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A Method for Preferential Delivery of Volatile Anesthetics to the In Situ *Goat Brain*

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Background: **As part of studies aimed at better defining the effects of anesthetics at different anatomic sites, we have developed a model of preferentially delivering inhaled anesthetics to the *in situ* goat brain, using a bubble oxygenator and roller pump. We tested the hypotheses that (1) this model excludes the cerebral circulation from the body; (2) the concentration of halothane in the oxygenator exhaust correlates with the concentration of halothane in the oxygenator arterial blood.**

Methods: **After ligation of the occipital arteries in six halothane-anesthetized goats, we used a bubble oxygenator to perfuse the brain preferentially (exclusive of the body) *via* a carotid artery, draining cranial venous blood back into the oxygenator *via* the isolated jugular veins. (In goats, the vertebral arteries do not directly contribute to the cerebral circulation, and internal jugular veins and extracranial internal carotid arteries are absent.) The extent of isolation was determined with radioactive microspheres injected into the left atrium during the following periods: (1) baseline; (2) during bypass when the blood pressure in the head equalled that in the body; (3) during bypass when the blood pressure in the body exceeded that in the head by approximately 30–35 mmHg; (4) when the bypass roller pump was stopped. We also measured the concentration of halothane in the arterial blood of the bypass unit. In three animals, systemic metocurine was administered during bypass to detect the presence of venous contamination.**

Results: **Baseline cerebral blood flow was 74 ± 32 ml·100 $g^{-1} \cdot min^{-1}$ (mean ± SD). During bypass, cerebral blood flow originating from the systemic circulation was less than 1 ml·100 $g^{-1} \cdot min^{-1}$, and isolation extended to the caudal medulla during periods 3 and 4, and to the first 1-cm segment of the spinal cord during period 2. The concentration of halothane in the oxygenator exhaust correlated reasonably well with the arterial halothane concentration ($r = 0.82$, $P < 0.001$). Systemic arterial metocurine concentrations peaked at 1 min (27 ± 3.7 μg/ml) and decreased to 10.6 ± 2.3 μg/ml at 10 min; head venous metocurine plasma concentrations gradually increased to 3.1 ± 0.4 μg/ml at 10 min.**

Conclusions: **This technique permits selective perfusion and delivery of inhaled anesthetics to the *in situ* goat brain, but is not adequate for selective delivery of fixed intravenous anesthetics. (Key words: Anesthetics, volatile: halothane. Brain: anesthetic delivery. Bypass: cerebral; cranial.)**

WE recently reported that when isoflurane was preferentially delivered to the brain, anesthetic requirements increased approximately 240%,[1] indicating that the spinal cord (and possibly the periphery) is important to suppression of purposeful movement in response to a painful stimulus. We used a goat model because other investigators have demonstrated that the arterial and venous cerebral circulations in the goat are easily isolated from the rest of the body.[2–6] In our prior study, the head and brain were perfused exclusive of the body by a bubble oxygenator–roller pump bypass unit. We measured isoflurane concentration from the exhaust of the bubble oxygenator and assumed that this was representative of the concentration of isoflurane in the arterial blood of the oxygenator.‡ This assumption was based in part on work by Nussmeier *et al.*[7] who demonstrated that the isoflurane concentration in the exhaust is a reasonable approximation of the arterial isoflurane concentration. We performed the present study to validate our methodology by injecting radioactive microspheres during the period of cerebral isolation to determine the extent of contamination and by measuring anesthetic concentration in the arterial blood of the oxygenator and correlating this with the concentration in the exhaust.

Materials and Methods

This study was approved by the Animal Care and Use Committee. Six goats aged approximately 3–4 yr and weighing 50.8 ± 9.2 kg (mean ± SD) were anesthetized

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‡ Unless otherwise noted, "anesthetic concentration in blood" denotes the concentration that would exist in gas equilibrated with the blood.

with halothane *via* mask. After tracheal intubation, a rumen tube was placed and catheters inserted into a femoral artery and a peripheral vein. Bilateral neck dissections were performed to isolate the external carotid arteries and external jugular veins (fig. 1). Heparin (4 mg/kg) was administered, and a Y cannula was placed in an external jugular vein so that cranial venous outflow could be alternated between the body and the oxygenator (B-10, Baxter, Irvine, CA). A cannula was inserted into the remaining external jugular vein to divert cranial venous blood to the bypass unit. Lactated Ringer's solution (2.5–3 l) was administered to the goat and the oxygenator primed with 400–500 ml blood drained from the animal. Oxygenator gas flows (95% oxygen/5% carbon dioxide) were 3–4 l/min, and roller pump flows were 300–650 ml/min. A halothane vaporizer was placed in line with the fresh gas flow to the oxygenator. Arterial blood was infused, *via* roller pump, into the left external carotid artery, which was permanently ligated. A small catheter was placed into the right carotid artery and directed toward the brain to measure "stump" pressures. The occipital arteries were ligated to prevent vertebral artery blood from entering the carotid system. (In goats, the vertebral arteries do not contribute blood directly to the cerebral circulation.) Cranial bypass was achieved by opening clamps 1–3, closing clamp 4, and temporarily occluding the right external carotid artery (fig. 1). Glucose (10–20 mg/min) was infused into the oxygenator during bypass. Thermistors were placed in the vena cava and nasopharynx to measure core and head temperatures, respectively. Core temperature was maintained at 36.6 ± 0.8°C and head temperature maintained at 36.6 ± 1.0°C. End-tidal halothane was measured with a calibrated Datex 254 agent analyzer (Datex Instrumentarium, Tewksbury, MA).

Via a left thoracotomy, a catheter was inserted into the left atrium, and baseline regional organ blood flow determined by injection of radioactive microspheres (see below). Cerebral bypass was initiated and when stable, regional blood flows were redetermined with microsphere injections during the following three periods: (1) with the head mean arterial pressure (MAP) and body MAP approximately equal; (2) with the body MAP approximately 30–35 mmHg greater than the MAP in the head; and (3) with the roller pump turned off. The order of the first two was randomized. During bypass, end-tidal halothane was generally decreased to 0.5–1.0%, and the halothane concentration in the oxygenator exhaust was between 1.3–3.4% (generally,

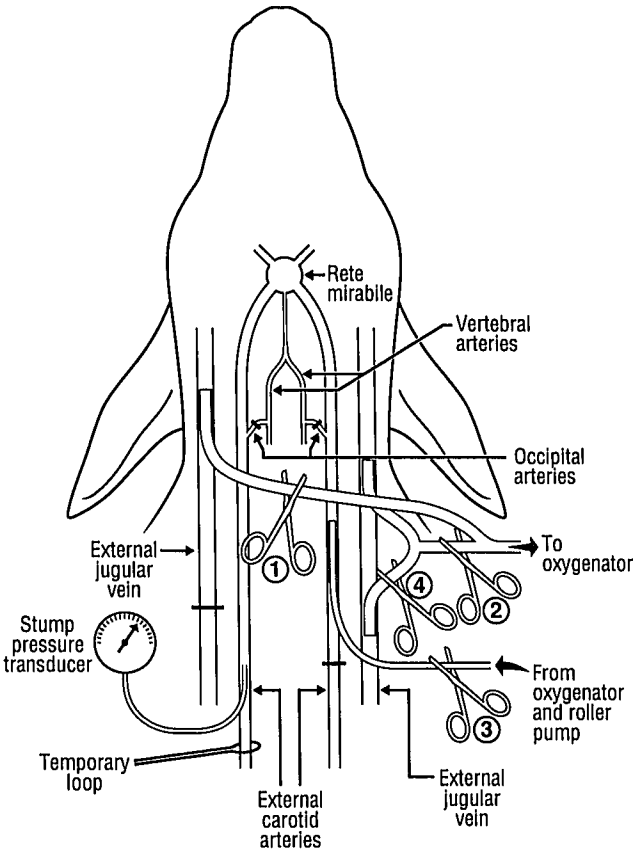

Fig. 1. The cranial bypass model. The vertebral arteries converge to form the basilar artery, which connects with the carotid rete; however, the basilar artery is small, and blood normally flows away from the rete. See text for details. (Reproduced from Antognini and Schwartz,[1] with permission.)

end-tidal halothane averaged 40% of the exhaust halothane). Minimum alveolar concentration for halothane in the goat is 1.3%.[8] Body MAP was altered by adjusting the inspired halothane, infusing phenylephrine and fluid administration. Head MAP was adjusted by altering the roller pump speed. Before and during the bypass period, arterial blood gases and hematocrit analyses were performed.

Radioactive microspheres were labeled with cerium 141, strontium 85, niobium 95, or ruthenium 103 and were suspended in 10% dextran in saline containing 0.01% Tween-80. Before injection, the suspension was mechanically and ultrasonically mixed to achieve complete dispersion, which was verified by microscopic examination. Four to six million microspheres were injected into the left atrial catheter for approximately 20 s and then the catheter flushed with normal

saline. Blood was withdrawn from the femoral arterial catheter 10–15 s before the injection and continuing for 2 min at a rate of 7.75 ml/min. At the end of the study, saturated potassium chloride was given intravenously and sections of the brain, spinal cord, kidney, heart, liver, masseter muscle, and tongue were taken. Sections of cerebral cortex were removed from the frontal, parietal and temporal region of each hemisphere. These samples also contained white matter beneath the cerebral cortex. Sections were taken of the midbrain (cerebral crux), cerebellar cortex, cephalad and caudal medulla and the first four 1-cm segments of the spinal cord. The tissue samples were weighed and counted for radioactivity. Tissue blood flow was determined from the ratio of radioactive counts of the sample to those of the reference blood sample.

During the bypass period, a calibrated Datex 254 monitor sampled the oxygenator exhaust and halothane concentration in the exhaust was held constant for approximately 15–20 min. Two 1.00-ml blood samples were withdrawn for measurement of halothane concentration. The blood was injected into 10.00 ml hexane that had toluene added as an internal standard. In four goats, the halothane in the exhaust was adjusted and maintained at a new concentration for 15–20 min and a second set of blood samples withdrawn for halothane analysis. Halothane was measured using high performance liquid chromatography with modifications of a previously described technique.[9] A 20-μl sample was injected into a Waters U6K manual injector that was connected to a Waters 510 high-performance liquid chomatography pump. Halothane was detected with a Waters 481 UV detector with wave length = 210 nm. A NOVA Pak C18, radial compression module 10 cm × 8 mm (ID) (4-μm particles) and μ Bonda Pak C18 guard column were used. The mobile phase consisted of 50:50 ratio of methanol to water. Retention times were 8 min for halothane and 17 min for toluene. Concentration of halothane was determined by comparison to standard curves. Analysis of the standard curves demonstrated little variability and correlation coefficients of 0.99 over the range of 10–40 ppm halothane. Blood halothane concentration (liquid phase) was converted to gas-phase concentration (expressed as volume percent) using published values[10] for the solubility coefficient (λ) corrected for the crystalloid dilution[11] ($\lambda = 2.2$).

In three goats, before terminating the study, metocurine 1.5 mg/kg was injected intravenously (body) during cranial bypass. Five-milliliter blood samples were withdrawn from both the femoral arterial catheter and the venous port of the oxygenator at 1, 2, 3, 4, 5, 8, and 10 min after metocurine injection. The blood was centrifuged and the plasma analyzed for metocurine using high performance liquid chromatography as previously described.[12]

The data are presented as the mean ± SD. Halothane concentration in the oxygenator exhaust was compared to halothane concentration in the oxygenator arterial blood using linear regression. Differences in regional blood flow were compared to baseline using analysis of variance. Statistical significance was achieved with $P < 0.05$.

Results

Regional blood flow is presented in table 1. These data indicate excellent arterial isolation during the bypass period. Baseline cerebral blood flow (CBF) for both hemispheres combined was 74 ± 32 ml · 100 $g^{-1} \cdot min^{-1}$. During bypass, systemic contribution to CBF was less than 1 ml · 100 $g^{-1} \cdot min^{-1}$. The highest regional CBF in any animal for all three periods was 4 ml · 100 $g^{-1} \cdot min^{-1}$. Baseline medullary blood flow was 59 ± 23 ml · 100 $g^{-1} \cdot min^{-1}$, and during bypass (all three periods combined) systemic contribution was 1.5 ± 3 ml · 100 $g^{-1} \cdot min^{-1}$. Significant blood flow contamination (> 3 ml · 100 $g^{-1} \cdot min^{-1}$) generally occurred in the first several 1-cm segments of the spinal cord. Thus, isolation extended to the level of caudal medulla; when head MAP equalled body MAP, isolation extended to the first 1-cm segment of the spinal cord. Baseline regional CBFs were numerically greater on the right than on the left, but this difference was not statistically significant. Extracranial tissue, such as muscle and tongue, were likewise excluded from the normal circulation.

Halothane concentrations are shown in figure 2, indicating significant correlation between the halothane concentration in the oxygenator exhaust and the arterial halothane concentration ($r = 0.82$, $P < 0.001$).

Arterial blood gas and hematocrit values are shown in table 2. There was a slight metabolic acidosis during bypass. Hematocrit decreased slightly, presumably because of the isotonic crystalloid administered in preparation for bypass, but this was not significant. The body metocurine concentrations decreased over the 10-min period, whereas there was a gradual increase in the head, indicating contamination from the body (table 3).

Table 1. Regional Blood Flows (ml · 100 g^{-1} · min^{-1})

	Baseline	$MAP_B = MAP_H$	$MAP_B > MAP_H$	CPB Off
MAP (mmHg)				
Body	68 ± 21	49 ± 5	75 ± 18	59 ± 12
Head	54 ± 7	45 ± 11	41 ± 11	20 ± 12
R temporal	81 ± 34	<1*	<1*	1 ± 2*
R frontal	83 ± 27	<1*	<1*	<1*
R parietal	91 ± 41	<1*	<1*	<1*
L temporal	65 ± 31	<1*	1 ± 1*	1 ± 1*
L frontal	72 ± 27	<1*	<1*	<1*
L parietal	76 ± 38	<1*	<1*	<1*
Midbrain	50 ± 20	<1*	<1*	1 ± 1*
Cerebellum	85 ± 51	<1*	<1*	<1*
Ceph med	55 ± 25	<1*	1 ± 1*	1 ± 1*
Caud med	63 ± 23	<1*	4 ± 6*	2 ± 2*
SC				
1	52 ± 43	<1*	10 ± 14*	3 ± 4*
2	35 ± 30	6 ± 8	18 ± 16	7 ± 10
3	24 ± 12	12 ± 12	25 ± 12	12 ± 6
4	20 ± 10	13 ± 14	32 ± 18	21 ± 12
Kidney	202 ± 71	186 ± 132	201 ± 137	135 ± 102
Epicardium	50 ± 17	106 ± 53*	138 ± 64*	162 ± 65*
Endocardium	59 ± 14	102 ± 34*	160 ± 59*	158 ± 74*
Masseter	4 ± 2	<1*	<1*	1 ± 2
Tongue	1 ± 1	<1*	<1*	2 ± 2

Values are mean ± SD, except when mean <1. n = 6. MAP = mean arterial pressure, subscript B or H for body or head; R = right; L = left; Ceph med = cephalad medulla; Caud med = caudal medulla; SC = spinal cord, designated by 1-cm segments; CPB Off = roller pump turned off.

* $P < 0.05$ *versus* baseline period.

Discussion

The technique of isolated perfusion of an animal brain is not a new concept. As early as 1858, investigators have attempted to perfuse the brain selectively.[13–16] These early attempts were complicated by incomplete isolation, nonphysiological perfusates and crude oxygenators. To our knowledge, none of these early scientists selectively anesthetized the brain, although there were attempts to inject anesthetics selectively to investigate gross anatomic sites of anesthetic action.[17] A similar preparation has been developed in our laboratory to deliver volatile anesthetics preferentially to the canine hind limb.[18]

The goat is an excellent animal to use for isolation of the cerebral circulation. Goats have external carotid arteries but no extracranial internal carotid artery. The vertebral arteries do not contribute blood directly to the cerebral circulation, but anastomose with the carotid arteries *via* the occipital arteries. Normally, blood flows away from the carotid rete to the vertebral artery system. In fact, when the external carotid arteries are ligated below the level of the occipital artery anastomosis, goats can maintain consciousness.[19] If, however, the carotid arteries are ligated above the occipital artery anastomosis, unconsciousness ensues. Therefore, it is important that the occipital artery be ligated to insure cerebral isolation. The goat has two external jugular veins; the internal jugular veins are absent.[6] Thus, the venous drainage and arterial blood supply to the head and brain of the goat are easily isolated. The vertebral venous plexus is a source of contamination[13] because blood may enter the bypass unit from the body, or *vice versa*. Complete isolation is possible, however, by ligating neck and facial tissues and by occluding the vertebral plexus.[13]

Our experimental preparation provides for selective perfusion of the brain with the addition of inhalational agents such as isoflurane or halothane to the bubble

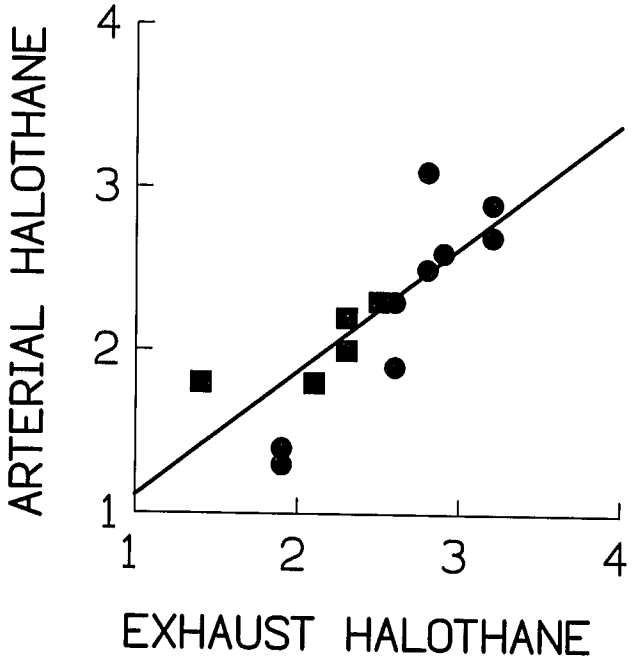

Fig. 2. The halothane concentration (volume percent) in the oxygenator exhaust is on the x-axis, and the halothane concentration (volume percent) in the oxygenator arterial blood is on the y-axis. Filled circles = single data points; filled squares = overlap of two data points. There is reasonable correlation ($r = 0.82$, $P < 0.001$; slope = 0.79 ± 0.13 [SE]).

oxygenator. Thus, the brain can be preferentially anesthetized. This is ideal for volatile inhaled anesthetics, especially those with a low blood–gas solubility (desflurane), because any contamination would be minimized by rapid equilibration with blood. For example, if a small amount of blood entered the cerebral circulation from the body, it would mix with blood from the head and, as it passed through the oxygenator, additional volatile anesthetic would be added to maintain a fairly stable concentration. Likewise, if blood were to leave the head and enter the body, it would mix with the large volume of blood in the body and be diluted considerably, and residual volatile anesthetic would subsequently be exhaled through the lungs. In our study, reservoir levels were stable, and changes were less than 20 ml/min when they occurred. With bypass pump flows of approximately 500 ml/min, this represents a 4% "shunt." Our experimental preparation is not ideal for intravenous drugs because these agents would accumulate and cannot be eliminated *via* the lungs. This is borne out by the metocurine data, which indicate some small contamination. Unfortunately, we did not inject a drug into the bypass circulation to determine leakage into the body. Conceivably, such a leak could be small enough such that the drug would be greatly diluted in the body. Thus, there could be sufficient concentration differences so that our preparation might be useful to investigate drugs selectively injected into the cranial circulation.

Because the head is isolated from the remainder of the body, glucose must be infused into the system to maintain normal glucose concentrations. During fasting, glucose is mobilized from glycogen in muscle and liver. During cranial bypass, however, the liver is excluded from the cerebral circulation and there are insufficient glycogen stores in the head to contribute to glucose homeostasis. For example, in a pilot study, we inadvertently failed to infuse glucose into the bubble oxygenator system and plasma glucose was 1 mg/dl. Brain metabolism is also dependent on uridine and cy-

Table 2. Acid–Base, Hematocrit, and Glucose Values

		Bypass		
	Baseline Pre-CPB	Body	CPB-Art	CPB-Ven
pH (units)	7.39 ± 0.04	7.34 ± 0.07	7.30 ± 0.04	7.26 ± 0.04
P_{O_2} (mmHg)	277 ± 141	222 ± 177	585 ± 25	326 ± 219
P_{CO_2} (mmHg)	37 ± 3	37 ± 4	40 ± 3	46 ± 2
BE (mM)	−2 ± 2	−5 ± 3	−6 ± 3	−7 ± 3
Hct (%)	30 ± 3	26 ± 6*	24 ± 5†	—
Glucose (mg/dl)	64 ± 20	97 ± 24	61 ± 13	60 ± 15

Values are mean ± SD; n = 6, except where noted. BE = base excess; Hct = hematocrit; Pre-CPB = prior to cranial bypass; CPB-Art = arterial sample from cranial bypass unit; CPB-Ven = venous sample from cranial bypass unit.

* N = 5.

† N = 4.

Table 3. Metocurine Plasma Concentrations (μg/ml)

	Time (min)						
	1	2	3	4	5	8	10
Systemic arterial	27 ± 3.7	23 ± 4.4	19 ± 3.4	16 ± 2.3	15 ± 2.8	12.0 ± 2.1	10.6 ± 2.3
Cranial venous	0.9 ± 0.6	1.1 ± 0.6	1.3 ± 0.6	1.8 ± 0.5	2.2 ± 0.4	2.9 ± 0.3	3.1 ± 0.4

Values are mean ± SD; n = 3.

tidine,[20] which we did not add to our perfusion apparatus. The small amount of contamination may have been sufficient to deliver these nucleosides.

Our demonstration of isolation is consistent with prior studies that used injected dyes.[4] These studies showed that the carotid arteries perfuse the brain and brain stem to the level of the caudal medulla, and below that perfusion is *via* the vertebral arteries. As we have demonstrated, however, when head MAP equals body MAP, this isolation extends down to the spinal cord itself.

We were unable to measure total CBF during bypass because of technical difficulties injecting radioactive microspheres into the bypass unit. Therefore, although we have compared the bypass CBF contamination to the baseline CBF, it would have been ideal to have made a comparison to the CBF arising from the bypass unit. The mean cranial venous oxygen tension is high for the following probable reasons: (1) one value is an outlier, most likely secondary to a technical problem with the blood gas machine; (2) pump flows are high relative to metabolic needs; and (3) cerebral oxygen consumption is moderately depressed by the high halothane concentrations. We cannot exclude the possibility of either greatly depressed cerebral function and oxygen extraction, or arterial–venous shunting; however, the normal arterial–venous carbon dioxide gradient suggests fairly normal metabolism.

In our prior study we used the oxygenator exhaust to estimate isoflurane concentration in the blood.[1] Isoflurane concentration in the bubble oxygenator correlates well with arterial isoflurane concentration.[7] Our correlation in the present study for halothane is slightly less than both that found in Nussmeier *et al.*[7] and that between end-tidal halothane and arterial halothane concentrations[21]; however, the range of halothane (1.3–3.4%) in our study was considerably greater compared to that of Nussmeier *et al.* (0.0–0.7%).[7] In one animal, we investigated higher concentrations of halothane (4%) in the exhaust but the arterial concentration was considerably less (data not reported).

We used halothane to determine if an anesthetic agent with a higher blood–gas solubility could be used in this preparation. We used bubble oxygenators because of efficient gas transfer, particularly with high gas–blood flow ratios.[22] We do not know what effect lower gas flows would have on our correlation. Because membrane oxygenators generally have less efficient gas transfer, particularly for large molecules such as inhalational agents, we speculate that membrane oxygenators may not be ideal for this preparation.

Our halothane data may have been affected by the absence of a measured λ. We were unable to measure λ because of technical difficulties measuring halothane in the gas phase. We used published values for λ,[10] corrected for the large bolus of crystalloid administered.[11] This correction was small because in goats, the concentrations of plasma constituents (lipids and proteins) that affect λ are similar to those in other species,[23,24] so that the normally low hematocrit in the goat should not significantly alter λ relative to other species, and also because the hematocrit change in our study is small.

In conclusion, we have described a method of preferentially anesthetizing and perfusing the brain of a goat, permitting determination of sites (brain *versus* spinal cord or periphery) of inhaled anesthetic action. This technique is not adequate when using an intravenous agent. Measurement of inhalational anesthetic concentration in the oxygenator exhaust is a convenient method to estimate the anesthetic concentration in the arterial blood.

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