

## Thiopental Amelioration of Brain Damage after Global Ischemia in Monkeys

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The authors studied the effect of thiopental in ameliorating permanent brain damage in monkeys after 16 min of global ischemia of the brain produced by a high-pressure neck tourniquet and systemic arterial hypotension. Intensive care and life support, including monitoring of physiologic variables, were provided for seven days after ischemia. Neurologic recovery was evaluated by scoring neurologic deficit and by histopathologic examination of the brain at sacrifice on day 7 after ischemia. Ten control monkeys had a mean neurologic deficit score of  $53 \pm 15$  per cent (mean  $\pm$  SEM). Thiopental, 90 mg/kg, administered 5 and 15 min after ischemia significantly improved neurologic recovery, with neurologic deficit scores of 0 ( $n = 5$ ) and  $18 \pm 8$  per cent ( $n = 5$ ), respectively. Improved neurologic recovery was not observed when therapy was delayed to 30 and 60 min after ischemia. However, thiopental, 120 mg/kg, improved recovery when administered 60 min (neurologic deficit score,  $7 \pm 6$  per cent), but not 30 min after ischemia. Histologic changes in the brain correlated with neurologic deficit scores. These results show that after 16 min of global ischemia of the brain, a major portion of the permanent brain damage occurs after restoration of circulation and is amenable to therapy with thiopental. There appears to be a dose- and time-related response to thiopental therapy, but the optimal values were not identified in this study. (Key words: Anesthetics, intravenous: thiopental. Brain: anoxia.)

EARLIER STUDIES showed that barbiturates, administered *before* hypoxia-ischemia, may be beneficial in minimizing neurologic deficit.<sup>1-3</sup> Yatsu *et al.*<sup>4</sup> reported that methohexital administered *during* cerebral hypoxia-ischemia improved neurologic recovery in rabbits. Smith *et al.*<sup>5</sup> demonstrated that large doses of thiopental were effective in decreasing the neurologic deficits and infarct sizes in dogs when administered not only before, but also *after*, focal ischemia produced by occlusion of the middle cerebral artery. Their findings were later confirmed in

primates by Hoff *et al.*,<sup>6</sup> Michenfelder *et al.*,<sup>7</sup> and Moseley *et al.*<sup>8</sup>

Major obstacles in proving the efficacy of any postischemic therapy have been the lack of reproducible neurologic deficit in animal models after global ischemia of the brain of comparable severity, and the lack of controlled postischemic life support. We recently developed a reproducible, controlled monkey model,<sup>9</sup> and have now demonstrated that large doses of thiopental can ameliorate neurologic deficit and brain histologic changes when given after global ischemia.

### Methods and Materials

Prequarantined female rhesus monkeys weighing 4 to 5 kg and fasted overnight, with water *ad libitum*, were used. All monkeys were observed for abnormalities in coordination, behavior, stool consistency, and feeding habits for two days prior to the study.

### ISCHEMIA

Procedures used in our model have been described in detail.<sup>9</sup> Briefly, the monkeys were anesthetized with halothane, 0.5-1.0 per cent/nitrous oxide, 66 per cent, and oxygen, 33 per cent, immobilized with pancuronium, 0.05 mg/kg, intravenously, and the lungs mechanically ventilated. End-tidal CO<sub>2</sub> was continuously monitored and maintained at about 5 per cent. A peripheral venous line was used for continuous infusion of dextrose, 5 per cent, in sodium chloride, 0.45 per cent (3-5 ml/kg body weight per hour); a transurethral bladder catheter, for continuous monitoring of urinary output; femoral-artery and femoral-vein catheters via cutdown for monitoring mean arterial pressure (MAP) and central venous pressure (CVP). EKG and rectal temperature were continuously monitored. Intracranial pressure (ICP) was monitored through a Silastic catheter inserted subdurally via a small burr hole over the parietal cortex. The electroencephalogram (EEG) was monitored bilaterally via supradural screw electrodes over the frontal, parietal and occipital cortices. After completion of surgical procedures, an arterial blood sample was drawn to verify normal blood gas, pH, and electrolyte values.

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The soft cuffed endotracheal tube was replaced with a stiff uncuffed Teflon tube to insure a patent airway during tourniquet inflation. A pediatric tourniquet was loosely wrapped around the neck. MAP was rapidly decreased to 50 torr by repeated injections of trimethaphan, 10 mg, intravenously, and the tourniquet immediately inflated to 1,500 torr. Throughout the 16-min ischemic episode MAP was maintained at about 50 torr by halothane, 4–5 per cent, in oxygen (nitrous oxide was discontinued) and by positive end-expiratory pressure (to as much as 20 cm H<sub>2</sub>O). Halothane was gradually discontinued after 5 min of ischemia. About 4 min before the end of ischemia, norepinephrine (0.08 mg/ml) was administered by intravenous infusion to increase MAP to about 80 torr to restore normal cerebral perfusion pressure (CPP).

The effectiveness of the neck tourniquet method for producing complete arrest of the circulation of the brain was verified by two methods: 1) brain scan with <sup>99m</sup>Tc (1 mCi/kg) injected intravenously after tourniquet inflation, which revealed no radioactivity above the tourniquet (13 monkeys); 2) intracarotid <sup>133</sup>Xe injection immediately before tourniquet inflation, which revealed no clearance of brain <sup>133</sup>Xe after tourniquet inflation (30 monkeys, 20 cats). Complete ischemia demonstrated by these methods was reproducibly correlated with an isoelectric EEG within 10–15 sec after tourniquet inflation and only a transient increase in ICP, whereas with incomplete ischemia, the isoelectric EEG occurred late or not at all, and the increase in ICP was sustained. Therefore, we used these changes in EEG and ICP to document complete ischemia in all studies.

The neck tourniquet alone, even when inflated to 1,500 torr, did not arrest the circulation of the brain reliably unless the hypertensive response to ischemia was prevented and MAP lowered to 50–80 torr. Therefore, an essential feature of our method is the maintenance of hypotension throughout ischemia, with systolic arterial pressures below 80 torr and MAP of about 50 torr.

#### TREATMENT GROUPS

A total of 62 monkeys was subjected to 16 min global ischemia of the brain; 19 were untreated and 43 received thiopental. Nine of the untreated monkeys<sup>9</sup> and 16 of the thiopental-treated monkeys were not included in the study because they died before seven days or complications arose due to errors in postischemic life support or for reasons not directly attributable to brain damage. Therefore, 53 per cent of the control and 63 per cent of thiopental studies

were successfully completed to seven days after ischemia, for an overall success rate of 60 per cent.

The ten untreated monkeys surviving for seven days after ischemia comprised the control group and were used for comparison with the thiopental-treated groups. Five monkeys each received thiopental, 90 mg/kg, 5, 15, or 30 min after ischemia and five each received thiopental, 120 mg/kg, 30 or 60 min after ischemia. Two monkeys received thiopental, 90 mg/kg, 60 min after ischemia.

#### POSTISCHEMIA

In all monkeys at the end of 16 min of ischemia, the neck tourniquet was rapidly deflated and removed. The norepinephrine infusion rate was increased to increase MAP as quickly as possible to above 75 torr without exceeding 125 torr. Controlled ventilation with 100 per cent oxygen was continued, with end-tidal CO<sub>2</sub> controlled at about 5 per cent. Positive end-expiratory pressure was maintained at 2 cm H<sub>2</sub>O. Between 4 and 5 min after ischemia, a blood sample was obtained for blood-gas and pH analysis.

Electroencephalogram (EEG), electrocardiogram (ECG), MAP, CVP, ICP, respiratory rate, heart rate, inspired O<sub>2</sub>, rectal temperature, and end-expiratory CO<sub>2</sub> were continuously recorded for seven days, unless the extent of recovery made restraining for maintenance of vascular catheters and leads hazardous. Variables monitored intermittently were arterial blood P<sub>O<sub>2</sub></sub>, P<sub>CO<sub>2</sub></sub>, pH, and hematocrit at 30 min, 2, 4, 6 and 12 hours, and every 12 hours thereafter; and serum and urinary sodium, potassium and osmolality at 12-hour intervals.

Several variables were controlled for seven days after ischemia. Rectal temperature was maintained at 37–39 C. End-expiratory CO<sub>2</sub> was maintained at 5 per cent during controlled ventilation and intermittent mandatory ventilation for weaning; when spontaneous breathing could maintain end-expiratory CO<sub>2</sub> at 5 per cent or less, it was not regulated even when spontaneous hyperventilation occurred. Base deficit exceeding –5 mEq/l was corrected with sodium bicarbonate. Arterial blood P<sub>O<sub>2</sub></sub> was maintained above 100 torr (above 80 torr after extubation) with 100 per cent oxygen during the first six hours and with 50 per cent thereafter, using positive end-expiratory pressure as needed. Serum electrolytes and osmolality were normalized by administration of sodium chloride, 0.9 per cent, dextrose, 5 per cent/sodium chloride, 0.45 per cent, and dextrose, 10 per cent in 0.25 per cent sodium chloride, intravenously. Fluid intake and output were regulated. MAP was main-

tained above 80 torr by infusion of norepinephrine. Control monkeys needed progressively less norepinephrine, and by an hour after ischemia, none was needed. Thereafter, MAP was allowed to increase to above 120 torr, unless cardiac dysrhythmias occurred, in which case trimethaphan was used to decrease MAP sufficiently to restore normal cardiac rhythm. During thiopental infusion, norepinephrine infusion was titrated to maintain MAP between 80 and 120 torr. As expected, the thiopental-treated monkeys needed more norepinephrine than the controls, and less norepinephrine was necessary with delayed thiopental infusion.

Procedures involved in respiratory care, including controlled ventilation and weaning via intermittent mandatory ventilation, were similar in the control and thiopental-treated groups. In the control group, weaning to spontaneous breathing was possible about two hours after ischemia, whereas the thiopental-treated monkeys had to be maintained on controlled ventilation for about 12 hours. The Teflon endo-

tracheal tube was replaced with a soft cuffed endotracheal tube two hours after ischemia. The endotracheal tube was suctioned every two hours, and changed every two days. Standard procedures and criteria for weaning from controlled ventilation and for extubation were followed.

General care included cleaning and disinfecting all surgical wounds, alcohol baths, rotation from one lateral position to the other, and rinsing the mouth with saline solution.

Caloric requirements were met by infusing dextrose, 10 per cent, in 0.25 per cent sodium chloride, intravenously, starting 24 hours after ischemia, until the appearance of bowel sounds permitted nasogastric feeding with Sustacal® (Mead-Johnson). Total caloric intake provided was about 200–300 Kcal/day.

Drugs occasionally used in the monkeys' life support, particularly for the controls, were: lidocaine, 1 per cent, in 10-mg doses, for dysrhythmias; Dramamine® (Searle), for vomiting during awakening; furosemide, 1 mg/kg, for oliguria. One of the

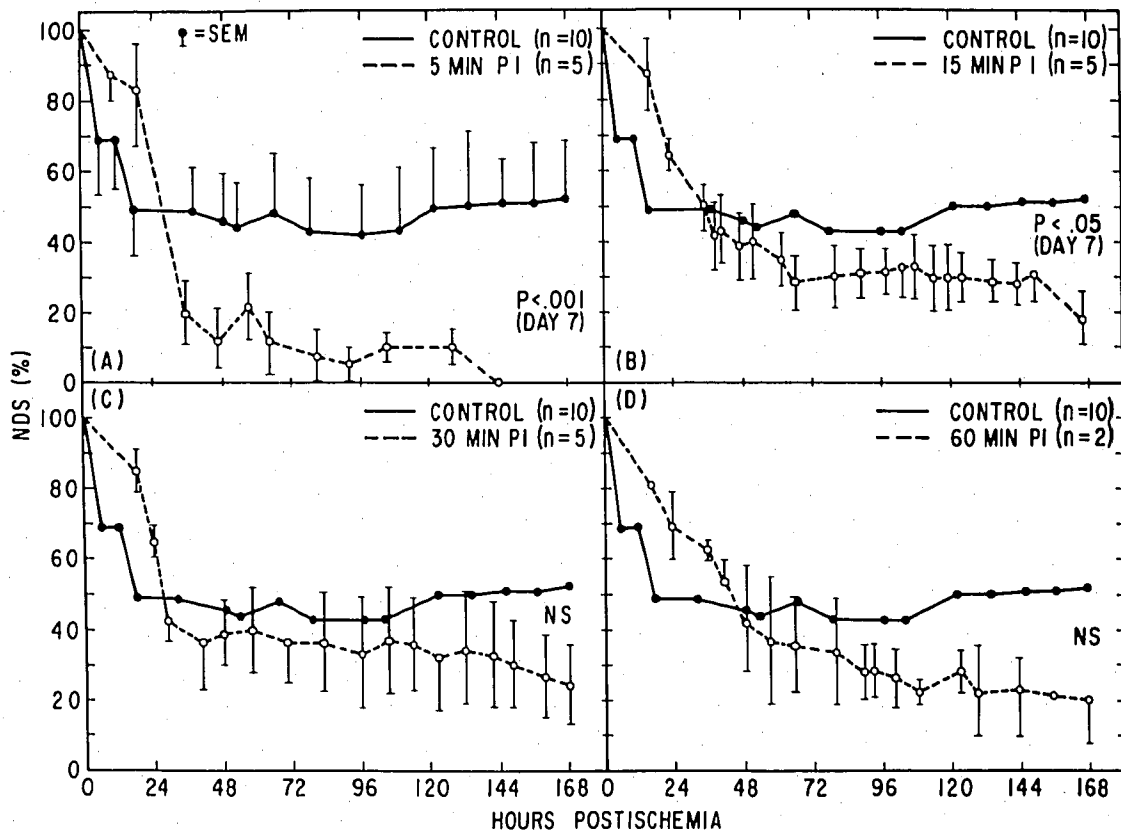


FIG. 1. Percentage neurologic deficit scores (NDS) in rhesus monkeys for seven days, after 16 min global ischemia of the brain produced by a high-pressure neck tourniquet and trimethaphan-induced hypotension. Ten untreated monkeys served as controls for four treatment groups (A–D). Treated monkeys received thiopental, 90 mg/kg, infused intravenously beginning 5 (A), 15 (B), 30 (C), and 60 (D) min postischemia, with a third of the total dose infused in the first 5 min and two thirds over the subsequent 55 min.

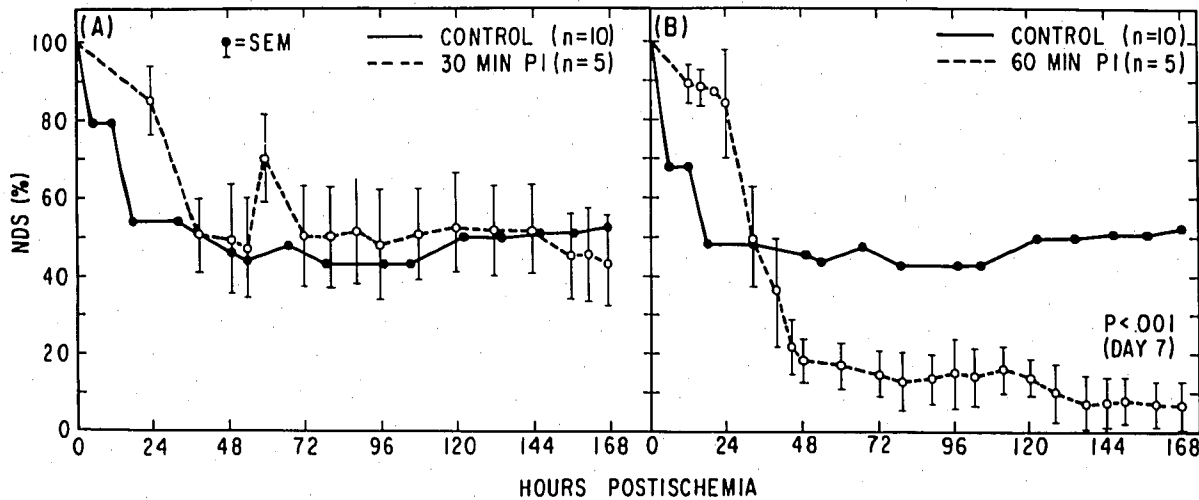


FIG. 2. Percentage neurologic deficit scores (NDS) in rhesus monkeys for seven days, after 16 min global ischemia of the brain produced by a high-pressure neck tourniquet and trimethaphan-induced hypotension. Ten untreated monkeys served as controls for two treatment groups (A, B). Treated monkeys received thiopental, 120 mg/kg, infused intravenously beginning (A) 30 and (B) 60 min postischemia, with a third of the total dose infused in the first 5 min and two thirds over the subsequent 55 min.

ten control monkeys was given diphenylhydantoin, 10 mg, and phenobarbital, 5 mg/kg, intramuscularly, for convulsions more than 12 hours after ischemia, and insulin-dextrose for hyperkalemia.

Thiopental was administered in 2.5 per cent solution by intravenous infusion for a total dose of either 90 or 120 mg/kg (Figs. 1 and 2). In all cases, a third of the total dose was infused in the first 5 min after the start of infusion (loading dose) and two thirds over the ensuing 55 min. The times of starting thiopental infusion, namely 5, 15, 30, and 60 min after ischemia for the different groups, were precisely adhered to in all studies. Transient minor adjustments of infusion rates were sometimes necessary to facilitate MAP support or reverse cardiac dysrhythmias. Problems were more frequently encountered in the group that received thiopental 5 min after ischemia than in the groups in which administration was delayed.

For scoring neurologic deficit, a system was developed.<sup>9</sup> Maximum neurologic deficit was given a score of 500 points (100 per cent), and normal neurologic status, zero points. The scoring sheet used for recording 17 different responses included: a) level of consciousness, 100 points; b) cranial nerve reflexes, 100 points; c) motor function, 200 points; d) respiration, 100 points. Throughout the seven days after ischemia, neurologic deficit scores were obtained every six hours for the first 24 hours and every eight hours thereafter.

A system for scoring histologic changes was also developed. On day 7 after ischemia, after induction of anesthesia with sodium pentobarbital, 30 mg/kg, the brain was perfused and fixed with paraformalde-

hyde, 4 per cent, infused into the left ventricle, with the thoracic aorta clamped and the right atrium incised. Two hours after perfusion, the brain and cervical spinal cord were removed and placed in buffered glutaraldehyde, 3 per cent, for two weeks prior to gross examination and histologic analysis with hematoxylin-eosin and, in some cases, Nissl and Bielschowsky silver stains. Microscopic slides of 20 brain regions were examined, and sizes and frequencies of three types of lesions were evaluated (*i.e.*, infarction, ischemic neuronal changes, and edema). The severity of each lesion was assessed on a four-point scale and multiplied by weighting factors (*i.e.*, infarction  $\times$  4, ischemic neurons  $\times$  2, and edema  $\times$  1) for quantitative histopathologic scoring. All histologic examinations and scoring were performed by two of us (JM and GR) who did not know whether the brains were from control or treated monkeys.

All data were tested by analysis of variance. Unless otherwise stated, statistical significance indicates *P* values of less than 0.05.

### Results

Physiologic variables were essentially the same in control and thiopental-treated groups. Before ischemia in all groups combined, MAPs ranged between  $76 \pm 6$  and  $88 \pm 8$  torr (mean  $\pm$  SEM) and ICPs between  $7 \pm 3$  and  $10 \pm 2$  torr, without significant differences among the groups. During ischemia, MAPs were decreased to about 50 torr in all groups (range,  $47 \pm 4$  to  $62 \pm 6$  torr). At zero minutes after ischemia (the moment of tourniquet deflation),

MAPs ranged between  $42 \pm 9$  and  $62 \pm 17$  torr, and were not significantly different among the groups. Immediately after ischemia, the rates of increase of MAP produced by norepinephrine infusion were slower in the control and thiopental, 120 mg/kg, groups than in the thiopental, 90 mg/kg, groups. By 15 minutes and for as long as two hours after ischemia, however, MAP, ICP and CPP values were similar in all groups. Beyond two hours after ischemia, MAP values were usually between 120 and 140 torr, but at times ranged between 90 and 150 torr. However, no consistent or sustained differences occurred among the groups. Beyond four days after ischemia, the number of monkeys with arterial catheters in place for blood pressure monitoring was too small for meaningful evaluation.

After ischemia, mean arterial oxygen tension ( $P_{a_{O_2}}$ ) values were between  $85 \pm 4$  (SEM) (breathing room air) and  $462 \pm 4$  (breathing oxygen) torr and arterial carbon dioxide tension ( $P_{a_{CO_2}}$ ) values were between  $24 \pm 4$  and  $37 \pm 5$  torr. Return of spontaneous respiratory efforts occurred approximately two hours after ischemia in the control group and 12 hours after ischemia in the thiopental-treated groups. In the control monkeys respiratory efforts were noticed as early as one to two hours after ischemia, but adequate spontaneous breathing when  $P_{a_{CO_2}}$  was 40 torr or less and  $P_{a_{O_2}}$  350 torr or more during breathing of oxygen was not restored in all monkeys until six hours after ischemia. All monkeys treated with thiopental 5 and 15 minutes after ischemia were breathing spontaneously 12 hours after ischemia, whereas some of those treated at 30 and 60 min needed controlled ventilation for 24 hours after ischemia. There was some variation among monkeys within each group. Thus, the thiopental-treated monkeys had controlled ventilation for longer periods after ischemia than the controls. For the first six hours,  $P_{a_{O_2}}$  values during mechanical ventilation with 100 per cent oxygen ranged between  $307 \pm 30$  and  $450 \pm 26$  torr;  $P_{a_{O_2}}$ s were above 100 torr thereafter during breathing of 50 per cent oxygen.  $P_{a_{CO_2}}$  values ranged from  $45 \pm 3$  to  $30 \pm 1$  torr ( $P < 0.05$ ). In all other groups  $P_{a_{CO_2}}$ s were similar among groups and ranged from  $33 \pm 7$  to  $37 \pm 9$  torr. When spontaneous breathing was fully restored,  $P_{a_{CO_2}}$ s were similar in all groups and ranged between  $25 \pm 2$  and  $31 \pm 3$  torr. Thus, control monkeys had lower  $P_{a_{CO_2}}$ s earlier after ischemia due to an earlier return of spontaneous hyperventilation.

Electroencephalographic (EEG) activity returned earlier after ischemia in control than in thiopental-treated monkeys. In control monkeys, EEG returned  $1.10 \pm 0.15$  hours after ischemia. EEG return was sooner

with thiopental, 90 mg/kg, at 5 min ( $12 \pm 0.67$  hours) than with thiopental, 120 mg/kg, at 30 min ( $15.8 \pm 1.16$  hours). Overall mean EEG return time for all thiopental-treated groups was 14 hours.

Neurologic recovery was significantly improved by thiopental, 90 mg/kg, given 5 or 15 min after ischemia and thiopental, 120 mg/kg, given 60 min after ischemia (figs. 1 and 2). Neurologic deficit scores of control monkeys (fig. 1A) had decreased to  $70 \pm 16$  per cent six hours after ischemia; by 36 hours after ischemia, the score had improved to  $49 \pm 14$  per cent, but it did not improve further. The final score was  $53 \pm 15$  per cent at seven days. On day 7, although cranial nerve reflexes were usually normal, all monkeys had severe motor deficits and were unable to sit, walk, or feed themselves. They appeared to be in a vegetative state, with levels of consciousness ranging from stupor (response to painful stimuli) to coma (no response to painful stimuli).

Monkeys treated with thiopental, 90 mg/kg, 5 min after ischemia (fig. 1A) had higher neurologic deficit scores ( $88 \pm 8$  per cent) six hours after ischemia than the control monkeys, presumably due to thiopental. Between 18 and 48 hours after ischemia, however, marked improvement occurred with neurologic deficit scores decreasing from  $83 \pm 16$  to  $12 \pm 8$  per cent. Gradually, over the next four days, the condition of the monkeys improved, until they were essentially normal neurologically 144 hours after ischemia (six days), with zero per cent neurologic deficit scores. Except for some cases of slight ataxia, consciousness was normal (fully alert) and the monkeys were able to sit, stand, walk, climb, and feed themselves.

Thiopental, 90 mg/kg, administered 15 min after ischemia resulted in improved recovery compared with control seven days after ischemia (fig. 1B). Neurologic deficit scores early after ischemia were higher than in the control monkeys, but rapidly improved between 18 and 48 hours after ischemia to  $39 \pm 9$  per cent and between 38 and 66 hours to  $29 \pm 7$  per cent. Neurologic deficit scores decreased in the last 24 hours to  $18 \pm 8$  per cent, which was significantly lower than the scores of the control monkeys. When administration of thiopental, 90 mg/kg, was delayed to 30 and 60 min after ischemia, the final neurologic deficit scores of  $25 \pm 12$  and  $22 \pm 13$  per cent, respectively, were not significantly lower than those of the controls (fig. 1, C and D).

Thiopental, 120 mg/kg, administered 30 min after ischemia did not significantly improve neurologic recovery compared with controls (fig. 2, A and B). Neurologic deficit was  $86 \pm 9$  per cent at 24 hours and decreased to  $50 \pm 14$  per cent by 48 hours. Little

further improvement occurred, and the final neurologic deficit was  $44 \pm 13$  per cent, which was not significantly different from control (fig. 2A). Thiopental, 120 mg/kg, given 60 min after ischemia, resulted in significantly improved recovery, namely,  $7 \pm 6$  per cent on day 7 (fig. 2B). In all groups the magnitude and rapidity of improvement occurring within the first 48 hours forecast the ultimate extent of neurologic deficit sustained by day 7 after ischemia.

Histologic changes in terms of infarction, ischemic neuronal changes, and total brain histopathologic scores correlated with the observations of neurologic deficit in every group. For controls, the mean infarction score was  $52 \pm 22$ , and was fourfold greater than that for the monkeys receiving thiopental, 90 mg/kg, at 5 min. Delays of thiopental, 90 mg/kg, administration to 15, 30, and 60 min after ischemia resulted in a stepwise increase in mean infarction scores relative to the values of the 5-min thiopental-treated group (fig. 3). Scores for ischemic neuronal changes were also decreased in 5 min thiopental-treated groups compared with controls, but a graded increase in scores was not observed with delayed therapy. Total histopathologic scores, which included an edema score in addition to the infarction and ischemic-neuron scores, also showed a progressive increase with delayed therapy. Brain histopathologic scores after thiopental, 120 mg/kg, with 30 and 60 min delays also correlated with neurologic deficit scores (fig. 4). Monkeys treated after a 60-min delay had histopathologic scores lower than that for the controls, whereas those treated after a 30-min delay had scores higher than that for the 60-min group.

### Discussion

In developing a long-term-survival monkey model of global ischemic anoxic damage to the brain, our objective was to evaluate the efficacies of therapies capable of markedly enhancing functional neurologic recovery. This required a model of severe permanent brain damage (which is also more easily evaluated), but with long-term survival, which necessitated development and standardization of intensive care and life support procedures.<sup>9</sup> Sixteen minutes of global ischemia achieved by the method described resulted in severe permanent neurologic deficit and survival until sacrifice seven days after ischemia. The final neurologic deficit score for untreated control monkeys was about 50 per cent at seven days, which, according to our speculation, falls on the steep portion of the S-shaped curve relating duration of ischemia to neurologic deficit.<sup>9</sup> Maximal sensitivity is obtained in this portion of the curve,

similar to the  $LD_{50}$  used in pharmacologic studies. The severity of the ischemic brain damage in the model used is important for evaluation of therapy. According to our hypothesized S-shaped curve, when the insult is too severe or not severe enough, the efficacy or detrimental effects of therapeutic procedures may not be revealed. For these reasons, we considered the severity of the insult in our model optimal. Obviously, our method of producing global ischemia of the brain does not completely simulate any clinical condition, but we selected a model of "pure" global ischemia to minimize variability due to failure of other organs such as the heart, lungs, and kidneys, and to simplify postischemic intensive care.

The quality of postischemic intensive care and life support is an important factor in determining final neurologic outcome. Early after ischemia, the condition of the severely brain-damaged monkey is unstable. Even slight hypoxic insults such as may occur from a partially obstructed endotracheal tube can precipitously lead to severe complications. Therefore, physiologic variables such as arterial pressure, blood-gas, and  $pH$  and serum and urinary electrolyte values were closely monitored to prevent deterioration attributable to factors other than those of neurologic origin.

Our studies and those of the previously mentioned investigators clearly demonstrate the efficacy of barbiturates in improving recovery of neurologic function after cerebral ischemic anoxia. However, none of these studies provides insight into the mechanism of action of barbiturates in attenuating ischemic brain damage. The necessity of having to provide postischemic intensive care and life support in our studies, however, emphasized possible factors that may influence recovery with barbiturate therapy. In our experiments, controlled ventilation with normal  $Pa_{CO_2}$  and  $pH_a$  values was maintained for 12 hours after ischemia in thiopental-treated monkeys, compared with two hours in the controls. Therefore, spontaneous breathing, with less hypocapnia and respiratory alkalosis, started later in thiopental-treated monkeys. The primary differences between the thiopental-treated and control groups that might have influenced recovery were normalization of arterial blood-gas and  $pH$  values and immobilization. The beneficial effects of normocapnia as opposed to hypo- or hypercapnia have not been clearly established.

The influence of immobilization after cerebral ischemic anoxia is also unclear. However, substantial evidence suggests that after anoxia, the brain continues in a state of borderline hypoxia, as evidenced by low cerebral venous oxygen content values, be-

cause cerebral blood flow (CBF) is decreased to approximately half normal.<sup>10</sup> On this basis, activation or stimulation of neurons may then lead to metabolic demands exceeding substrate and oxygen delivery to the brain. Indeed, in recent unpublished observations,<sup>††</sup> we showed that controlled ventilation and immobilization with pancuronium without barbiturate therapy for 48 hours after ischemia decreased the neurologic deficit from 50 to 20 per cent. The latter values were significantly higher, however, than the zero per cent neurologic deficit observed in monkeys treated with thiopental, 90 mg/kg, 5 min after ischemia. The protective effects of immobilization and anesthesia may attenuate ischemic brain damage by decreasing sensory input and motor activation, thus decreasing "stress" injury of the brain.

Another factor of possible importance to final neurologic outcome is the adequacy of cerebral perfusion pressure (CPP) in the first two hours after ischemia. Although protracted severe arterial hypotension (MAP 35–40 torr) impedes neurologic recovery, the importance of CPP in the range of 70–125 torr or more has not been defined. Severe repetitive arterial hypertension with MAP elevated to 150–190 torr for 2–4-min periods during the first two hours after ischemia worsened neurologic recovery after 16 min of global ischemia of the brain

in our monkeys.<sup>††</sup> On the other hand, Hossmann *et al.*<sup>11</sup> reported that immediate postischemic hypertension is important for recovery of neurologic function after global ischemia of the brain and that there is also a range of CPP values resulting in optimal functional neurologic recovery.<sup>‡‡</sup> In our studies, CPP values were essentially the same among all groups after ischemia. MAP values were similar throughout the seven days in all groups, except for a somewhat delayed restoration of MAP in the control and thiopental, 120 mg/kg, 60 min groups. Thus, there was no correlation between final neurologic deficit and CPP values among the groups. In fact, improved recovery occurred in the group treated with thiopental, 120 mg/kg, at 60 min, in which restoration of CPP 5 min after ischemia was delayed. Thus, the results of our studies and those of other investigators demonstrate that barbiturates administered after the insult can attenuate ischemic brain damage. This attenuation may be attributable to "anesthesia,"

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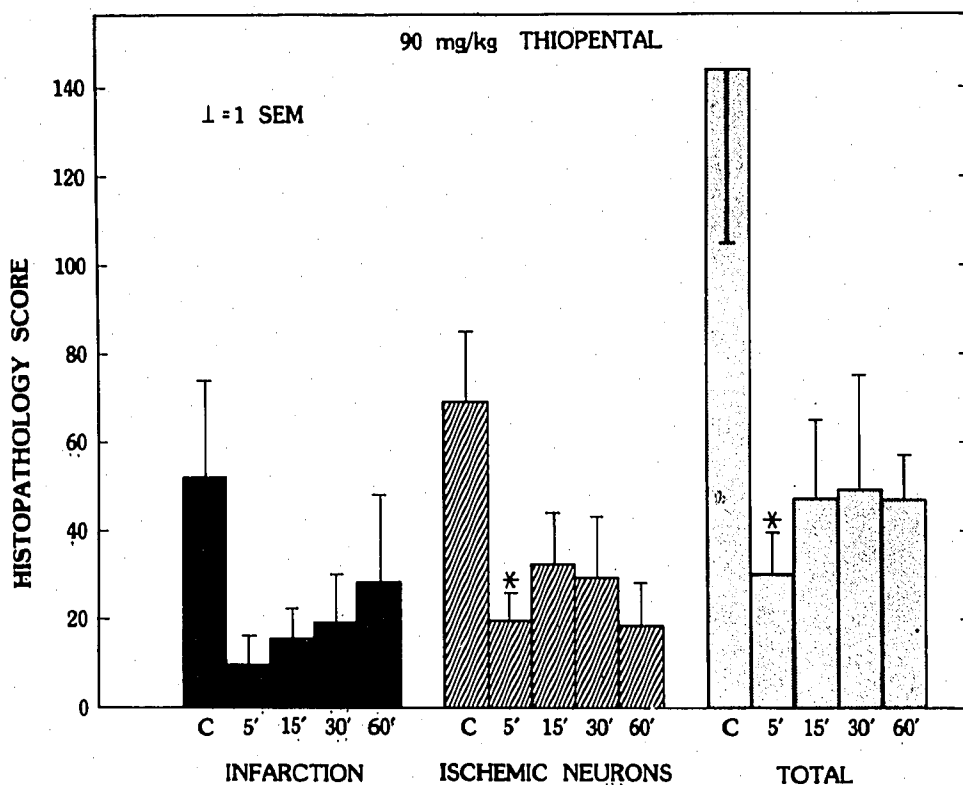
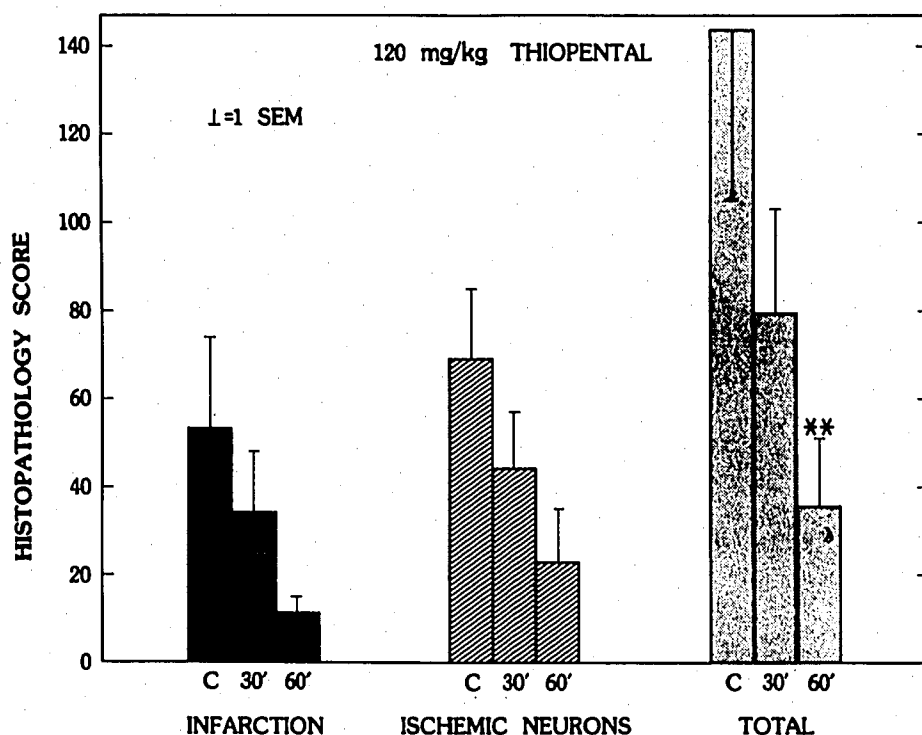


FIG. 3. Histologic changes in the brains of rhesus monkeys seven days after ischemia: after 16 min global ischemia of the brain produced by a high-pressure neck tourniquet and trimethaphan-induced hypotension in nine untreated control monkeys (C) and in those treated with thiopental, 90 mg/kg, intravenously beginning 5 (n = 5), 15 (n = 5), 30 (n = 4), and 60 min (n = 2) after ischemia. Maximum possible histopathologic scores were: infarction, 672; ischemic neuronal changes, 336; total score, 1,092. \* $P < 0.05$  compared with controls.

FIG. 4. Histologic changes in the brains of rhesus monkeys seven days after ischemia: after 16 min global ischemia of the brain produced by a high-pressure neck tourniquet and trimethaphan-induced hypotension in nine untreated control monkeys (C) and in those treated with thiopental, 120 mg/kg, intravenously beginning 30 (n = 5), and 60 min (n = 5) after ischemia. Maximum possible histopathologic scores were: infarction, 672; ischemic neuronal changes, 336; total score 1,092. \*\* $P < 0.01$  compared with controls.



immobilization, and controlled ventilation, in addition to the so-called direct effects of anesthetics on membrane stabilization and ion transport.

For clinical application of barbiturate therapy, it is important to know the optimal time and dose for maximal efficacy. That thiopental administered after ischemia ameliorates ischemic brain damage provides definitive proof that much of the brain damage sustained after global ischemia of the brain is due to postischemic pathologic processes that can be treated or prevented. We observed improved recovery when thiopental, 90 mg/kg, was administered 5 or 15 min after ischemia, but not at 30 or 60 min, implying that a major portion of the pathologic processes occurred within the first 30 min after ischemia. However, the improved recovery observed with thiopental, 120 mg/kg, at 60 min, but not at 30 min, after ischemia is not consistent with this interpretation. This apparent paradox may be because thiopental, 120 mg/kg, actually impedes recovery when administered during the reactive hyperemic phase (within 30 min after ischemia), thus attaining higher brain concentrations than when administered after 60 min during the low-flow state. Similarly, thiopental, 90 mg/kg, may be ineffective 30 min after ischemia or later because the concentration attained in the brain is less than that necessary for therapeutic effectiveness.

With regard to the optimal time of thiopental

administration, it is logical to assume that ischemic brain damage occurs progressively during the ischemic insult and, as we now know, into the early and perhaps even late postischemic period. It is difficult to compare the relative extents of recovery observed with pre- and posttreatment in the different studies of focal or global ischemic anoxia, since different barbiturates, doses, animal models, and evaluation systems were used. In general, the effect of delayed thiopental therapy on neurologic deficit scores was corroborated in our studies by histologic analyses of the brain. That is to say, with increasingly delayed administration of thiopental, neurologic recovery was progressively less and the extent of histologic damage to the brain progressively greater.

The dose of 90 mg/kg selected was based on the findings of Hoff *et al.*<sup>6</sup> They used increasingly larger doses of pentobarbital administered an hour prior to occlusion of the middle cerebral artery in baboons, and found the first significant decrease in cerebral infarct size at a dose of 90 mg/kg. At 120 mg/kg, infarction was decreased to 15 per cent of control. Smith *et al.*<sup>5</sup> administered thiopental, 40 mg/kg, after focal ischemia in dogs and reported a decrease in infarct size to 10 per cent of control with complete neurologic recovery. Moseley *et al.*<sup>8</sup> administered pentobarbital, 4 mg/kg/hr, for 12 hours, beginning 30 min after embolization of the middle cerebral artery



with silicone, and found infarction size decreased to 30–75 per cent of control. Michenfelder *et al.*<sup>7</sup> tested the effect of pentobarbital in Java monkeys with an initial loading dose of 14 mg/kg, followed by 3.5 mg/kg/hr for 48 hours starting 30 min after occlusion of the middle cerebral artery, and found significant decreases in infarct size and neurologic deficit. The extents of neurologic recovery with barbiturate therapy in the studies by Michenfelder *et al.*<sup>7</sup> and Moseley *et al.*<sup>8</sup> were apparently not so remarkable as that reported by Smith *et al.*<sup>5</sup> However, the doses used by both investigators were clearly smaller than those we have used or that used by Smith *et al.*<sup>5</sup> In summary, these studies suggest that within the dose ranges tested, there is improvement in neurologic recovery with increasing doses of barbiturate.

The mechanism of barbiturate amelioration of ischemic brain damage is not yet understood, but it is probably attributable to a number of factors. The most obvious hypotheses are those suggesting that the mechanism is a result of decreased cerebral oxidative metabolism,<sup>7,12,13</sup> cerebral blood flow,<sup>5</sup> and intracranial pressure.<sup>14</sup> Other hypotheses suggesting more specific mechanisms have also been proposed.

In conclusion, we have demonstrated the effectiveness of thiopental in ameliorating ischemic brain damage when applied early after global ischemia of the brain in doses exceeding that necessary for surgical anesthesia. We have also shown that delaying thiopental administration after ischemia results in a progressive loss of efficacy, suggesting that most of the pathophysiologic and biochemical changes adding to ischemic brain damage occur early after ischemia and can be treated with appropriate therapy. The dose and duration of barbiturate administration for effective therapy are yet to be determined.

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