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IN URINE**

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# A METHOD FOR DETERMINING INERT GAS ("N<sub>2</sub>") SOLUBILITY IN URINE \*

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The present interest in the physiology of inert gases makes desirable a more accurate and reproducible method for determining the inert gas content of biological fluids than is currently available (2). This communication describes such a method. The method has been tested on water and used to determine the solubility of the inert gas of air in human urines of different solute concentrations at 37° C.<sup>1</sup> Empirical relationships of solubility to osmolality, chloride concentration, and specific gravity are given. Preliminary studies indicate that the method can also be applied to blood. Another communication (3) will describe the method's application to human urine in a manner believed to enable the calculation of the inert gas tension of arterial blood within  $\pm 2$  mm Hg.

## METHOD

The method to be described is similar to one employed by Van Slyke, Dillon and Margaria (4). It consists essentially in determining the total content of gas other than CO<sub>2</sub> and O<sub>2</sub> in a 25 ml sample of fluid, using a Van Slyke manometric apparatus. This gas will be referred to as nonabsorbable gas. A more complete description and discussion of the method than can be given here appears elsewhere (5). The basic procedure is outlined below.

*Special reagents and apparatus.* 1) CO<sub>2</sub>-O<sub>2</sub> absorber. Twenty g of sodium hyposulfite (Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>) is dissolved in 100 ml 3 N KOH and filtered rapidly through gauze to remove any insoluble impurity. Three N KOH was

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<sup>1</sup> Since the inert gas of air is often treated as being entirely N<sub>2</sub>, its solubility in a fluid has been referred to as "atmospheric nitrogen" or "N<sub>2</sub>" solubility.

chosen arbitrarily because it provides enough hydroxide to ensure alkalinity of any 25 ml urine sample. No sodium anthraquinone-B-sulfonate is included because the red color produced makes critical adjustment of the fluid meniscus to the 0.5 ml mark more difficult. 2) *Ostwald-Van Slyke pipets.* Each pipet was equipped with rubber tip and stopcock and custom-built to deliver 25 ml.

*Evacuation of reagent and introduction of sample.* Three to four drops of caprylic alcohol and CO<sub>2</sub>-O<sub>2</sub> absorber to make 7 ml are drawn into the Van Slyke extraction chamber and doubly extracted (2 minutes per extraction). Five ml of the alcohol-absorber reagent is left in the extraction chamber and the bore of the stopcock leading to it after the second extraction. The sample is admitted from the Ostwald-Van Slyke pipet, with the rubber tip of the pipet sitting tightly at the bottom of the cup. A drop or two of Hg is added to the cup to prevent any of the excess reagent from entering the extraction chamber. Great care is taken to prevent any small air bubbles from entering the chamber with the sample. Bubbles frequently lodge at the pipet stopcock. Their number may be minimized by having the stopcock and adjacent pipet bore scrupulously clean and by manipulating the stopcock under visual observation during filling. This manipulation is easier when pipets are filled in an "inverted" fashion; in addition, dislodged bubbles do not pass through the 25 ml sample. Bubbles also lodge at the rubber tip of the pipet. Their number may be minimized by not adjusting the meniscus to the upper calibration mark until just before the sample is introduced, by keeping the pipet vertical after this adjustment, and by not dislodging any small drop of sample extending below the rubber tip.

*Extraction and reading.* The Hg in the extraction chamber is brought to the 50 ml mark and the reaction mixture (reagent + sample) is shaken vigorously for 8 minutes (by magnetic stirrer). Care is taken to readjust the Hg to the 50 ml mark during the first 1 to 2 minutes. The fluid meniscus in the chamber is brought to the 0.5 ml mark and a manometer reading is taken. The extraction process is repeated for 3 minutes, and another manometer reading is taken at the 0.5 ml mark. The Hg is returned to the 50 ml mark and kept there for 45 minutes without shaking, except for three manometer readings at the 0.5 ml mark at 15, 30 and 45 minutes. At the 45 minute reading, which is designated P<sub>1</sub>, the temperature of the water jacket surrounding the extraction chamber is recorded to the nearest 0.1° C. After reading P<sub>1</sub>, another 3 minute extraction is given, fol-

lowed by another manometer reading at the 0.5 ml mark. The extracted gas is then ejected quantitatively without loss of any reaction mixture, as described by Peters and Van Slyke (6). The fluid meniscus is again brought to the 0.5 ml mark and manometer reading  $P_2$  is taken. The analysis is then complete.

A definitive  $P_1$  reading can be made only under three conditions: when there has been complete absorption of  $\text{CO}_2$  and  $\text{O}_2$  and partial pressure equilibrium of the non-absorbable gas between the gas and liquid phases, and when the thermometer in the water jacket represents the true temperature within the extraction chamber. The initial 8 and 3 minute shaking periods are intended to satisfy the first two conditions; the agreement of the first two manometer readings indicates that this is the case. The further serial readings at 15-minute intervals are believed necessary to satisfy the third condition. Many factors can raise the temperature within the extraction chamber above its pre-analysis level. Other factors suggest that the secondary increase in water jacket temperature will be delayed, smaller and more protracted; of great importance may be the unfavorable ratio of fluid surface to volume when 30 ml of reaction mixture is contained in a 50 ml extraction chamber. This situation is proposed as the explanation for changes in manometer readings and water jacket temperatures we have regularly observed. The manometer reading decreases a few millimeters of Hg during the first 15 to 25 minutes of the 45 minute waiting period. The water jacket temperature increases ( $\sim 1^\circ \text{C}$ ) during the 11 minutes of shaking and during the first 5 to 15 minutes of the 45 minute waiting period; it then slowly returns to room temperature. The importance of this temperature problem was alluded to by Peters and Van Slyke (6) several years ago. With the method reported,  $P_1$  and the reading preceding it almost always agree within 1 mm Hg (an error of only 0.0015 ml in setting the meniscus at the 0.5 ml mark could account for such a 1 mm pressure difference). The final 3 minute extraction period is included to be sure no nonabsorbable gas has been redissolved into the fluid phase whenever the volume of the gas phase has been reduced from 20 to 0.5 ml.

*Calculations.* The general equation for the gas content ( $V$ ) of a fluid sample analyzed with a manometric apparatus has been developed by Van Slyke, Neill and Stadie (7, 8). With the method reported, four factors in this equation become constants ( $a = 0.5$  ml,  $S = 30$  ml,  $A = 50$  ml and  $s = 25$  ml).  $P$ , the observed partial pressure (in millimeters of Hg) exerted by the gas when reduced to fixed volume at known temperature, is taken as  $(P_1 - P_2)$ . No "c" (blank analysis) correction is required (7). The original Van Slyke equation therefore simplifies to:

$$V = (P_1 - P_2) \times \frac{0.002632 i}{(1 + 0.00384 t)} (1 + 1.50 \alpha') \quad [1]$$

where  $V$  = nonabsorbable gas content (volumes per cent, STPD);  $i$  = experimental correction factor for reabsorption of gas from gas into fluid phase when volume of gas phase is reduced to 0.5 ml;  $t$  = temperature ( $^\circ \text{C}$ ) of wa-

ter jacket at time of reading  $P_1$ ;  $\alpha'$  = the ratio in which the nonabsorbable gas mixture being analyzed distributes itself between equal volumes of the gas and fluid phases when extraction is complete (Ostwald coefficient). The factor,  $i$ , is dependent upon several other factors, the most important of which is the solubility of the gas mixture being measured in the reaction mixture being used. Determinations of  $i$  for common gases have been described (7, 8);  $\alpha'$  can be calculated for any given gas mixture and temperature (8, 9).

#### EVALUATION OF METHOD ON EQUILIBRATED WATER

The accuracy and reproducibility of the method were tested by analyzing 46 successive distilled water samples equilibrated with compressed air at  $37^\circ \text{C}$ .

*Equilibration technique.* Several of the equilibration techniques investigated proved unacceptable. For example, when compressed air is simply bubbled through a column of water, the total pressure in each bubble exceeds  $P_B$  because of the hydrostatic pressure. The amount of this excess pressure varies with the depth and size of the bubble and is difficult to determine. The exact value of  $P_{\text{H}_2\text{O}}$  may also be uncertain, since there is no assurance that each bubble is 100 per cent saturated. While these considerations are usually unimportant in the equilibration of fluids with  $\text{CO}_2$  and  $\text{O}_2$  in their physiological ranges, they are extremely important for  $\text{N}_2$ . Every 1.2 mm error in water vapor tension or total gas pressure causes a 1 mm error in the usual calculation of inert gas tension. The increased inert gas constant resulting from this equilibration technique is sufficient to be detected by the method reported.

The modification of the spherical tonometer of Laue, described by Finley and co-workers (10), was finally adapted to obtain rapid, accurate equilibration of the water. The only change was the omission of the syringe used to withdraw the equilibrated fluid; the center arm of the distilling flask was instead occluded with a rubber stopper. A minimal continuous flow of saturated gas was maintained from a tank of compressed air. Distilled water, 35 to 50 ml (containing less than 0.2 parts per million NaCl) was added to the flask and swirled 30 minutes, the speed of rotation being adjusted so that the water formed a thin film more than half-way up the walls of the flask. The pressure within the flask never exceeded atmospheric by more than 0.5 cm  $\text{H}_2\text{O}$ . The

temperature of the water bath did not vary more than  $\pm 0.1^\circ \text{C}$ .

Equilibrium was found to be approached logarithmically by analyses of inert gas-free water kept in a tonometer for various periods of time. Ninety per cent of equilibrium content was reached in 3 minutes and 99 per cent in 6 minutes. To transfer the equilibrated water to an Ostwald-Van Slyke pipet, the rubber stopper was removed and the long upper stem of the pipet inserted to the bottom of the distilling flask. The pipet was thus filled in the inverted fashion previously described. It is interesting that no change in nonabsorbable gas content could be detected after a filled pipet had been allowed to stand at room temperature for as long as 8 hours.

*Predicted inert gas content.* Since the amount of gas physically dissolved in a fluid is the product of its partial pressure and its solubility at the temperature of the fluid, the predicted inert gas content of any sample was given by the following equation. In order to simplify the nomenclature, the usual subscript  $\text{N}_2$  (designating the  $\text{N}_2$ -A mixture that is the inert gas of air) will be omitted.

$$V_P = \frac{\alpha_{w37}}{760} \times F_I(P_B - 47.1) \times 100 \quad [2]$$

where  $V_P$  = predicted inert gas content (volumes per cent, STPD);  $\alpha_{w37}$  = volume of the inert gas of air (STP) absorbed by 1 vol of distilled water at  $37^\circ \text{C}$  when the dry pressure of the gas is 760 mm Hg (Bunsen coefficient);  $F_I$  = inert gas fraction in the compressed air used for equilibration; and  $P_B$  = ambient pressure (mm Hg) during equilibration. The inert gas of the compressed air was assumed to be 98.8 per cent  $\text{N}_2$  and 1.2 per cent A (11);  $\alpha_{w37}$  was calculated to be 0.01244, using this equation:

$$\alpha_{w37} = 0.988\alpha_{w\text{N}_{237}} + 0.012\alpha_{w\text{A}_{37}} \quad [3]$$

Values for  $\alpha_{w\text{N}_{237}}$  and  $\alpha_{w\text{A}_{37}}$  were obtained by interpolating values in standard tables (12-14). A study of the literature (4, 13-16), showed that the values for  $\text{N}_2$  solubility in standard tables represent  $\text{N}_2$  solubility alone, and not the solubility of the  $\text{N}_2$ -A mixture found as the inert gas of atmospheric air.  $F_I$  was checked with a Scholander gas analyzer (17), and always taken as 0.7903 (11).  $P_B$  varied from 733.6 to 749.4.

*Determined nonabsorbable gas content.* The nonabsorbable gas content,  $V_d$  (volumes per cent, STPD), of each water sample was measured with the method reported, using one of three extraction chambers. The nonabsorbable gas measured was presumably 97.5 per cent  $\text{N}_2$  and 2.5 per cent A, since A is over twice as soluble in water as  $\text{N}_2$ . In applying Equation 1,  $i$  was always taken to be 1.000 (4);  $\alpha'$  values were obtained from the usual equation (8, 9) after determining the appropriate Bunsen coefficients for a 97.5 per cent  $\text{N}_2$ -2.5 per cent A mixture in water (see above). This assumed that the solubility of nonabsorbable gas in the reaction mixture was the same as that in water. Since it is actually less than in water (7),  $\alpha'$  has in each case been overestimated by a small but definite constant amount. Temperatures of analyses varied between  $24.4$  and  $28.8^\circ \text{C}$ .

*Composition of nonabsorbable gas.* The question arises as to whether the "nonabsorbable gas" in the extraction chamber at the time of reading  $P_1$  was entirely  $\text{N}_2$  and A, in the ratio expected from their relative concentrations in air and solubilities in water. The composition of the gas has therefore been checked by mass spectrometer analysis. To collect the gas, the stopcock at the top of an extraction chamber was replaced with a high vacuum stopcock. Distilled water equilibrated with compressed air at  $37^\circ \text{C}$  was then analyzed in the usual fashion. After reading  $P_1$ , the nonabsorbable gas was ejected (through an all glass evacuated system) into a special mass spectrometer sampling tube equipped with a high vacuum stopcock. The nonabsorbable gas from four successive water analyses was collected in one sampling tube and found to have the composition shown in Table I. The sample was scanned from mass 2 to mass 200.

TABLE I  
Mass spectrometer analyses of nonabsorbable gas in Van Slyke extraction chamber at time of reading  $P_1$ \*

Specimen	$\text{N}_2$	A	$\text{CO}_2$	$\text{O}_2$	"Hydro-carbon"
	%	%	%	%	%
Equilibrated water	97.4	2.3	0.11		0.15
Equilibrated urine (lab worker)	98.0	1.9	0.04		0.03
Equilibrated urine (emphysema patient)	97.1	2.4	0.33	0.06	0.06

\* Results have been calculated on a water-free basis.

TABLE II

Comparison of predicted inert ( $V_p$ ) and determined nonabsorbable ( $V_d$ ) gas content of 46 successive distilled water samples equilibrated with air at 37° C

Extraction chamber	$V_p/V_d$	
	Mean	SD*
1 (n = 14)	0.9904	0.004
2 (n = 21)	0.9976	0.003
3 (n = 11)	0.9897	0.002

\* Standard deviation.

It is significant that 99.7 per cent of the nonabsorbable gas was  $N_2$  and A. There was no significant difference between the observed  $N_2$  to A ratio (97.4/2.3) and the predicted  $N_2$  to A ratio (97.5/2.5). There was no mass 12 peak to indicate that any of the " $N_2$ " measured was CO. The 0.15 per cent "hydrocarbon" has not been identified specifically.

*Discussion.* The ratio of  $V_p$  to  $V_d$  was determined for all 46 analyses. The results are summarized in Table II. The ratios consistently fall 0.2 to 1.0 per cent below unity, i.e., determined nonabsorbable gas contents regularly exceed predicted inert gas contents by 0.2 to 1.0 per cent. This constant "excess" nonabsorbable gas is at least partly due to the overestimation of  $\alpha'$  already described. It could conceivably also result from repeated setting of the fluid meniscus slightly above the 0.5 ml mark, incomplete absorption of  $CO_2$  and/or  $O_2$ , evolution of unknown gas during the analyses, or from some other cause not yet apparent. Regular setting of the fluid meniscus just above the 0.5 ml mark is possible, since one of us (F.J.K.) performed all analyses. Incomplete absorption of  $O_2$  or evolution of unknown gas during analyses seems unlikely in view of our mass spectrometer analysis. Incomplete absorption of  $CO_2$  remains a possibility.

The amount by which unity exceeds the ratio of  $V_p$  to  $V_d$  varies significantly with extraction chambers. The mean ratio of  $V_p$  to  $V_d$  with chamber 1 was 0.9904. The same mean ratio with chambers 2 and 3 was 0.9976 and 0.9897. When these results are used to compare chamber 2 with chamber 1 or 3,  $p$  values well under 0.01 are obtained. When chambers 1 and 3 are compared,  $p$  is between 0.2 and 0.3. This variation among extraction chambers is explainable by the fact that

standard chambers are calibrated at 0.5 ml with an accuracy of  $\pm 0.003$  ml. This is  $\pm 0.6$  per cent of the 0.5 ml, and could provide as much as a 1.2 per cent difference between any two chambers. Our three mean ratios are all within a range of 0.8 per cent.

To obtain maximum accuracy with our technique then, an average correction factor must be derived for each extraction chamber. For any chamber, this factor is obviously the mean ratio of  $V_p$  to  $V_d$ . For the chambers we have used, it is 0.9904, 0.9976 and 0.9897, respectively. It should be emphasized that the use of these factors in effect standardizes this application of our technique against accepted values for  $N_2$  and A solubility in water. These factors "compensate" for the possibilities mentioned above, as well as for slight inaccuracy in calibrating the 0.5 ml mark and any other constant factor we have overlooked.

The particular correction factors given here apply to a particular analyst's determination of a single gas mixture contained in a given liquid phase, namely 97.5 per cent  $N_2$ -2.5 per cent A in water. One may wish to apply the method reported to other gas mixtures in water, to the same gas mixture in other solvents, or to other gas mixtures in other solvents. If maximum accuracy is necessary, the individual analyst must determine other similar correction factors.

The reproducibility of our technique is assessed by examining the reproducibility of results with each of the chambers used, i.e., by examining the scatter of the ratios of  $V_p$  to  $V_d$  around the mean ratio of  $V_p$  to  $V_d$ . For chambers 1, 2 and 3, 1 standard deviation was 0.004 (0.4 per cent), 0.003 (0.3 per cent) and 0.002 (0.2 per cent), respectively. The improvement in reproducibility from the original group of analyses to the second, and from the second to the most recent, is to be noted. The standard deviation of 0.2 per cent is the maximum reproducibility achieved. A reasonable, reliable estimate of reproducibility is suggested as being the over-all standard deviation for all 46 water analyses, 0.3 per cent. This indicates that the mean corrected nonabsorbable gas content of a water sample analyzed in duplicate has a 95 per cent chance of being within 0.4 per cent of the true inert gas content (standard error = standard deviation of mean = 0.3 per cent/ $\sqrt{2}$  for duplicate analyses).

SOLUBILITY OF THE INERT GAS OF AIR IN URINE AT 37° C

This method has been studied with the particular aim of determining the inert gas content of urine samples. In the process, the solubilities of a 98.8 per cent N<sub>2</sub>-1.2 per cent A gas mixture in 50 urines at 37° C have been determined. Thirty-nine of the urines were from laboratory personnel, and 11 from patients with severe, diffuse, obstructive emphysema. A majority of samples was produced during slight or moderate water diuresis. The equilibration and transfer technique described for water was employed without modification. P<sub>B</sub> varied between 739.5 and 752.0 mm Hg. Each urine was analyzed in duplicate with the method reported. Analysis temperatures were between 23.3 and 32.0° C. Urine osmolality, specific gravity, and chloride concentration were never altered by the equilibration process. Mass spectrometer analyses of the nonabsorbable gas being measured (Table I) showed it to be 99.5 to 99.9 per cent N<sub>2</sub> and A.

In applying Equation 1, the water analyses values of *i* and  $\alpha'$  were again employed. Each nonabsorbable gas content was then multiplied by the water analysis correction factor for the chamber used, to give the corrected nonabsorbable or "determined inert" gas content for the analysis.

These analyses were performed by the same individual who performed the water analyses, using the same extraction chambers. The validity of using the same *i*,  $\alpha'$ , and chamber correction factors as in water analyses therefore depends on two assumptions: 1) that the nonabsorbable gas being measured is 97.5 per cent N<sub>2</sub>-2.5 per cent A, and 2) that  $\alpha'$  is not overestimated by a significantly different amount than that used in water analyses. Our mass spectrometer analyses support the first assumption. The second seems reasonable when one considers that a 20 per cent greater overestimation of  $\alpha'$  in urine analysis would affect determined inert gas content by only 0.2 per cent (see Equation 1).

The inert gas solubility for each analysis was obtained from this rearrangement of Equation 2. The subscript N<sub>2</sub> is again omitted.

$$\alpha_{u37} = \frac{V_{dcorr.} \times 760}{F_I(P_B - 47.1) \times 100} \quad [4]$$

where V<sub>dcorr.</sub>, the corrected nonabsorbable gas content, has been substituted for V<sub>p</sub>.  $\alpha_{u37}$  is the Bunsen coefficient of a 98.8 per cent N<sub>2</sub>-1.2 per cent A mixture for the urine sample at 37° C. The average difference between duplicate  $\alpha_{u37}$  determinations was 0.4 per cent. The average difference between duplicate water analyses was also 0.4 per cent. The mean  $\alpha_{u37}$  of duplicate analyses should therefore have a 95 per cent chance of being within 0.4 per cent of the true  $\alpha_{u37}$ .

The variation of the mean  $\alpha_{u37}$  of duplicate analyses with osmolality, chloride concentration

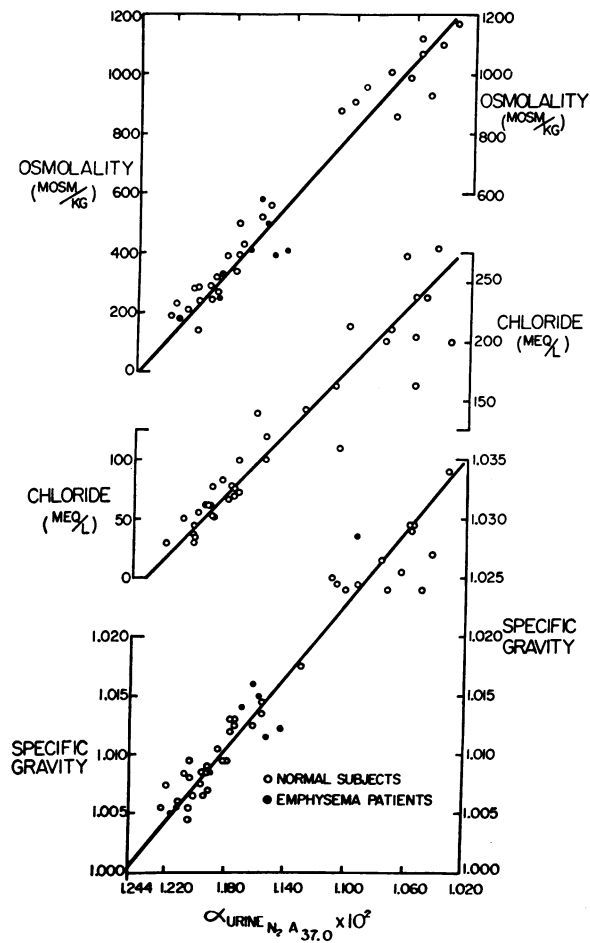


FIG. 1. THE ABSCISSA SHOWS THE SOLUBILITY OF THE INERT GAS OF AIR IN URINES OF NORMAL SUBJECTS AND EMPHYSEMATOUS PATIENTS AT 37° C. This solubility is expressed as volume gas (STP) per volume fluid per 760 mm Hg dry pressure of gas and has been plotted against osmolality, chloride concentration and specific gravity. See text for equations of regression lines and further analysis of relationships shown.

and specific gravity has been studied empirically. The results appear in Figure 1. There is no apparent difference between urines of normal subjects and those of emphysematous patients. The least squares regression lines shown have the following equations and correlation coefficients of 0.95, 0.89 and 0.94:

$$\alpha_{u_{37}} = 0.01246 - (1.76 \times 10^{-6}) \cdot (\text{mOsm per kg}) \quad [5]$$

$$\alpha_{u_{37}} = 0.01236 - (7.57 \times 10^{-6}) (\text{mEq Cl per L}) \quad [6]$$

$$\alpha_{u_{37}} = 0.01250 - (6.48 \times 10^{-2}) (\text{sp gr} - 1.0000). \quad [7]$$

In Equation 7, specific gravity is expressed to the nearest 0.005 unit. An analysis of variance of each line shows that none of the abscissa intercepts (0.01246, 0.01236 or 0.01250) is significantly different from 0.01244, the solubility of a 98.8 per cent N<sub>2</sub>-1.2 per cent A gas mixture in water. Further analysis indicates that  $\alpha_{u_{37}}$  can be predicted from one or more of these parameters with an accuracy of only  $\pm 2$  per cent (and then only 95 per cent of the time).

#### APPLICATION OF METHOD TO BLOOD

Application of the method reported to whole blood has recently been attempted. Preliminary results seem promising. A larger amount of caprylic alcohol (10 drops) is used to minimize foaming, and a less alkaline CO<sub>2</sub>-O<sub>2</sub> absorber is necessary. Twenty g of Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> and 2 g of sodium anthraquinone-B-sulfonate are dissolved in 100 ml of 1 N KOH and filtered rapidly through gauze. KOH, 0.5 N, does not absorb all the CO<sub>2</sub> in 25 ml of blood. KOH, 1.5 N or stronger, causes formation of precipitates that are extremely difficult to remove. Critical adjustment of the fluid meniscus to the 0.5 ml (or 50 ml) mark is more difficult with blood than when analyzing water or urine. Immediately after the blood sample has been introduced, the Hg in the extraction chamber is slowly raised and lowered several times. A precipitate forms as the sample and reagent are mixed. The slow mixing helps avoid this precipitate's forming in large lumps. For the same reason, the initial 1 to 2 minutes of mechanical shaking are done at low speeds.

#### SUMMARY

A method for determining the inert gas content of a fluid within 0.004 volumes per cent (0.4 per cent) is described. A Van Slyke manometric apparatus is used to measure the amount of gas other than CO<sub>2</sub> and O<sub>2</sub> in a 25 ml sample of the fluid. The method has been evaluated by analyzing distilled water equilibrated with air at 37° C. The solubility of the inert gas of air in various human urines has been determined at 37° C. Empirical relationships of solubility to osmolality, specific gravity, and chloride concentration are described. Preliminary results indicate that the method can be applied to blood.

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