

## Quantitative Analysis of Microvascular Diameters during Pentobarbital and Thiopental Anesthesia in the Bat

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The effects of intraperitoneal injection of physiologic saline solution, pentobarbital, 50 mg/kg, or thiopental, 20 or 50 mg/kg, upon small arteries (35–45  $\mu$ ) and small veins (70–100  $\mu$ ) in the wings of unanesthetized bats were studied. The rate of venous vasomotion and the diameters of the artery and vein were recorded for 20 minutes before, and for 50 minutes after, each injection. Dilatation of the artery to  $129.3 \pm 6$  per cent of control ( $P < 0.02$ ) and the vein to  $124.1 \pm 7.3$  per cent of control ( $P < 0.02$ ) occurred 10 minutes following pentobarbital. Thiopental (50 mg/kg) increased venous vasomotion ( $108 \pm 1.3$  per cent,  $P < 0.02$ ), but neither dosage of thiopental produced a significant change in the diameters of the artery and vein. It is concluded that pentobarbital and thiopental have different effects upon the small blood vessels of the subcutaneous tissues in the bat wing. (Key words: Microcirculation; Pentobarbital; Thiopental; Barbiturate anesthesia; Vasomotion; Television-microscopy.)

PENTOBARBITAL and its sulfur analogue, thiopental, alter the cardiovascular system when administered in anesthetic doses. Most of these

cardiovascular changes have been defined at the macrocirculatory level, *i.e.*, changes in cardiac output, stroke volume, heart rate, venous pressure, and peripheral vascular resistance.<sup>1-3</sup> Some data describing the effects of pentobarbital and thiopental on small arteries and veins are available. However, in these studies it has been necessary to use indirect techniques,<sup>4</sup> artificial tissue chambers<sup>5, 6</sup> or prior anesthesia in the preparations.<sup>7</sup>

In this study, the responses of subcutaneous small arteries and veins in the bat wing were observed before and after the administration of thiopental or pentobarbital in anesthetic doses. The objective of this investigation was quantitation of vascular diameters and venous vasomotion in subcutaneous tissue during barbiturate anesthesia without the need for mechanical chambers, previous anesthesia, or surgical trauma.

### Methods

In these studies we utilized closed-circuit television microscopy to quantitate vascular responses in the bat wing.<sup>3</sup> In essence, an unanesthetized bat (7–14-g *Myotis*) was restrained with wings extended on a microscope stage. An image of a subcutaneous small artery (35–45  $\mu$ ) and small vein (70–100  $\mu$ ) was selected for display on a television monitor with a calibrated magnification of approximately 1,000 $\times$ . The diameters of the artery and vein were measured at 30-second intervals while the numbers of venous contractions was counted over successive 1-minute periods. As previously described,<sup>9</sup> the raw data for each of these three variables were smoothed by a five-point digital computer filter and normalized to give data as per cent of control for each experiment. Subsequently, the mean

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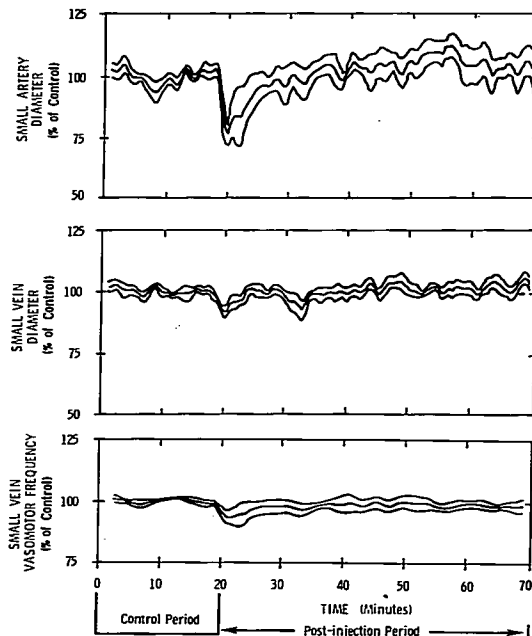


FIG. 1. Average values for five bats which received intraperitoneal injections of saline solution. The abscissa is time in minutes, with 0 and 20 minutes corresponding to the start of the control and postinjection periods, respectively. Ordinates from top to bottom are small-artery and small-vein diameters and frequency of venous vasomotion as percentages of their respective mean values during the first 20 minutes. Ordinate values greater than 100 per cent represent vasodilatation, while values less than 100 per cent indicate vasoconstriction. The upper and lower tracings in each panel are average values  $\pm$  SE.

and standard error of the mean (SE) were calculated for each point in time to provide the graphs presented here. Data obtained 10 minutes after injection of pentobarbital or thiopental were compared with data obtained after injection of saline solution by group *t* tests.

One experiment was performed on each of 23 bats. The protocol for each experiment consisted of: 1) animal preparation and microscopic selection of comparable vessels according to landmarks in wing structure<sup>9</sup>; 2) a waiting period of at least 20 minutes in a darkened room to achieve a quiet condition; 3) a 20-minute control period for data collection; 4) an intraperitoneal injection of sodium pentobarbital, thiopental, or saline solution; 5) a 50-minute postinjection period of data collection. The animal was observed for spontaneous head movements and was tested for a biting reflex in response to tactile stimulation of the nose. The barbiturate dosages were 50

mg/kg body weight for pentobarbital and 20 or 50 mg/kg for thiopental in concentrations to give injection volumes of 10  $\mu$ l/g body weight. The 50 mg/kg doses were selected because they produced surgical anesthesia in the bat and because they approximate the doses needed for surgical anesthesia in mice (90 mg/kg) and rats (40 to 50 mg/kg).<sup>10</sup>

In another group of ten animals, mean blood pressures in the radial artery were measured for 10 minutes prior to, and for 35 minutes after, intraperitoneal injection of pentobarbital, 50 mg/kg.

## Results

The average vascular responses to intraperitoneal injection of saline solution ( $N = 5$ ), pentobarbital, 50 mg/kg ( $N = 6$ ), thiopental, 50 mg/kg ( $N = 6$ ), and thiopental, 20 mg/kg ( $N = 6$ ) are given in figures 1-4. In each figure, small-artery diameter, small-vein diameter, and frequency of small-vein con-

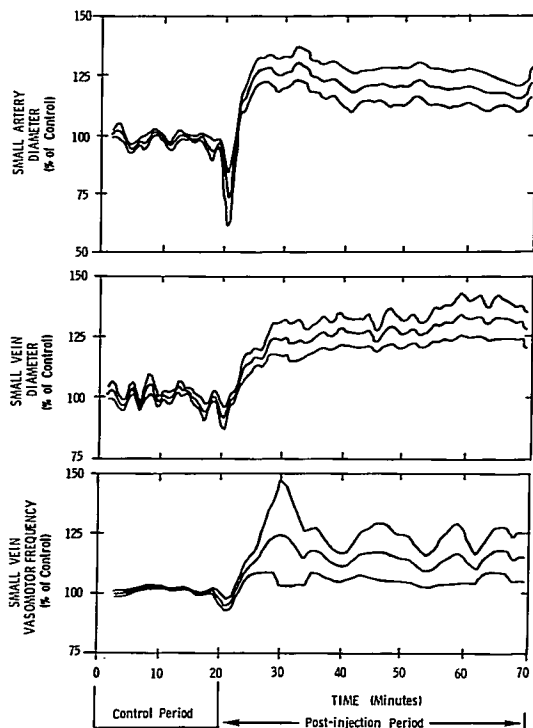


FIG. 2. Average values for six bats which received intraperitoneal injections of pentobarbital, 50 mg/kg, at 20 minutes. Otherwise, the data were collected according to the protocol described in the legend to figure 1.

tion appear on the ordinates in the top, middle, and bottom panels, respectively. Data are presented as percentages of mean preinjection values. The abscissa is time of vascular observation, with injection at 20 minutes. In each panel, the middle tracing is the mean curve for all bats in one of the four experimental groups; the upper and lower tracings represent means  $\pm$  SE.

Other than the apparent changes in vessel diameters and vasomotion at the time of injection, there was little change in the vascular variables following injection of saline solution (fig. 1). In contrast, pentobarbital, 50 mg/kg (fig. 2), produced significant dilatation of the small artery to  $129.3 \pm 6.0$  per cent of the control value ( $P < 0.01$ ) and the small vein

to  $124.1 \pm 7.3$  per cent of the control value ( $P < 0.02$ ) at 30 minutes (10 minutes after injection). Small-vein vasomotion appeared to increase in frequency to  $123.8 \pm 21.8$  per cent of the control value ( $P > 0.05$ ), but this could not be demonstrated statistically since the amplitudes of vasomotion in some experiments decreased to the point that frequencies could not be counted. Mean arterial blood pressure decreased to  $75 \pm 4.0$  per cent ( $P < 0.01$ ) of the control value 10 minutes after pentobarbital, and this hypotension persisted throughout the observation period. Within five minutes after pentobarbital, the bats appeared to be anesthetized, since they did not have spontaneous head movements or a biting reflex in response to tactile stimulation of the

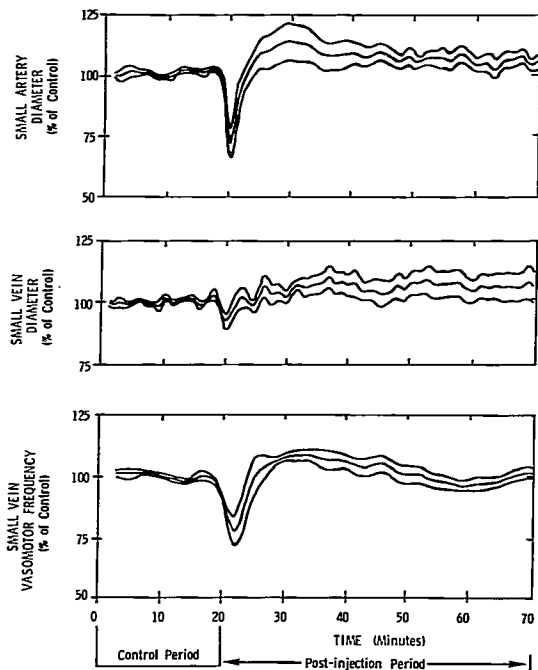


FIG. 3. Average values for six bats which received thiopental, 50 mg/kg. (See legend to fig. 1.)

nose. These two qualitative indicators of anesthesia were present throughout the postinjection period.

Thiopental, 50 mg/kg, also appeared to anesthetize the bats, since spontaneous head movements and the biting reflex were absent. However, these indicators persisted for only the initial 12 to 20 minutes after thiopental injection. Thiopental, 50 mg/kg, did not produce significant changes in small-artery or small-vein diameters ( $113.5 \pm 7.4$  per cent and  $102.5 \pm 2.6$  per cent, respectively,  $P > 0.05$ ), but it did increase the frequency of small-vein vasomotion ( $108.3 \pm 1.3$  per cent,  $P < 0.02$ , fig. 3). Thiopental, 20 mg/kg, did not produce anesthesia. Ten minutes following thiopental, the vascular responses to the 20 mg/kg (fig. 4) and the 50 mg/kg (fig. 3) doses were almost identical ( $P > 0.05$ ) (small artery,  $103.7 \pm 3.3$  per cent vs.  $113.5 \pm 7.2$

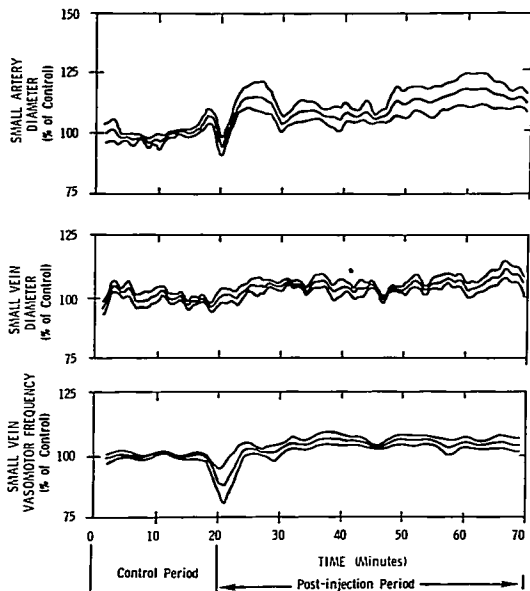
per cent; small vein,  $104.5 \pm 1.5$  per cent vs.  $102.5 \pm 2.6$  per cent; venous vasomotion frequency,  $101.1 \pm 3.4$  per cent vs.  $108.3 \pm 1.3$  per cent).

### Discussion

Almost 30 years ago, Seldon<sup>5</sup> suggested that the bat wing would provide an excellent preparation for observation of the effects of anesthetic drugs on the skin microvasculature. The bat can be gently restrained on a microscope stage and will become calm shortly after this maneuver. Thus, the microvasculature in the wing membrane can be observed without surgery and/or anesthesia.

In our preparation, an anesthetic dose of pentobarbital was associated with dilatation of the small arteries (fig. 2). In dogs, there have been numerous but inconsistent descriptions of the effect of pentobarbital on total periph-

FIG. 4. Average values for six bats which received thiopental, 20 mg/kg. (See legend to fig. 1.)



tral resistance. Increases,<sup>1</sup> decreases,<sup>11</sup> or no change<sup>2</sup> in this variable have been reported. Although the present study is concerned with one organ system only, the presence of significant hypotension coupled with significant small-artery dilatation suggests a decrease in total peripheral vascular resistance. In addition, the present findings are similar to those of Forsyth and Hoffbrand,<sup>2</sup> who observed decreases in both total peripheral and skin resistance, an increase in skin blood flow, and a decrease in arterial blood pressure in the monkey anesthetized with pentobarbital.

Limited information about the venous circulation suggests dilatation in response to pentobarbital. For example, Shubin and Weil<sup>12</sup> postulated that the hypotension in patients with barbiturate overdosage results from expansion of the capacitance bed with intravascular pooling of blood. Our data demonstrate postcapillary dilatation following pentobarbital anesthesia (fig. 2), and thus tend to support this postulate.

Small veins in the bat wing have a rhythmic pattern of contraction, referred to as "vasomotion." Our data regarding frequency of vasomotion cannot be compared with those of others, since previous investigators have not observed this variable in their preparations. The significance of venous vasomotion is unknown, although it appears to be related to intravascular pressure in the small veins.<sup>13</sup>

Small-vessel responses to an anesthetic dose of thiopental were considerably different from those to pentobarbital. The arterial response to thiopental (fig. 3) could not be distinguished from the response to intraperitoneally injected saline solution (fig. 1). These observations differ from those of Seldon and Lundy<sup>5</sup> and van den Brenk *et al.*,<sup>6</sup> who found arterial dilatation in the rabbit ear chamber after thiopental. In addition, Thomson<sup>14</sup> reported a decrease in skin resistance and an increase in "apparent blood flow" in patients following intravenous administration of thiopental. Acheson<sup>15</sup> postulated a similar response

in the cat, but MacCannell<sup>11</sup> could not demonstrate significant alterations in resistance or flow after injection of thiopental into the dog mesenteric artery. Hershey *et al.*<sup>7</sup> found that light thiopental anesthesia had little effect on vascular diameters in the dog omentum. However, progressive arterial dilatation occurred as anesthetic depth was increased. This dose-response relationship may explain the apparent contradictions regarding the effects of this drug on small arteries. Clinically, our bats appeared to be lightly anesthetized, since they recovered from thiopental anesthesia within 20 minutes after injection.

In this study, an anesthetic dose of thiopental had no effect on small-vein diameters (fig. 3). Eckstein *et al.*,<sup>4</sup> using the indirect technique of plethysmography, observed a decrease in "venous tone" following intravenous administration of thiopental in patients. They postulated that thiopental had a direct vasodilator effect on the veins of the forearm. It is difficult to relate their findings to those reported here because of variations in techniques, anesthetic depth, and species.

Moderate sedation without hypnosis was achieved with thiopental, 20 mg/kg, but did not cause significant alterations in small-artery or small-vein diameters (fig. 4). These data support our subjective observation that the bats were not excited or hyperactive during these studies. The lack of vasodilatation following hypnotic doses of thiopental also supports the concept that our bats were not in a state of high sympathetic activity prior to barbiturate administration.

Saline solution was administered to determine the effects of intraperitoneal injection on the microcirculation. The injection technique produced only a brief small-artery constriction. Therefore, the microvascular responses to pentobarbital and thiopental apparently are results of the drugs rather than the injection technique.

The rats of absorption of these drugs from the intraperitoneal cavity could not be controlled during these experiments. However, the techniques used and volumes of drugs administered were similar in all experiments. The durations of anesthesia and the patterns of vascular responses were similar for all experimental groups, suggesting that drug absorption was adequately uniform among the

animals. Physical limitations of vein size and current lack of a venipuncture technique precluded intravenous drug administration in this study.

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