Tissue solubility of four volatile anesthetics in fresh and frozen tissue specimens from swine

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Objective—To determine tissue solubilities of desflurane, sevoflurane, enflurane, and halothane in swine and to evaluate the effects of freezing specimens on tissue solubility.

Sample Population—Arterial blood samples and specimens of brain, heart, liver, kidney, muscle, and subcutaneous fat from 5 healthy female adult Chinese Meishan pigs.

Procedure—Each tissue specimen was divided into 2 parts. One part was used to measure tissue-gas partition coefficients immediately after collection. The other part was frozen at –20 C for 6 days prior to determination of tissue-gas partition coefficients. Tissue-gas and blood-gas partition coefficients were measured by use of gas chromatography, and tissue-blood partition coefficients were calculated. Regression analysis was performed to determine whether fat-gas partition coefficients were correlated with lean tissue-gas partition coefficients.

Results—Tissue-gas and blood-gas partition coefficients of halothane were greater than those of enflurane followed by coefficients of sevoflurane and desflurane. However, the order of anesthetic agents with the greatest to smallest tissue-blood partition coefficients was sevoflurane, halothane, enflurane, and desflurane. Muscle-gas partition coefficients of sevoflurane and enflurane, liver-gas partition coefficients of desflurane and halothane, and the kidney-gas partition coefficient of enflurane were significantly greater in frozen specimens, compared with fresh specimens. Lean tissue-gas partition coefficients of all 4 volatile anesthetics correlated directly with fat-gas partition coefficients.

Conclusions and Clinical Relevance—The fat content of lean tissue is an important factor in determining the tissue solubility of volatile anesthetics. Freezing specimens before determination of tissue-gas partition coefficients may result in a false increase in tissue solubility. (*Am J Vet Res* 2002;63:74–77)

The tissue solubility of volatile anesthetics is determined by the tissue-blood partition coefficient of that anesthetic. This coefficient determines the duration of the uptake and elimination periods of volatile anesthetics into and out of tissue. Anesthetics with a high tissue-blood partition coefficient have a prolonged duration of uptake and elimination, compared with those with a low partition coefficient. Thus, knowing the tissue-blood partition coefficients of various volatile anesthetics may be important for determining which agent to use.

Although swine have been used to study the pharmacokinetics of volatile anesthetics,[1,2] to our knowledge there is only 1 published study[2] on tissue solubility of volatile anesthetics in swine, and solubility of enflurane was not reported in that study. Thus, the purpose of the study determined here was to simultaneously measure blood-gas and tissue-gas partition coefficients of desflurane, sevoflurane, enflurane, and halothane, which are widely used in clinical and laboratory settings, in the brain, heart, liver, kidney, muscle, and fat of swine and to calculate the respective tissue-blood partition coefficients of these volatile anesthetics.

Materials and Methods

Specimens—This study was approved by the Committee on Laboratory Animal Research in the First University Hospital. Five healthy adult female Chinese Meishan pigs (12 to 15 months old) were anesthetized with sodium pentobarbital (100 mg/kg of body weight, IM) and euthanatized by IV injection of 1.5% potassium chloride (50 ml) within 1 minute. Arterial blood samples and tissue specimens of the brain, heart, liver, kidney, psoas major muscle, and subcutaneous fat were collected immediately after euthanasia. After collection, fascial structures and visible fat were removed from the psoas major muscle. Pericardial and endocardial membranes were discarded, and a specimen of the left ventricle was collected. The capsule, large vessels, and ducts of the liver and kidney were removed. The arachnoid and pial membranes were stripped from the brain, and vascular structures were removed from fat specimens. Each tissue specimen was then sliced into small cubes and divided into 2 parts. One part was used to determine tissue-gas partition coefficients immediately after specimen collection (ie, partition coefficients for fresh tissue). The other part was frozen at –20 C for 6 days prior to measurement of tissue-gas partition coefficients (partition coefficients for frozen tissue). Blood-gas partition coefficients were determined, using fresh arterial whole blood only.

Determination of tissue-gas partition coefficients—Total tissue volume of fresh and frozen specimens was determined by volume displacement, using saline (0.9% NaCl) solution. Tissue cubes were put into a glass container in which the 20-ml mark was precisely calibrated. The container with tissue was weighed, and saline solution was added to the 20-ml mark. The container was weighed again, and the tissue volume was calculated as the difference between the first and second weight. Frozen specimens were thawed at 20 C before use. Each specimen was then homogenized[a] (12,000 rpm for 3 minutes) in a volume of saline solution 2 to 3 times (6 to 8 times for fat) the specimen volume. The homogenate was filtered through a 4-mm^2 stainless steel mesh to remove

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extraneous fascia. The net volume of tissue in the homogenate was calculated as the difference between the total volume of the tissue specimen and the volume of extraneous fascia, which was also measured by use of saline solution volume displacement.

Homogenate-gas partition coefficients were determined immediately after homogeneization. A 20-ml (50-ml for fat specimens) gas-tight glass syringe capped with a 3-way stopcock was sealed by coating the plunger with a thin layer of silicone grease. The internal volume (4- and 20-ml marks of 20-ml syringes, and 5- and 50-ml marks of 50-ml syringes) of the syringes had been precisely calibrated by use of water displacement. In addition, we had determined that the anesthetics evaluated were not lost from or absorbed by these syringes. We found that concentrations of anesthetic vapors in the syringes decreased by no more than 2% over 8 hours.

Approximately 7 ml of each tissue homogenate was added to a sealed syringe, and an anesthetic gas mixture (1.3% desflurane, 0.4% sevoflurane, 0.3% enflurane, or 0.6% halothane in air) was added to the 18-ml mark of the 20-ml syringe, or for fat specimens, the 45-ml mark of the 50-ml syringe. The stopcock was closed, and the filled syringe was shaken vigorously and immersed in a waterbath set at 37 C. The syringe was shaken vigorously for 5 to 10 seconds every 15 minutes for 2 hours (first equilibration period). After the third shaking, the plunger of the syringe was withdrawn to the 20- or 50-ml mark with the stopcock closed, thereby creating a slight negative pressure. The stopcock was then opened briefly, allowing entry of ambient gases and equilibration with ambient pressure. The concentration of anesthetic in the gas phase of the syringe after the first equilibration period was analyzed by use of gas chromatography. [3] All remaining gas was then expelled from the syringe, and the tissue homogenate was expelled to the 4-ml mark (5-ml mark of the 50-ml syringes). Vapor-free air was drawn into each syringe to the 18-ml mark (45-ml mark of 50-ml syringes). The syringe was shaken vigorously and immersed in a waterbath set at 37 C for 2 hours (second equilibration period). The second equilibration period followed the same sequence of shaking, volume adjustment to 20 ml (50 ml for fat), and timing as described for the first equilibration period. The concentration of anesthetic in the gas phase at the end of the second equilibration period was again analyzed by use of gas chromatography.

The total amount of anesthetic (volume in the liquid phase + volume in the gas phase) at the end of the second equilibration period equaled the amount in the liquid phase retained in the syringe after the first equilibration period. This relationship was expressed as:

$$C_2 \times V_G \times C_{H2} \times V_H = C_{H1} \times V_H$$

where C_2 is the anesthetic concentration in the gas phase at the end of the second equilibration period, V_G and V_H are the gas and homogenate volumes in the syringe for the second equilibration period, respectively, and C_{H1} and C_{H2} are the anesthetic concentrations in the homogenate at the end of the first and second equilibration periods, respectively.

The homogenate-gas partition coefficient ($\lambda_{H/G}$) was defined as the ratio of anesthetic concentration in the homogenate phase to that in the gas phase (ie, $C_{H1} = \lambda_{H/G} \times C_1$ and $C_{H2} = \lambda_{H/G} \times C_2$). Substituting these into equation 1 yields:

$$C_2 \times V_G + \lambda_{H/G} \times C_2 \times V_H = \lambda_{H/G} \times C_1 \times V_H$$

Rearranging this equation yields:

$$\lambda_{H/G} = (V_G/V_H) \times (C_2/[C_1 - C_2])$$

where C_1 is the anesthetic concentrations in the gas phase at the end of the first equilibration period. The final equation was used to calculate $\lambda_{H/G}$.

Tissue-gas partition coefficients ($\lambda_{T/G}$) for each agent in fresh and frozen specimens was then determined according to the formula:

$$\lambda_{T/G} = \lambda_{H/G} + (V_S/V_T) \times (\lambda_{H/G} + \lambda_{S/G})$$

where V_S and V_T are the volume of saline solution and tissue in the homogenate, respectively, and $\lambda_{S/G}$ is the saline solution-gas partition coefficient for each agent.[b]

Determination of blood-gas partition coefficients— Freshly collected whole blood was used for determination of blood-gas partition coefficients. Blood-gas partition coefficients were determined, using the same method described for determination of tissue-gas partition coefficients.

Calculation of tissue-blood partition coefficients— Once tissue-gas and blood-gas partition coefficients were determined, tissue-blood partition coefficients were calculated by dividing the tissue-gas partition coefficient by the blood-gas partition coefficient.

Determination of anesthetic concentrations— Anesthetic concentrations in the gas phase of each syringe after the first and second equilibration periods were measured by use of gas chromatography.[c] The system used comprised a 6-m-long stainless steel column (internal diameter, 0.32 cm) packed with 10% SF96 on 60/80 mesh[d] maintained at 75 C. A nitrogen carrier stream (10 ml/min) was delivered through the column to a flame ionization detector supplied by hydrogen at 35 ml/min and air at 300 ml/min. Output from the gas chromatograph was collected, using an integrator,[e] and peak areas were calculated automatically. Under these conditions, the peaks for desflurane, sevoflurane, enflurane, and halothane were completely separated.

Primary and secondary (compressed gas tanks) standards were used to calibrate the gas chromatograph system. Primary standards were produced by injection of aliquots of each volatile anesthetic from a syringe into a glass flask of known volume. Because of the high-saturated vapor pressure of desflurane and to ensure that no desflurane was lost when producing the primary and secondary standards, li quid desflurane and the syringe were kept in a refrigerator (4 C) before use. Liquid desflurane was drawn into the cool syringe in the refrigerator and transferred into the flask or tank immediately. The primary standards (glass flask) were used to calibrate the secondary standards, and the secondary standards (tank) were used to calibrate the gas chromatograph system each day. Known concentrations in each standard were compared with peak areas on the chromatogram by use of linear regression; R^2 for each analysis was > 0.9995. The regression equation was used to convert peak area to concentration, and peak areas were proportional to concentrations over the entire range of the concentrations tested.

Statistical analyses—Tissue-gas, blood-gas, and tissue-blood partition coefficients were compared among agents by use of ANOVA and the Student-Newman-Keuls method of multiple comparisons. Tissue-gas partition coefficients determined for fresh specimens were compared with coefficients determined for frozen specimens by use of paired *t*-tests. Regression analysis was used to determine whether the fat-gas partition coefficients of the 4 anesthetics correlated with each lean tissue-gas partition coefficient. For all tests, significance was set at $P < 0.05$.

Results

Blood-gas and tissue-gas partition coefficients were significantly different among agents (**Table 1**). Tissue-gas and blood-gas partition coefficients of

Table 1—Tissue-gas partition coefficients of 4 volatile anesthetic agents determined in freshly prepared and frozen (–20 C for 6 days) tissue specimens from 5 healthy adult pigs

Tissue	Tissue specimen preparation	
	Fresh	Frozen
Brain		
Desflurane	0.83 ± 0.09[a]	0.75 ± 0.09[a]
Sevoflurane	1.43 ± 0.06[b]	1.47 ± 0.12[b]
Enflurane	2.75 ± 0.24[c]	2.81 ± 0.29[c]
Halothane	4.88 ± 0.42[d]	4.39 ± 0.39[d]
Heart		
Desflurane	0.47 ± 0.04[a]	0.47 ± 0.07[a]
Sevoflurane	0.99 ± 0.06[b]	0.88 ± 0.10[b]
Enflurane	2.09 ± 0.17[c]	1.83 ± 0.21[c]
Halothane	3.16 ± 0.15[d]	2.98 ± 0.31[d]
Kidney		
Desflurane	0.48 ± 0.06[a]	0.46 ± 0.04[a]
Sevoflurane	0.91 ± 0.13[b]	0.97 ± 0.06[b]
Enflurane	1.56 ± 0.24[c]	1.81 ± 0.15[c,1]
Halothane	2.72 ± 0.32[d]	2.48 ± 0.21[d]
Liver		
Desflurane	0.60 ± 0.09[a]	0.73 ± 0.12[a,1]
Sevoflurane	1.30 ± 0.17[b]	1.37 ± 0.17[b]
Enflurane	2.69 ± 0.28[c]	2.80 ± 0.36[c]
Halothane	4.37 ± 0.47[d]	4.78 ± 0.51[d,1]
Muscle		
Desflurane	0.56 ± 0.04[a]	0.59 ± 0.08[a]
Sevoflurane	1.17 ± 0.07[b]	1.32 ± 0.14[b,1]
Enflurane	2.38 ± 0.17[c]	2.77 ± 0.28[c,1]
Halothane	4.43 ± 0.25[d]	4.65 ± 0.42[d]
Fat		
Desflurane	11.85 ± 1.63[a]	11.80 ± 1.10[a]
Sevoflurane	29.32 ± 4.28[b]	28.73 ± 4.27[b]
Enflurane	60.18 ± 8.57[c]	57.72 ± 7.73[c]
Halothane	110.74 ± 16.26[d]	114.62 ± 11.63[d]
Blood		
Desflurane	0.40 ± 0.02[a]	ND
Sevoflurane	0.48 ± 0.00[b]	ND
Enflurane	1.31 ± 0.06[c]	ND
Halothane	2.19 ± 0.15[d]	ND

Data reported as mean ± SD.
ND = Not determined.
[a-d]Within a column, values with different superscript letters are significantly ($P < 0.05$) different from other values for the same group (tissue).
[1]Significantly ($P < 0.05$) different from value for freshly prepared specimens.

Table 2—Tissue-blood partition coefficients* of 4 volatile anesthetic agents determined in tissue specimens collected from 5 healthy adult pigs

Coefficient	Desflurane	Sevoflurane	Enflurane	Halothane
Brain-blood	2.12 ± 0.20[a]	2.99 ± 0.12[b]	2.16 ± 0.20[a]	2.20 ± 0.06[a]
Heart-blood	1.19 ± 0.08[a]	2.06 ± 0.12[b]	1.54 ± 0.17[c]	1.55 ± 0.11[c]
Kidney-blood	1.14 ± 0.23[a]	1.90 ± 0.25[b]	1.19 ± 0.13[a]	1.20 ± 0.10[a]
Liver-blood	1.53 ± 0.22[a]	2.72 ± 0.33[b]	2.06 ± 0.12[c]	2.07 ± 0.11[c]
Muscle-blood	1.43 ± 0.13[a]	2.44 ± 0.13[b]	1.82 ± 0.04[c]	1.96 ± 0.02[d]
Fat-blood	30.25 ± 3.74[a]	61.12 ± 8.48[b]	45.95 ± 4.55[c]	48.96 ± 5.17[c]

Data reported as mean ± SD. *For each pig, specific tissue-blood partition coefficients were calculated by dividing the tissue-gas partition coefficient determined for fresh specimens by the blood-gas partition coefficient.
[a-d]Within a row, values with different superscript letters are significantly ($P < 0.05$) different.

halothane were the highest of all agents evaluated, followed by coefficients of enflurane, sevoflurane, and desflurane. Most of the tissue-blood partition coefficients were also significantly different among agents (**Table 2**). However, brain-blood and kidney-blood partition coefficients of halothane, enflurane, and desflurane were not significantly different. In addition, heart-blood, liver-blood, and fat-blood partition coefficients of halothane were not significantly different from those of enflurane. The tissue-blood partition

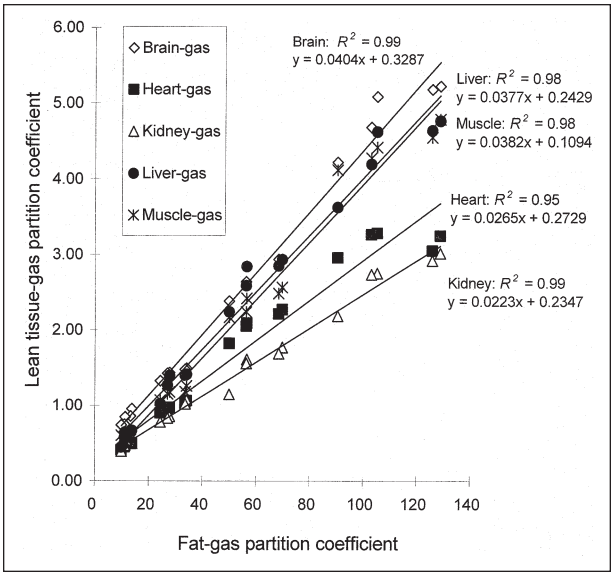

Figure 1—Fat-gas partition coefficients versus lean tissue-gas partition coefficients of halothane, enflurane, desflurane, and sevoflurane determined in tissue specimens collected from 5 healthy adult pigs. Each point represents the mean value determined for 1 agent. Fat-gas partition coefficients were significantly ($P < 0.05$) correlated with lean tissue-gas partition coefficients.

coefficients of sevoflurane were highest, followed by coefficients of halothane, enflurane, and desflurane.

Muscle-gas partition coefficients of sevoflurane and enflurane, liver-gas partition coefficients of desflurane and halothane, and the kidney-gas partition coefficient of enflurane were significantly greater in frozen specimens, compared with fresh specimens (Table 1). For all 4 volatile anesthetics evaluated, brain-gas, heart-gas, kidney-gas, liver-gas, and muscle-gas partition coefficients of all 4 volatile anesthetics evaluated correlated directly with the fat-gas partition coefficients (**Fig 1**).

Discussion

The blood-gas partition coefficients of desflurane and halothane that we determined were 14% greater and 16% less, respectively, than those reported by Yasuda et al.[2] In addition, the brain-gas partition coefficients of desflurane, sevoflurane, and halothane were 66, 38, and 11% greater, respectively, than those reported previously.[2] The brain-gas partition coefficient of halothane in white matter is 1.5 to 2 times greater than that in gray matter.[4-6] Because we did not deliberately control the proportion of white and gray matter in our study, we cannot confirm whether this is the reason for the discrepancies between our results and the results of Yasuda et al.[2]

However, differences in preparation of brain specimens could not have accounted completely for the differences in results between our study and that of Yasuda et al,[2] because the fat-gas partition coefficient of desflurane, heart-gas partition coefficients of desflurane and sevoflurane, and kidney-gas partition coefficient of desflurane were greater and the fat-gas and kidney-gas partition coefficients of halothane were less in the present study than those previously reported. Differences may

have been attributable to the age of pigs used. We used 12- to 15-month-old pigs, whereas Yasuda et al[2] used 3- to 4-month-old pigs. Lerman et al[7,8] found that the tissue solubilities of isoflurane, enflurane, halothane, and methoxyflurane in sheep and humans increased with age. In addition, we filtered tissue homogenates through a 4-mm^2 stainless steel mesh to remove extraneous fascia before determination of tissue-gas partition coefficients determination. Because solubility of anesthetics is low in fascia, any fascia remaining in the tissue homogenate will result in measurement of a low tissue-gas partition coefficient.

We found that tissue-gas and blood-gas partition coefficients of halothane in swine were greater than those of enflurane, followed by coefficients of sevoflurane and, finally, desflurane. This was the same order found for these agents in humans.[8,9] However, because of the low blood-gas partition coefficients of sevoflurane and enflurane in swine, compared with humans, the order of tissue-blood partition coefficients differed. In pigs, the order of agents with the greatest to smallest tissue-blood partition coefficients was sevoflurane, halothane, enflurane, and desflurane, whereas in humans the order was halothane, sevoflurane, enflurane, and desflurane.[8,9] The small blood-gas and tissue-blood partition coefficients of desflurane indicate that this agent moves more rapidly from the alveolar space to the tissue space than the other agents. This result was confirmed by results of the study of Yasuda et al.[2]

We found that tissue-gas partition coefficients significantly increased in some tissue specimens frozen at –20 C for 6 days, compared with values obtained for fresh tissues. As water within cells freezes, intracellular volume increases. This increase may result in disruption of cell membranes, which allows for the loss of intracellular water as specimens are thawed. For all modern volatile anesthetics, saline solution-gas partition coefficients are greater than tissue-gas partition coefficients.[2,7-12] Thus, loss of intracellular water after freezing and thawing may result in an increase in measured tissue-gas partition coefficients. On the basis of the results of the present study, we recommend that tissue-gas partition coefficients be determined, using freshly isolated tissue specimens. If tissue specimens must be preserved before determination of partition coefficients, specimens should be refrigerated in the same saline solution used to determine tissue volume, or, alternatively, specimens should be homogenized and the homogenate stored at –70 C. Freezing homogenized tissue specimens does not affect tissue-gas partition coefficient measurements.[7-9]

Previously, we found good correlations between fat-gas and lean tissue-gas partition coefficients in humans.[f] This finding indicates that, in humans, fat content is the most important factor determining the solubility of volatile anesthetics in lean tissue. In the present study, we also found good correlations (R^2 ranging from 0.95 for heart to 0.99 for brain and kidney) between fat-gas and lean tissue-gas partition coefficients in swine. This finding allowed us to estimate lean tissue-gas partition coefficients from fat-gas partition coefficients obtained for the same animals and to predict the pharmacokinetic characteristics of volatile anesthetics more precisely by determining the percentage of distribution of the total fat mass in different tissues. However, analysis of the regression lines for fat-gas versus lean tissue-gas partition coefficients revealed different slopes and intercepts depending on the lean tissue assessed. This suggests that amount of fat is not the only factor determining tissue solubility of volatile anesthetics in swine.

[a]Changhong 12B homogenizer, Shanghai Instrument Co, Shanghai, China.

[b]Zhou JX, Liu YQ, Liu J. The combined effect of hypothermia and crystalloid hemodilution on the blood solubility of volatile anesthetics (abstr). *Anesthesiology* 1998;89:A504.

[c]GOW-MAC 580 gas chromatography, GOW-MAC Instrument Co, Bethlehem, Pa.

[d]Chromosorb P, Bafangshiji Scientific Instrument Co, Chengdu, SiChuan, China.

[e]TAI SSC922 integrator, Qilu Instrument Co, Shangdong, China.

[f]Zhou JX, Liu J. Effect of temperature on the solubility of volatile anesthetics in human tissues (abstr). *Anesthesiology* 1998;89:A503.

References

1. Laster MJ, Taheri S, Eger EI II, et al. Visceral losses of desflurane, isoflurane, and halothane in swine. *Anesth Analg* 1991;73: 209–212.

2. Yasuda N, Targ AG, Eger EI II, et al. Pharmacokinetics of desflurane, sevoflurane, isoflurane, and halothane in pigs. *Anesth Analg* 1990;71:340–348.

3. Jian-Xin Zhou, Jin Liu. The effect of temperature on solubility of volatile anesthetics in human tissues. *Anesth Analg* 2001;93: 234–238.

4. Larson CP Jr, Eger EI II, Severinghaus JW. The solubility of halothane in blood and tissue homogenates. *Anesthesiology* 1962; 23:349–355.

5. Lowe HJ. Determination of volatile organic anesthetics in gases, blood, and tissues. In: Kroman HS, Bender SR, eds. *Theory and application of gas chromatography in industry and medicine*. New York: Grune & Stratton, 1968;194–209.

6. Lowe HJ, Hagler K. Determination of volatile organic anaesthetics in blood, gases, tissues and lipids: partition coefficients. In: Porter R, ed. *Gas chromatography in biology and medicine, Ciba Foundation Symposium*. London: Churchill Livingstone Inc, 1969; 86–112.

7. Lerman J, Gregory GA, Willis MM, et al. Age and the solubility of volatile anesthetics in ovine tissues. *Anesth Analg* 1985; 64:1097–1100.

8. Lerman J, Schmitt-Bantel BI, Gregory GA, et al. Effect of age on the solubility of volatile anesthetics in human tissues. *Anesthesiology* 1986;65:307–311.

9. Yasuda N, Targ AG, Eger EI II. Solubility of I-653, sevoflurane, isoflurane, and halothane in human tissues. *Anesth Analg* 1989;69:370–373.

10. Weathersby PK, Homer LD. Solubility of inert gases in biological fluids and tissue: a review. *Undersea Biomed Res* 1980;7: 277–296.

11. Eger EI II. Partition coefficients of I-653 in human blood, saline, and olive oil. *Anesth Analg* 1987;66:971–973.

12. Strum DP, Eger EI II. Partition coefficients for sevoflurane in human blood, saline, and olive oil. *Anesth Analg* 1987;66:654–656.