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Diffusion of Nitrous Oxide, Cyclopropane, and Halothane through Human Skin and Amniotic Membrane

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Rates of diffusion of nitrous oxide, cyclopropane, and halothane through isolated sheets of human skin were determined. For each per cent concentration, the diffusion rate of nitrous oxide (0.122 ml/min/m^2) was five times that of cyclopropane (0.023 ml/min/m^2) or halothane (0.028 ml/min/m^2). These diffusion rates are similar to those found in man *in vivo*, suggesting that diffusion plays the major role in limiting percutaneous loss of anesthetics. Diffusion rates decreased approximately 1 per cent per degree C decrease in temperature. This small change *in vitro* suggests that the large temperature-related changes in diffusion *in vivo* reported previously may have resulted from alterations in cutaneous blood flow. Rates of diffusion through human amniotic membrane of nitrous oxide (1.344 ml/min/m^2/%) and halothane (1.231 ml/min/m^2/%) exceeded that of cyclopropane (0.386 ml/min/m^2/%). After correction for the difference between the thicknesses of skin and amniotic membrane, diffusion rates through the two barriers are comparable. (Key words: Nitrous oxide; Cyclopropane; Halothane; Diffusion; Skin; Amniotic membrane; Intertissue diffusion.)

IN A STUDY of anesthetized patients, Stoelting and Eger[1] determined that nitrous oxide passes through skin faster than cyclopropane or halothane. At 1 per cent alveolar concentration, the rate of percutaneous transfer of nitrous oxide was 0.051 ml/min/m^2, while that of cyclopropane was 0.015 ml/min/m^2 and that of halothane was 0.0084 ml/min m^2. Nitrous oxide loss increased fivefold when skin temperature increased from 20 to 40 C. Stoelting and Eger suggested that diffusion was the most important factor limiting the percutaneous loss of these anesthetics, although differences in cutaneous blood flow and intertissue diffusion of anesthetics from dermis to subcutaneous fat were considered as alternative explanations. To distinguish among these alternatives, we determined the rates of diffusion of nitrous oxide, cyclopropane, and halothane through human skin *in vitro*. Our technique eliminated the factors of blood flow and intertissue diffusion. To determine whether skin is a unique barrier, we compared the results with those determined for diffusion through another tissue barrier, human amniotic membrane.

Methods

Seven specimens of skin were obtained from limbs amputated for cancer or ischemic vascular disease. Care was taken to obtain skin from uninvolved portions of the amputated limbs. During the amputations two patients received halothane and five patients received nitrous oxide anesthesia. No patient received cyclopropane. Immediately upon removal of a section of skin from the amputated limb, the subcutaneous fat was dissected from the dermis. The average (±SE) thickness of the prepared specimens was 1.02 ± 0.07 mm, as measured with a micrometer.

Each specimen was placed between halves of a cylindrical glass chamber connected to nylon tubing (fig. 1). The chamber diameter of 3.1 cm allowed exposure of 7.55 cm^2 of skin on either side. No inward or outward gas leaks were tolerated. Humidified nitrous oxide (85.1 per cent), cyclopropane (12.4 per cent) and halothane (1.76 per cent) were simultaneously passed through the side of the

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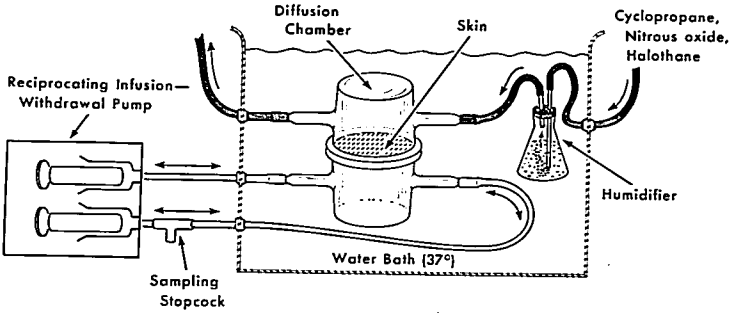

FIG. 1. System used to measure diffusion of nitrous oxide, cyclopropane, and halothane through human skin.

chamber facing the dermal surface at a rate of 1 l/min. The anesthetic gases diffused through the skin into the opposite side of the chamber, which contained nitrogen. A three-way stopcock and two 50-ml glycerinized syringes set in a reciprocating infusion–withdrawal pump were attached to the chamber by nylon tubing. The total volume of the system was 89.7 ml. Gas within this system was continually mixed through the reciprocal movement of the syringes. At 30-minute intervals 10 ml of gas were withdrawn from this system into a glycerinized syringe and analyzed. The gas withdrawn was immediately replaced with an equal volume of nitrogen. Gas samples were analyzed simultaneously for nitrous oxide, cyclopropane, and halothane by gas chromatography.

Three skin specimens were studied only while immersed in the constant-temperature water bath at 37 C. Two other specimens were studied for three hours at 37 C and then for an additional two hours at room temperature (24 C). Last, two specimens were studied for three hours at room temperature and then for two hours in the bath at 37 C.

Amniotic membrane (0.27 ± 0.4 mm thick) was selected as another type of human tissue

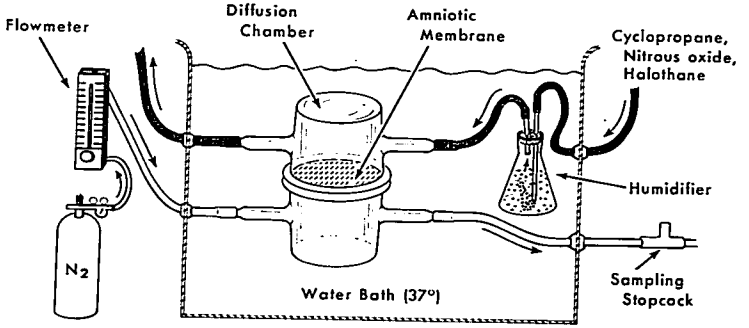

FIG. 2. System used to measure diffusion of nitrous oxide, cyclopropane and halothane through human amniotic membrane.

TABLE 1. Diffusion of Nitrous Oxide, Cyclopropane, and Halothane through Skin at 37 C

	Nitrous Oxide	Cyclopropane	Halothane
Mean (± SE) concentration to which skin was exposed (per cent)	85.1 ± 1.1	12.4 ± 1.2	1.76 ± 0.20
Mean (± SE) diffusion rate (ml/min/m²/%)	0.122 ± 0.020	0.023 ± 0.003	0.028 ± 0.007
Fraction of N_2O diffusion rate (per cent)	100.0	18.9	22.9
Diffusion coefficient (cm²/min)	$1.23 \pm 0.20 \times 10^{-4}$	$0.23 \pm 0.03 \times 10^{-4}$*	$0.27 \pm 0.06 \times 10^{-4}$*

* Significantly different from nitrous oxide, $P < 0.01$.

TABLE 2. Effect of Temperature on the Diffusion Rate (ml/min/m²/%) of Nitrous Oxide through Skin

	(1) Diffusion Rate at 37 C	(2) Diffusion Rate at 24 C	(1) Minus (2)	Per Cent Change in Diffusion Rate per Degree C
Specimen A	0.1268	0.0888	0.0380	2.31
Specimen B	0.2113	0.1765	0.0348	1.27
Specimen C	0.1267	0.1100	0.0167	1.01
Specimen D	0.1838	0.1576	0.0262	1.10
Mean ± SE	0.1622 ± 0.0212	0.1332 ± 0.0407	0.0290 ± 0.0047	1.42 ± 0.26

membrane for study because it was readily available, it has relatively constant thickness, it does not need to be separated from adjacent tissues, and it lacks a keratin layer. The amniotic membrane was obtained from normal parturients immediately post partum. Specimens were placed in the glass chamber and connected to nylon tubing (fig. 2). The rapidity of diffusion of gases through amniotic membrane necessitated modification of the system used for the study of diffusion through skin. Nitrous oxide (85.6 per cent), cyclopropane (12.3 per cent) and halothane (2.46 per cent) were simultaneously delivered through one side of the chamber at 1 l/min. Nitrogen was delivered to the opposite (diffusate) side of the chamber at a rate of 50 or 100 ml/min. Every 15 minutes, 10 ml of gas were obtained from a stopcock located distal to the chamber and analyzed for anesthetic gas concentrations. All amniotic membrane studies were performed with the chamber in a water bath at 37 C.

Diffusion coefficients (D) were calculated utilizing Fick's equation in the form described by Kety.[2] That is,

$$\frac{dQ}{dt} = \frac{-DS}{H}(C_2 - C_1)$$

where dQ/dt = the diffusion rate, S = the membrane surface area, H = the membrane thickness, and C_1 and C_2 = the concentrations of gas on either side of the membrane. For simplicity, we assumed that diffusion occurred in parallel streams, that there was a linear concentration gradient through the membrane, that there was no laminar flow barrier to diffusion at the surface of the membrane, and that the concentrations of gases on either side of the membrane were uniform at any instant.

Data were analyzed using Student's paired t test. Values were considered significant when $P < 0.05$.

Results

Rates of diffusion are reported in terms of ml gas passing through the membrane, per minute, per square meter surface area, per per cent concentration of gas to which the

TABLE 3. Diffusion of Nitrous Oxide, Cyclopropane, and Halothane through Amniotic Membrane at 37 C

	Nitrous Oxide	Cyclopropane	Halothane
Mean (± SE) concentration to which membrane was exposed (per cent)	85.6 ± 0.2	12.3 ± 0.9	2.46 ± 0.02
Mean (± SE) diffusion rate (ml/min/m²/%)	1.344 ± 0.124	0.386 ± 0.031	1.231 ± 0.163
Fraction of N_2O diffusion rate (per cent)	100.0	28.7	91.7
Diffusion coefficient (cm²/min)	$3.48 \pm 0.32 \times 10^{-4}$	$1.00 \pm 0.18 \times 10^{-4}$*	$3.09 \pm 0.22 \times 10^{-4}$

* Significantly different from nitrous oxide and halothane, $P < 0.001$.

membrane was exposed. Diffusion of nitrous oxide through skin at 37 C was significantly greater than diffusion of cyclopropane or halothane. The mean rates of diffusion were 0.122 ml/min/m²/% for nitrous oxide, 0.023 ml/min/m²/% for cyclopropane, and 0.028 ml/min/m²/% for halothane (table 1). These diffusion rates were obtained by averaging all determinations for each specimen and then taking the mean of those averages. Examination of the mean rates of diffusion for each anesthetic at 30-minute intervals demonstrated that the system rapidly came to equilibrium and that there was no significant change in rate of diffusion with time.

Temperature affected the mean rate of nitrous oxide diffusion slightly (table 2). There was a mean difference of 0.029 ml/min/m²/% between 37 C and 24 C, or slightly more than a 1 per cent decrease per degree C decrease in temperature (last column of table 2). The variation in absolute diffusion rates among specimens correlated with differences in membrane thickness.

As a control, diffusion through a minute hole in a thin nylon membrane was measured. Rates of diffusion of nitrous oxide, cyclopropane, and halothane through this perforated membrane differed by less than 1.5 per cent.

Of the three anesthetics tested, nitrous oxide was the most diffusible through amniotic membrane. The mean rate of diffusion for nitrous oxide was 1.344 ml/min/m²/% (table 3). Amniotic membrane, however, was also highly permeable to halothane. The mean diffusion rate for halothane was 1.231 ml/min/m²/%. Cyclopropane diffused at a rate of 0.386 ml/min/m²/%. The diffusion coefficients for halothane and nitrous oxide were not significantly different.

Discussion

We conclude that percutaneous loss of inert anesthetic gases in man is limited primarily by diffusion. Nitrous oxide, cyclopropane, and halothane pass through an isolated sheet of human skin at rates which are in the same proportion as those reported for skin *in vivo* by Stoelting and Eger.[1] Nitrous oxide diffuses through skin at a rate five times that of cyclopropane or halothane. Thus, it is unnecessary to invoke intertissue diffusion of gas to subcutaneous fat to explain the slower rates of percutaneous loss of cyclopropane and halothane *in vivo*. This does not deny the possibility of intertissue diffusion, as suggested by Perl[3] and demonstrated for xenon by Sejrsen.[4] It only suggests that such intertissue diffusion does not materially affect percutaneous loss of anesthetics. That diffusion is the primary limiting factor in percutaneous gas transfer has also been demonstrated for argon, nitrogen and helium.[5-7]

Diffusion coefficients calculated for nitrous oxide, cyclopropane, and halothane are of the order of 10^{-4} cm²/min, and are comparable to those reported by Krough[7] and Fitzgerald[8] for oxygen. These values for diffusion coefficients are only approximate, however, because of the uneven geometry of the skin specimens and because of the assumptions made regarding linearity of the concentration gradients and the effects of laminar flow near the sur-

face of the membrane (*i.e.*, the surface and bulk gas concentrations might be different).

The reasons for the differences in diffusivity among anesthetic gases are not readily apparent. There is no correlation between diffusion rates and solubility. The halothane fat/blood partition coefficient is four times the cyclopropane coefficient, yet the two gases have nearly identical diffusion coefficients. Diffusivity is also an inverse function of the square root of molecular weight, but here again, we find no correlation. Nitrous oxide and cyclopropane have nearly identical molecular weights, yet their diffusion rates through skin differ fivefold.

Diffusion of nitrous oxide, like that of other gases,[7] increased approximately 1 per cent for each degree C increase in temperature (table 2). This small increase with temperature cannot explain the fivefold increase in percutaneous loss of nitrous oxide *in vivo* when skin temperature was increased from 20 to 40 C.[1] We conclude, as did Klocke,[5] that changes in temperature *in vivo* effect large changes in percutaneous loss of gas, not through altered diffusivity, but rather through concomitant changes in cutaneous blood flow.

All three gases had much faster rates of diffusion through amniotic membrane relative to the rates of diffusion through skin. This is attributable, in part, to the difference between the thicknesses of the two types of membranes, although there was a significantly greater diffusivity of halothane through amniotic membrane compared with the other two anesthetics. If diffusion through a membrane varies as an exponential function, the difference between diffusivity of these gases through skin and amniotic membrane is less apparent. For example, if the diffusion rates through amniotic membrane are divided by 4 squared (skin specimens being four times as thick as amniotic membrane), the corrected diffusion rates are:

	N_2O	C_3H_6	Halothane
Diffusion through skin	0.122	0.023	0.028
"Corrected" amniotic membrane diffusion	0.084	0.024	0.077

Thus, the nitrous oxide and cyclopropane values are very close, and the halothane rates differ by a factor of less than 3 only. As was true for skin, there was no clear correlation between amniotic-membrane diffusion rates and the molecular weights or solubility characteristics of the anesthetic gases.

Perl[3] and Rackow[9] reported that when nitrous oxide and cyclopropane were administered simultaneously to man the whole-body uptake of nitrous oxide approached equilibrium more rapidly than that of cyclopropane. They proposed that part of this difference was due to the greater lipid solubility of cyclopropane and its faster diffusion from lean to neighboring adipose tissue. Assuming that intertissue diffusion would entail transfer of gas through membranes (*e.g.*, fascia, peritoneum, etc.) and that such membranes would behave like skin or amniotic membrane, our data would dispute this hypothesis. In other words, the effect of the eightfold difference between the lipid solubilities of the two gases would be countered by the fivefold difference between their rates of diffusion through membranes. Another explanation for the more rapid increase in alveolar nitrous oxide might be that cyclopropane is more soluble in blood[10,11] and body tissues,[12] particularly at lower concentrations.

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Respiration

LUNG PARTICLE CLEARANCE—SMOKERS AND NONSMOKERS Transport of mucus by the cilia of the tracheobronchial epithelium is a vital mechanism for removal of inhaled particulate matter. Normal mucociliary activity is essential for the maintenance of healthy lungs, especially in an environment in which noxious materials exist in dangerous quantities. Deposition and clearance of inhaled particles of iron oxide labeled with ^{198}Au were studied in 19 normal subjects (10 nonsmokers and 9 smokers). Monodisperse aerosols of particles with a 2-μ diameter were produced in a spinning-disc atomizer. Thoracic counts and obtaining of images with a scintillation camera were begun immediately after inhalation of the aerosol and continued for six hours. In all subjects, smokers and nonsmokers, deposition of particles was uniform throughout both lung fields, with about half of the particles deposited in the ciliated airways (tracheobronchial deposition) and half in the nonciliated airways (alveolar deposition). In nonsmokers, tracheobronchial clearance occurred immediately after inhalation, first at a fast rate for particles deposited in the largest, central airways, and then at a slower rate for particles in the smaller and more peripheral airways. Photoscintigrams indicated that particle clearance occurred steadily, with no retention in any area. The general pattern of clearance was similar to the multiple-conveyor-belt model, with speed of removal increasing from the periphery to the central portions in a manner intended to prevent excessive accumulation at airway confluence points. In smokers, tracheobronchial clearance was delayed for periods of 1–4 hours after inhalation. Furthermore, in contrast to nonsmokers, many smokers had significant clearance still going on during the fifth and sixth hours after inhalation. Photoscintigrams showed abnormal accumulations of particles in the large airways several hours after inhalation of the aerosol. This clearance delay is considered important in the genesis of the bronchitis frequently present in persons who smoke cigarettes. (*Lourenco, R. V., Klimek, M. F., and Borowski, C. J.: Deposition and Clearance of 2 μ Particles in the Tracheobronchial Tree of Normal Subjects—Smokers and Nonsmokers, J. Clin. Invest. 50: 144, 1971.*) EDITOR'S COMMENT: An elegant analysis that would have been additionally valuable if the authors had considered studying the clearance rate in smokers after a few days of abstinence. Obviously, we need to know whether impairment of clearance of particulate matter after chronic inhalation of cigarette smoke is rapidly reversible or permanent.