inner areas</td>
</tr>
<tr>
<td>Control</td>
<td>1 - + + + + +</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
</tr>
<tr>
<td>2</td>
<td>- + + + + +</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
</tr>
<tr>
<td>3</td>
<td>- - + + + +</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
</tr>
<tr>
<td>4</td>
<td>- - + + + +</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
</tr>
<tr>
<td>P B T 3</td>
<td>1 - + + + + +</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
</tr>
<tr>
<td>2</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
</tr>
<tr>
<td>4</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
</tr>
<tr>
<td>5</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
</tr>
<tr>
<td>Y-9179 3</td>
<td>1 - - - - -</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
</tr>
<tr>
<td>2</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
</tr>
<tr>
<td>4</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
</tr>
<tr>
<td>5</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
</tr>
</tbody>
</table>

- : No change, + : Mild, ++ : Moderate, +++ : Severe

The fact that PBT caused a remarkable $\ell$CBF increase in the area with a P-$\ell$CBF lower than 40 ml/100g/min. It is noteworthy that the cortical area invariably underwent infarction in spite of this increase in the $\ell$CBF.

Discussion

1. The Infarction Ratio

In the control group, there was a linear correlation between the infarction ratio and the mean of the P-$\ell$CBF. The points in the PBT and Y-9179 groups were located mostly below and to the left of the regression line (fig. 7). This fact in itself is indicative of the protective action of both drugs. Nevertheless, the statistical analysis revealed no significant difference when the infarction ratios of the three groups were compared. This was due to the fact that the control group included a few animals with high P-$\ell$CBFs and, subsequently, very low infarction ratios. Considering that the four animals that died during the observation period were already excluded from the control group, it seemed reasonable to make a further selection of animals so that all the experimental groups had comparable means and variances in respect to the P-$\ell$CBF. Therefore, the upper limit of the mean P-$\ell$CBF was arbitrarily determined as 50 ml/100g/min, and the animals with higher mean P-$\ell$CBFs were excluded from all the groups. Subsequent analysis revealed a significant difference between the control and Y-9179 groups (fig. 7 below). Such a procedure may appear artificial, but it is still believed rational as well as necessary when the present MCA occlusion model in cats is employed for the evaluation of a drug’s effects on the infarction ratio.

2. Effects of the drugs on the courses of the $\ell$CBF following MCA occlusion

The present study revealed that a spontaneous redistribution of the $\ell$CBF took place in the control group during the first three hours following MCA occlusion. In the Y-9179 group, an $\ell$CBF redistribution similar to that in the control group was observed. Hence, it was shown that Y-9179 did not possess any specific effect on the $\ell$CBF. On the other hand, a remarkable change in the $\ell$CBF was observed in the PBT group. Following drug administration, a striking $\ell$CBF increase oc-

![Figure 8. The courses of the $\ell$CBF in areas of different histological outcomes are shown with each experimental group.](http://stroke.ahajournals.org/)}
occurred in the electrodes the P-\(\bar{C}B\)F of which were below 40 ml/100g/min. As shown in figure 5, the flow increase was greater in electrodes with P-\(\bar{C}B\)F lower than 20 ml/100g/min.

In the experiments by Branston et al., the \(\bar{C}B\)F increased in poorly perfused areas (in which the P-\(\bar{C}B\)F or clip flow in their report was less than 20 ml/100g/min) and it decreased in well-perfused areas (P-\(\bar{C}B\)F greater than 25 ml/100g/min) following administration of methohexital. Although the flow changes following drug administration are considered essentially similar, there are some discrepancies between the results of the present study and that of Branston et al. First, the P-\(\bar{C}B\)F value at which the regression line crossed the horizontal axis in figure 4 (60 ml/100g/min) was considerably greater than the corresponding value obtained in their study (20 ml/100g/min). This may be partly due to differences in experimental conditions, such as the kind of anesthetic, the control of the systemic arterial pressure and respiratory conditions, the kind of animal, the amount of the drug administered and so on. Since a significant flow change can occur during the first 30 minutes following MCA occlusion, the difference in the time interval between the first \(\bar{C}B\)F measurement and MCA occlusion may also be an important factor. In the present study, the P-\(\bar{C}B\)F was obtained 30 minutes after MCA occlusion, whereas in their study it was obtained immediately thereafter. Therefore, it is thought that the P-\(\bar{C}B\)F values in the present study were generally greater than the clip flow values in their study, reflecting the rapid collateral blood supply to the ischemic area. Second, they suggested that the flow increase in poorly perfused areas might result from a reduction in flow occurring in relatively well-perfused areas following the reduction in metabolic rate and vasoconstriction by the barbiturate. In the present experiment, however, the flow in well-perfused areas in the PBT group was not so remarkably reduced as to be statistically significant. On the other hand, the border-zone electrodes showed wide variations in the \(\bar{C}B\)F. In the Y-9179 group, no electrodes were obtained in the central zone. In the normal area, the mean \(\bar{C}B\)F following MCA occlusion was lower than that in the control group, but the difference was not statistically significant. In the other two areas, the courses of the \(\bar{C}B\)F were not significantly different from those in the control group. In the PBT group, the \(\bar{C}B\)F in the normal area was almost the same as in the control group. However, there was a remarkable \(\bar{C}B\)F increase following drug administration in the severely affected (reactive zone) area. The border-zone electrodes showed similar \(\bar{C}B\)F increases. From the above results, it may be said that the alteration in the \(\bar{C}B\)F distribution as a result of PBT administration occurred almost selectively in areas with later histological damages.

4. The Mechanisms of Cerebral Protection By PBT and Y-9179

The present study disclosed that PBT caused a remarkable \(\bar{C}B\)F increase in the areas with low P-\(\bar{C}B\)Fs. The histological examination indicated that the flow increase occurred almost selectively in severely damaged areas. Therefore, the cause of flow redistribution by PBT in the present study may be tentatively ascribed to the drug’s specific action on the vasculature of the affected hemisphere. It has been believed that PBT and other barbiturates exert direct constrictive effects on cerebral vessels. However, the recent report of Marin et al. suggests that PBT decreases cerebrovascular reactivity and inhibits contractions elicited by various vasoconstrictor agents, such as noradrenaline, potassium chloride and 5-hydroxytryptamine, in isolated human cerebral arteries. As a possible explanation for the mechanism of the PBT-induced fall in ICP, they suggested that PBT relaxed the major cerebral arteries, but not the arterioles, thus triggering a vasoconstriction of the latter due to the increased hydrostatic pressure (the Bayliss effect). If this also holds true in the present MCA occlusion model in cats, the observed flow increase as a result of PBT administration may be explained as follows: PBT relaxes the major cerebral arteries and increases the intraluminal hydrostatic pressure in the collateral circulation to the ischemic region where the normal vascular reactivity has been lost; subsequently, the increased pressure head will cause an increase in blood flow. Although the results of the present study favor the above hypothesis, further studies are needed to elucidate the mechanism of flow redistribution by PBT.

Given that PBT increases blood flow in severely ischemic areas, what beneficial effect did it exert on
the subsequent evolution of pathological changes? So far as the correlation between the course of the CBF and the final histological changes was examined, no evidence supporting the thesis that the increased CBF was effective in relieving the corresponding cortical area from infarction was obtained. Nevertheless, it must be pointed out that PBT was administered 30 minutes after MCA occlusion in the present study. It is still possible that the area with an extremely low CBF had undergone irreversible change before drug administration. The effect of an earlier administration of PBT would merit further investigation.

In the Y-9179 group, there were no differences from the control group in the CBF distribution or its time courses following MCA occlusion. Therefore, it may be concluded that the CBF change is not involved in its protective action. On the other hand, the histological examination suggested that Y-9179 might lessen cerebral edema of the vasogenic type. The above effect was observed in the center of the severely affected area and this indicates a direct anti-edema action of Y-9179, which might be related to the mechanism of its cerebral protection. Such an effect of Y-9179 would certainly deserve further investigation. A similar study in the PBT group revealed that the extent of the perivascular fluid accumulation was almost equal to the control group, indicating that the mechanisms of cerebral protection by PBT and Y-9179 are again different in this respect.

In summary, the present study revealed that both PBT and Y-9179 exerted protective actions against permanent regional ischemia in cats. PBT caused a significant CBF increase in the severely ischemic areas, but this action of PBT did not seem to be involved in its protective action. Y-9179 showed no significant action on the course of the CBF but its anti-edema effect may be related to its protective action. Finally it may be stated that yet unidentified mechanisms not related to the CBF are involved in cerebral protection by PBT and Y-9179.

References
Representative pathological appearances of the severely affected areas in each group (C: Central zone, R: Reactive zone). In the control and the pentobarbital groups (Photo 1, 2. HE stain, × 306), the serum leakage as shown in the Photos (black arrows) was prominent. In the Y-9179 group (Photo 3. HE stain, × 612), serum leakage was almost absent in the otherwise severely affected areas.
Mechanisms of cerebral protection by pentobarbital and nizofenone correlated with the course of local cerebral blood flow changes.
C Ochiai, T Asano, K Takakura, T Fukuda, H Horizoe and Y Morimoto

Stroke. 1982;13:788-796
doi: 10.1161/01.STR.13.6.788

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/13/6/788

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/