THE EFFECTS OF HEPARIN ON THE RESULTS OF BLOOD GAS ANALYSIS

by

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INTRODUCTION

Arterial blood gas analysis has been performed for over 75 years. Prior to the development of modern electrodes the method of analysis was difficult and time consuming. Since the development of precision electrodes accurate and efficient methods of measuring pH, pCO_2, and pO_2 are now possible (Dowd, 1975; Goodwin & Schreiber, 1979).

The use of blood gas analysis in the management of cardiopulmonary disease and metabolic disorders has increased significantly in the last fifteen years. The measurement of pH, pCO_2, and pO_2 routinely guides the assessment of the patient's acid-base and cardiopulmonary status. With these data decisions are made concerning the appropriate therapy for critically ill patients. For example, serial arterial blood gas analyses are essential for the proper supportive care of the patient receiving ventilatory assistance (Shapiro, 1979).

Nurses in many settings, especially in the critical care units, are now directly involved with the use of arterial blood gas analysis in the assessment of the patient. The nurse's participation may include any of the following:

1) interpretation of the results in order to provide appropriate intervention for the patient;

2) timing of repeating arterial blood gas analysis when a change in the patient's status warrants the procedure;

3) actual collection of the blood sample; and

4) preparation of the sample for temporary storage prior to analysis.

The nurse's role is crucial in maximum utilization of this assessment tool. The nurse may be one of many links in the chain between the patient and the final blood gas results, but at some hospitals she is the only link between the patient and the person in the clinical laboratory who analyzes the blood sample. Any error made in the initial steps will affect the results of the analysis. Therefore, the nurse must be sure that the blood gas analysis procedure is performed properly to obtain accurate results.

There is considerable trust placed in the accuracy of the results of blood gas analysis. Yet there are many steps in the process where error may be introduced. The procedure of arterial blood gas analysis can be divided into two major areas:

1) blood collection and temporary storage until analysis;

2) analysis using a blood gas analyzer.

Under the first area some of the potential sources of error are:

1) type of syringe used (e.g., glass vs. plastic);

2) size of syringe used for sampling blood;

3) presence of air bubbles in the sample;

4) uneven distribution of anticoagulant in the blood sample prior to analysis;

5) changes in CO_2 and O_2 content by exposure of the sample to atmospheric air;

6) dilution from addition of anticoagulants;

7) effects of the gas composition of the anticoagulant on blood pH, pCO_2, pO_2, and $[HCO_3^-]$;

8) metabolism by red blood cells during storage (Siggaard-Andersen, 1961).

The second area is concerned with the assurance of quality control in the operation of the blood gas analyzer. A few examples of important variables influencing the proper functioning of the blood gas analyzer are precision in calibration, regulation of the temperature of the electrodes, adequate humidification, updated and uncontaminated reagents, and proper cleaning of the sample chambers (Moran, 1979). To prevent introduction of error all aspects of quality control must be carefully considered.

Statement of the Problem

The purpose of this research is to investigate the effects of altering various steps in the collection of the blood sample on the final results obtained from blood gas analysis. Heparin is routinely chosen as the anticoagulant

for arterial blood samples. Yet the methods of handling heparin vary from hospital to hospital as well as from nurse to nurse within the same hospital. Three of these methods will be studied in this research. They are:

1) choice of volume of heparin used in the sample;

2) use of a multidose vial of heparin with injection of air to facilitate withdrawal of heparin versus use of single dose ampule;

3) storage of the heparin in the refrigerator versus storage on a shelf at room temperature.

The first technique is not concerned with the concentration but rather with the volume of heparin and its dilutional effect on the results from blood gas analysis. The volume of heparin remaining in the syringe just prior to the collection of the blood sample depends on the nurse's previous instruction on the proper procedure for collecting an arterial blood gas specimen. A sampling of the literature concerning the technique of collection of blood gas samples is shown in Table 1. It is obvious that there is a difference in opinion on the proper handling of heparin for arterial blood gas samples. This research examined the effects of increasing volumes of heparin in the blood sample on the final analysis obtained from blood gas analysis.

The other two techniques that will be explored are concerned with factors that alter the pO_2 and pCO_2 of the heparin solution prior to its addition to the blood sample.

Table 1

Recommended Strength and Volume of Heparin and Volume of Blood for Blood Gas Samples

Author, Source, Year	Concentration of Heparin	Volume of Heparin	Volume of Blood
1. Barber & Budassi, Mosby's manual of emergency care, 1979	1000 u/ml	0.5 ml	3-5 ml
2. Barry, Emergency nursing, 1978	1000 u/ml (10 mg/ml)	Dead space in 5-10 ml syringe	0.5 to 2 to 4 ml depending on analyzer available
3. Bech-Jansen & Beck, Acta Anaesthesia Scandinavia, 1972	1000 IU/ml	0.1 ml	0.6 ml
4. Burgess, The nurse's guide to fluid and electrolyte balance, 1979	---	1 ml	8-10 ml
5. Cissik, et al., CVP, 1977	1000 USP units/ml	0.1 ml	3-4 ml
6. Cotes, Lung Function, 1975	5000 IU/ml	Dead space in a 10 ml glass syringe	7 ml
7. Holloway, Nursing the critically ill adult, 1979	1000 u/ml	Dead space	Dependent on laboratory
8. Hudak, Lohr, & Gallo, Critical Care Nursing, 1977	1000 u/ml	Dead space	5-10 ml
9. Kacmanek, Dimas, & Mack, The essentials of respiratory therapy, 1979	1000 u/ml	0.05 ml	1 ml
10. Lanros, Assessment and intervention in emergency nursing, 1978	1000 USP u/ml	Dead space in 10 ml syringe	---
11. Petty, Intensive and rehabilitative respiratory care, 1974	1000 USP u/ml	Dead space in 10 ml glass syringe	---

Frequently hospitals have available both or either multi-dose vials of heparin or 1 ml single dose, single use ampules of heparin. With the multidose vial the accepted method of removal of heparin with a syringe is to inject air into the vial to occupy the space of the heparin that is to be removed. All too often, though, a greater volume of air is injected into the vial than the volume of heparin withdrawn. This creates an increased pressure within the vial. It is of concern whether this increased pressure changes the gas composition of the heparin sufficiently to influence the blood gas values of the blood sample. With the single dose, 1 ml ampule of heparin, the top is snapped off the ampule, and the heparin drawn immediately into the syringe through a needle. In this study the gas composition of heparin from a single dose ampule was compared to the gas composition of heparin under increased pressure from a multidose vial.

In the third step heparin vials and ampules have been observed to be stored in the refrigerator as well as on the shelf at room temperature. The question of the influence of temperature on the gas composition of the heparin and eventually on the results of the blood gas analysis needs to be examined. Gases are more soluble in cold liquids than warm liquids. Thus it is probable that more CO_2 and O_2 will dissolve in cold heparin than warm. If cold heparin is then used when obtaining an arterial blood sample, the

pO_2 and pCO_2 may be increased. The goal of this research is to gather information concerning the effect, if any, that these factors have on the final results obtained from blood gas analysis.

Hypotheses

1. Increasing the ratio of volume of heparin to volume of blood sample will have the effects of:

    a) not changing the pH significantly,

    b) decreasing the pCO_2,

    c) increasing the pO_2

of the final results obtained from blood gas analysis. This will hold true regardless of the baseline blood gas composition of the blood sample.

2. Blood samples equilibrated with identical gas mixtures and containing heparin stored under increased pressure will have higher pO_2 and pCO_2 than those containing heparin stored under atmospheric pressure.

3. Blood samples equilibrated with identical gas mixtures and containing refrigerated heparin will have a higher pO_2 and pCO_2 than those containing heparin at room temperature.

Review of the Literature

To prevent an arterial blood sample from clotting in the syringe prior to analysis an anticoagulant is required.

Several different types of anticoagulants have been studied for this function such as heparin, trisodium citrate, potassium oxalate, tetrasodium, ethylenediamine-tetracetate (EDTA) and sodium fluoride (Yoshimura, 1935; Goodwin & Schreiber, 1979). However, heparin has gained the greatest popularity.

Heparin is an acid mucopolysaccharide whose anticoagulant properties were first described in 1916. By 1935 heparin became available for commercial use. The main industrial source of heparin since 1960 has been intestinal mucosa of pigs, sheep, and ox (Ballus, 1978). It is postulated that heparin originates in the mast cells of connective tissue. Heparin has been found in beef, hog and dog livers, beef muscle, adipose tissue and spleen, hog mucin and alimentary canal, skins of rats, fish plasma, sea clams, whales, and scales of carp (Ehrlich, 1973).

Heparin varies in chemical, physical and pharmacological properties depending on the source and methods of extraction. The British Pharmacopoeia, the International Standard, and the United States Pharmacopoeia all have established standards of preparation. The need for one reference preparation of heparin has been acknowledged for over 40 years, but the debate still continues as to which source of heparin, what assay, which substrate, and which reference preparation should be used (Brozovic, 1975; Gallus, 1978). The United States Pharmacopoeia (USP) unit has been defined as the quantity of heparin that will prevent 1 ml of citrated sheep

plasma from clotting for 1 hour after the addition of 0.2 ml of a 1:100 $CaCl_2$ solution. The anticoagulant potency of USP heparin is standardized so that 1 mg has an activity of at least 120 units (Gallus, 1978; Levine, 1975). From the review of pertinent literature it is clear that no single reference preparation of heparin has been consistently used in the research.

Although heparin is the anticoagulant of choice for blood gas samples, only a limited number of studies have been conducted to evaluate the effects of heparin on the results of the blood gas analysis. In 1935 Yoshimura investigated the effects of five different anticoagulants on the pH value of the blood. For each anticoagulant concentration tested two samples of arterial blood from rabbits were collected. One syringe contained a designated concentration of anticoagulant solution while the other had no anticoagulant and served as a control. The two blood samples were transferred into separate glass electrodes and the pH of the treated sample was measured first within 4-6 minutes of collection of the blood at 37°C.

The anticoagulant solution of varying concentrations was added in the proportion of 1:9 to the blood. In all control samples the dead space in the syringe and needle were filled with saline which was found to have no effect on the pH value of the blood. Heparin was dissolved in physiological saline solution. In four experiments two

concentrations of heparin (0.5% and 1%) were used. The pH decreased 0.005 units for both concentrations of heparin. The maximum concentration of heparin in the blood that did not cause a detectable change in pH was 0.1%.

In Yoshimura's experiments the activity of the heparin used was not specified. Therefore, it is difficult to compare these results with later studies.

In 1961 Siggaard-Andersen explored the changes in pH, pCO_2 and base excess resulting from sampling errors. The three most common errors were 1) loss of CO_2 by exposure, 2) dilution of the sample by anticoagulant, and 3) effects of the anticoagulant itself.

In his experiments the pH was measured by a thermostated capillary glass electrode at 38°C. The pCO_2 and base excess in mEq/L of blood were calculated from the actual pH value and from the pH value of the blood equilibrated with a known CO_2 tension. The different concentrations of anticoagulant were prepared from powdered heparin.

A blood sample was first stabilized with 0.1 mg/ml heparin to prevent coagulation. Then in four experiments adding powdered heparin to achieve concentrations of 2, 4, and 10 mg/ml, a nearly linear relationship between the concentration of heparin and the effect on pH, pCO_2, and base excess was observed. The calculated effect from the results of these experiments was that a concentration of heparin 1 mg/ml would cause a decrease in pH of 0.003 units, an

increase in pCO_2 of 0.1 mmHg, and a decrease in base excess of 0.2 mEq/L. These changes are considered minimal, and Siggaard-Andersen states that heparin in concentrations below 1 mg/ml will produce insignificant error.

Siggaard-Andersen also investigated the effect of 12% dilution with physiological saline in four experiments with plasma and eleven experiments with whole blood. The reported changes are summarized in Table 2.

Table 2

Mean Changes in Blood Gas Parameters of Plasma and Whole Blood After 12% Dilution with Physiological Saline

	Plasma	Whole Blood
pH	Increase 0.003	Increase 0.006
pCO_2	Decrease 14%	Decrease 16%
Bicarbonate	Decrease 13%	Decrease 15%
Base Excess	Decrease 3.5 mEq/L	Decrease 3.3 mEq/L

These results confirmed Yoshimura's study that small dilutions with physiological saline (10% to 12%) do not significantly affect the blood pH value. The actual bicarbonate concentration and pCO_2, however, decreased in direct proportion to the percentage of dilution, and the base excess declined about 0.3 mEq/L per percent dilution.

Bech-Jansen and Beck (1972) investigated the influence of heparin on the pH of arterial blood samples. Blood samples from 15 randomly selected anaesthetized patients were tested. A 20 ml glass syringe with 2,500 International Units (IU)/ml heparin in the dead space (0.1 ml) was used to collect blood from the femoral artery. This method provided a concentration of 12.5 IU of heparin per ml of blood which is considered near the minimum concentration necessary to prevent coagulation. From this main heparinized blood sample, 30 different samples (3 sets of 10 each) were withdrawn into 1 ml Mantoux plastic syringes in increments of volume beginning with 0.1 ml and ranging to 1.0 ml. The dead space of the Mantoux plastic syringe (0.1 ml) contained a different concentration for each set (5,000 IU/ml, 1000 IU/ml, and 100 IU/ml). The concentrations of 1000 IU/ml and 100 IU/ml were achieved by dilution of the standard solution of heparin 5,000 IU/ml with physiological saline. The graphed results of pH to blood volume in 0.1 ml increments showed the slope of the mean curve to reduce to a value of zero as the concentration of the heparin decreased. The authors found that a concentration of heparin of 100 IU/ml in the syringe dead space of 0.1 ml with a volume of 0.6 ml or greater is acceptable for clinical use in arterial blood sampling.

While engaged in research requiring the analysis of small blood samples, Bradley (1972) concluded that the decrease in measured pCO_2 due to dilution of the sample by

heparin was greater than had been reported by Siggaard-Andersen in 1961. Therefore, Bradley sought to reinvestigate the effect of dilution with heparin on the pCO_2 of the blood sample.

Using a bubble tonometer, heparinized blood was equilibrated with a certified gas mixture at a pCO_2 of 37 mmHg and pO_2 of 71 mmHg. In four experiments a sample of the equilibrated blood was withdrawn into a glass syringe containing a known volume of physiological saline as diluent. The percentages of dilution for the four experiments were 4.0, 6.6, 7.5, and 15.0. The results showed a greater decrease in pCO_2 than reported previously by Siggaard-Andersen. The author suggested that the discrepancy might have been due to the use of a pCO_2 electrode in his experiment compared to Siggaard-Andersen's use of the interpolation techniques.

Hansen and Simmons (1977) also studied the effects of heparin on the pH, pCO_2, and bicarbonate concentration of the blood. Initially the dead space volume of various syringes ranging in size from 1 ml to 10 ml was measured and found to range from 0.07 to 0.26 ml. Therefore it becomes apparent that the volume of heparin diluting the blood sample can vary even though the heparin may only occupy the dead space of a syringe.

In this experiment a 20 ml syringe with 0.2 ml of 1000 IU/ml heparin to stabilize the blood sample was filled with venous blood. The blood gas composition of this sample

served as the control values. Then six 3 ml plastic syringes with known volumes of 1000 IU/ml heparin were filled to the 3 ml mark with blood and analyzed. To obtain a sufficient number of samples this procedure was repeated on four different days in the same hospital. The results revealed that the pH of all samples varied less than 0.01 units, and the pCO_2 and bicarbonate concentration declined in nearly direct proportion to the percentage of heparin in the sample.

Hansen and Simmons postulated that since the pH of the blood sample remained stable during the dilution with heparin, the ratio of pCO_2 and bicarbonate will remain constant. Decreases in both pCO_2 and bicarbonate concentration will be directly in proportion to the percentage of dilution. From their data the authors developed a table for correction of the dilutional effect of heparin on the pCO_2 and bicarbonate concentration of a blood sample analyzed in vitro.

Cissik, Salustro, Patton and Louden (1977) were the first to explore the pH, pCO_2, and pO_2 measurements of the heparin used as an anticoagulant. The influence of known concentrations of heparin on the results of blood gas analyses of five patients was investigated as well. The heparin used in the experiment was 1000 USP units/ml made from intestinal mucosa. Each desired volume of heparin was drawn into a plastic syringe and allowed to warm to room temperature for twenty minutes with the cap of the syringe in place. Two IL pH/blood gas analyzers were used to measure the pH, pCO_2,

and pO_2 of the blood sample and heparin. In each case for blood a minimally heparinized sample was collected from the patient and placed on ice until analyzed. This original reading served as a control. In the next step a predetermined volume of heparin was drawn into a syringe; the syringe was filled with the blood of known blood gas composition and reanalyzed. Eleven different ratios of heparin to blood ranging from 0.05 ml heparin:1:0 ml blood to 1.0 ml heparin:1.0 ml blood were evaluated.

The results of the analyses of the blood sample with the varying volumes of heparin were as follows: 1) There was no change in pH, 2) The pO_2 increased linearly with increasing volume of heparin, and 3) There was a linear decrease in pCO_2, $[HCO_3^-]$, total CO_2, and base excess (BE) with increasing volume of heparin. Based on their results the authors suggest that the minimum ratio of heparin to blood be no more than 0.1 ml heparin:3-4 ml of blood.

The results of Cissik et al. on the analysis of 10 vials of heparin with the same lot number revealed: 1) the pO_2 ranged from 170 mmHg to 210 mmHg, 2) the pH ranged from 6.47 to 6.65 units, 3) the pCO_2 ranged from 3 to 7 mmHg, 4) $[HCO_3^-]$ ranged from 0.30 to 0.50 mEq/L, 5) total CO_2 from 0.50 to 0.70 mEq/L, and 6) base excess from -42 to -46 mEq/L. When compared to the normal arterial blood gas values, the heparin values showed an extremely elevated pO_2 with low pH, pCO_2, HCO_3, total CO_2 and base

excess values.

Scheinhorn (1978) investigated the effects of excessive amounts of heparin on the pH, pCO_2, and pO_2 of arterial blood samples. Twenty-five samples of arterial blood collected from 25 patients were withdrawn into 10 ml syringes containing heparin in a concentration of 1,000 USP units/ml in a dead space volume of 0.2 ml. All blood samples were kept on ice prior to analysis. After measurement of the initial values of pH, pCO_2, and pO_2, the samples were diluted in increments of 0.5 ml with heparin 1000 USP units/ml and reanalyzed.

Scheinhorn found that the pH did not change even with 50% dilution with heparin. The pCO_2, though, declined 1% for each 1% of dilution as predicted by Siggaard-Andersen (1961). The pO_2 increased slightly but not significantly.

Hamilton, Crockett, and Alpers (1978) were concerned that excessive volumes of heparin increased the value of pO_2 obtained from blood gas analysis and that these erroneous data could cause premature removal of supplemental oxygen from patients. In their study the blood gas values of heparin as well as blood samples with different volumes of heparin were analyzed using a Radiometer ABL fully automated blood gas analyzer.

Thirty ml of venous blood was collected from a young, healthy male each morning and stabilized with 0.1 ml of "mucous" sodium heparin (Allen and Hansbury heparin 1 ml

ampoule of 1000 u/ml). Aliquots of 6 ml of the heparinized sample were equilibrated with a gas mixture of 15.6% O_2, 6.3% CO_2, and 78.1% nitrogen for 25 minutes in an IL 237 Tonometer at 37° ± 0.2°C. The tonometered blood was transferred to a 10 ml plastic syringe and placed on ice for 30 minutes. In the next step a 5 ml glass luerlock syringe with a designated amount of heparin was filled to the 2 ml mark with the equilibrated blood and analyzed. The volumes of heparin used were 0.1, 0.2, 0.3, 0.4, 0.5 and 1 ml.

The results of this research are unclear. Changes in blood gas values were discussed in relation to "percentages of heparin concentration." Yet the authors did not specifically state how the percentages were calculated. Under the discussion of pO_2 changes it was stated that "a blood sample of 0.8 ml plus sufficient heparin to fill the dead space of the syringe" would produce a heparin concentration of 25%. It appears the authors used the ratio of volume of heparin to volume of blood sample to calculate the percentage. This is possible only by assuming that the authors used the dead space volume (0.21 ml) of the 5 ml syringes used in this research in their calculation. Yet in the discussion of the changes seen in the pCO_2 a heparin concentration of 15% was supposedly achieved by dead space plus 1.5 ml of blood. If the same method as reported above is used to calculate the percentage dilution, the value should be 7%, not 15%.

Bearing this in mind, the authors reported the results

as follows. There is an increase in the pO_2 of samples with the addition of heparin. The increase in pO_2 did not become statistically significant until the heparin concentration was greater than 15%. The pCO_2 decreased linearly as the concentration of heparin increased. A statistically significant decrease in pH was reported at heparin concentrations as low as 10%, but the authors did not believe that the pH fall was clinically significant until heparin concentrations reached 2.5%.

Hamilton, et al. (1978) also measured the pO_2, pCO_2, and pH of various brands of heparin available in Australia. The reported values are summarized in the Table 3. These are the mean values of five vials ± 1 SD of each type of heparin tested. The large difference between the gas composition of heparin and the tonometered blood in this experiment were not noted by the authors.

Goodwin and Schreiber (1979) studied the effects of excessive volumes of heparin on the results of analysis of blood gas samples. Arterial blood samples were collected from four patients. Five ml glass syringes with different concentrations of heparin in the dead space or in the dead space plus 0.5 ml were filled to 2 ml and analyzed on an IL413 blood gas analyzer. The concentrations of heparin were 1,000 IU/ml, 5,000 IU/ml, and 25,000 IU/ml. The four patients were reported as having relatively normal blood gas values.

Table 3

pH, pCO_2, and pO_2 of Various Brands of Heparin Available in Australia

	CSL (100u/ml) 5 ml ampoule	CSL (1000u/ml) 5 ml multidose vial	Allen & Hanbury (5000u/ml) 5 ml ampoule	Weddel (1000u/ml) 5 ml multi-dose vial	Evans (1000 u/ml) 5 ml ampoule
pO_2 (mmHg)	182.9 ± 6.5	188.7 ± 2.6	164.9 ± 10.7	223 ± 11.3	183.1 ± 4.8
pCO_2 (mmHg)	4.6 ± 0.2	7.4 ± 0.3	4.1 ± 0.4	4.3 ± 0.2	5.3 ± 0.1
pH	6.774 ± 0.025	6.588 ± 0.044	6.993 ± 0.014	6.796 ± 0.046	6.555 ± 0.042

The results were reported as the pH was "within the accuracy of our machine" for heparin 1000 IU/ml and heparin 5000 IU/ml. When heparin 25,000 IU/ml was used the pH was described as excessively low. All pO_2 values were within the range of accuracy of the analyzers used. The pCO_2 decreased 16% with a 20% dilution of heparin in concentrations of 1000 IU/ml or 5000 IU/ml. When heparin in concentration of 25,000 IU/ml was used, the pCO_2 declined only 4%.

In summary, there remains some controversy about the effects of heparin on the results of blood gas analysis. The majority of research has shown that heparin does have a dilutional effect. The influence is most noticeable in the pCO_2 measurement which usually decreases linearly in proportion to the percentage of heparin added (Cissik, et al., 1977; Hamilton, et al., 1978; Scheinhorn, 1978; Siggaard-Andersen, 1961). Goodwin and Schreiber (1979) reported a greater decrease in pCO_2 than that reported by Siggaard-Andersen (1961).

The pH value is generally considered to be unaffected by the addition of heparin to the blood sample (Bech-Jansen & Beck, 1972; Cissik, et al., 1977; Hansen & Simmons, 1977; Scheinhorn, 1978). Two authors, though, have reported a significant decrease in pH values. Goodwin and Schreiber (1979) found a markedly reduced pH when high concentrations of heparin (25,000 IU/ml) were used. Hamilton, et al. (1978)

also reported a decrease in pH with addition of heparin. Their results are not easily explained since the analysis of his Australian heparin was almost identical to the American heparin analyzed by Cissik, et al. (1977).

Changes in pO_2 have not been extensively investigated. Three authors have reported a slight but not significant increase in the pO_2 (Goodwin & Schreiber, 1979; Hamilton, et al., 1978; Scheinhorn, 1978). Cissik, et al. (1977) found a linear increase in pO_2 values with increased heparin volume.

Cissik, et al. (1977) and Hamilton, et al. (1978) reported almost identical results from heparin analysis, even though the heparin from one study was produced in America and the other in Australia.

In only two research projects were the original blood gas values controlled by equilibrating the blood sample with a known gas mixture. Even in these cases the mixtures were not of normal arterial blood gas composition. Normal arterial blood gas values range from pH 7.35-7.45, pO_2 80-100 mmHg, pCO_2 35-48 mmHg (Davenport, 1974). Bradley (1972) used a gas mixture containing a pO_2 of 71 mmHg which is below the normal range of 80 to 100 mmHg and a pCO_2 of 37 mmHg. Hamilton, et al. (1978) used tonometered blood which had a pO_2 of 118 mmHg and a pCO_2 of 48 mmHg. Both of these latter values are greater than the normal range obtained from arterial blood gas analysis.

In this investigator's literature review no research was found that reported the effect of repeated use of multidose vials of heparin versus single-use ampules of heparin as a source of anticoagulant for arterial blood gas analysis. In the clinical setting both are commonly used.

Another practice observed in the clinical setting is the refrigeration between uses of the stock multidose vial of heparin. Cissik, et al. (1977) refrigerated their stock supply of multidose vials of heparin but allowed the heparin to warm to room temperature prior to mixing with the blood sample. Again no research was found that reported the effects of refrigerated heparin on the final results of blood gas analysis.

Chapter II

METHODS

Design

There were four independent variables: 1) volume of heparin remaining in the syringe prior to filling with the blood sample, 2) temperature of the heparin, room temperature versus refrigerated, 3) pressure within the multidose vial of heparin, as received from the manufacturer versus increased after air injection into the vial, and 4) the initial values of pO_2, pCO_2, and pH of the blood sample prior to the addition of heparin. The dependent variables were the measured values of pO_2, pCO_2, and pH of the blood sample.

Subject

The same healthy adult female volunteer with no history of chronic illness donated the venous blood used in this research. The blood was collected on the day of testing.

Equipment

All blood samples and heparin were analyzed on the Radiometer BMS3 MK2 blood-gas analyzer. The Dynex Gas/Liquid Equilibration System was used to equilibrate all blood samples with a gas mixture prior to analysis. The gas mixture

for that day's experiment was connected to the tonometer and its flow adjusted and maintained between 50 and 60 ml/min. throughout the day. Three gas mixtures were chosen for this research: 1) O_2 10%, CO_2 5%, Nitrogen 85%, 2) O_2 5%, CO_2 10%, Nitrogen 85%, and 3) Air 97%, CO_2 3%. Each machine was allowed to warm for a minimum of two hours prior to use.

All equipment was calibrated and operated according to their respective instruction manuals. A reliability study was completed to familiarize the investigator with the equipment and the techniques necessary for use of the tonometer and the blood gas analyzer.

Preparation

While the equipment warmed, numerous preparatory steps were completed. The plunger and barrel of all glass syringes were lubricated with stopcock grease to reduce the possibility of air entry or exit around the barrel.

To assure proper functioning of the blood gas analyzer, the calibration of the pO_2 and pCO_2 electrodes was assessed, and when necessary, the membranes of the electrodes were changed.

Treatment of the Blood

On each day of analysis 20 ml of venous blood was collected into a 20 ml plastic syringe with sodium heparin (Panheprin^R 1000 USP units/ml) in the dead space. After thorough mixing by rocking the syringe gently, this blood was transferred into a greased 20 ml glass syringe with a

16 gauge needle. All excess air was expelled through the needle, and the needle inserted into a rubber stopper to prevent entry of air. The syringe was stored in the refrigerator except when temporarily removed to place blood samples in the blood cups of the tonometer. Each blood sample was equilibrated individually for 25 to 30 minutes. The volume of blood equilibrated each time was between 2.5 and 3.0 ml. The first and last control samples without additional heparin were withdrawn directly from the tonometer into the 2 ml test glass syringe and analyzed. The remaining samples which were mixed with a designated volume of heparin were first drawn up into a five ml glass sryinge through its attached 3-way stopcock with the sideport closed. This stopcock was necessary for the transfer of the tonometered blood into the two ml glass syringe. After the tonometered blood was withdrawn into a 5 ml syringe, the control on the stopcock was adjusted to open only the ports to the 5 ml syringe and the side port. Excess tonometered air and a small amount of blood to fill the side port were expelled from the 5 ml syringe. The two ml glass syringe with its heparin was immediately attached to this side port. Gently the blood in the 5 ml glass syringe was transferred to the 2 ml glass syringe to the 2 ml mark. This transfer was performed by exerting equal pressure against the plunger of the 5 ml syringe while pulling on the plunger of the 2 ml syringe. Each sample with heparin was mixed by

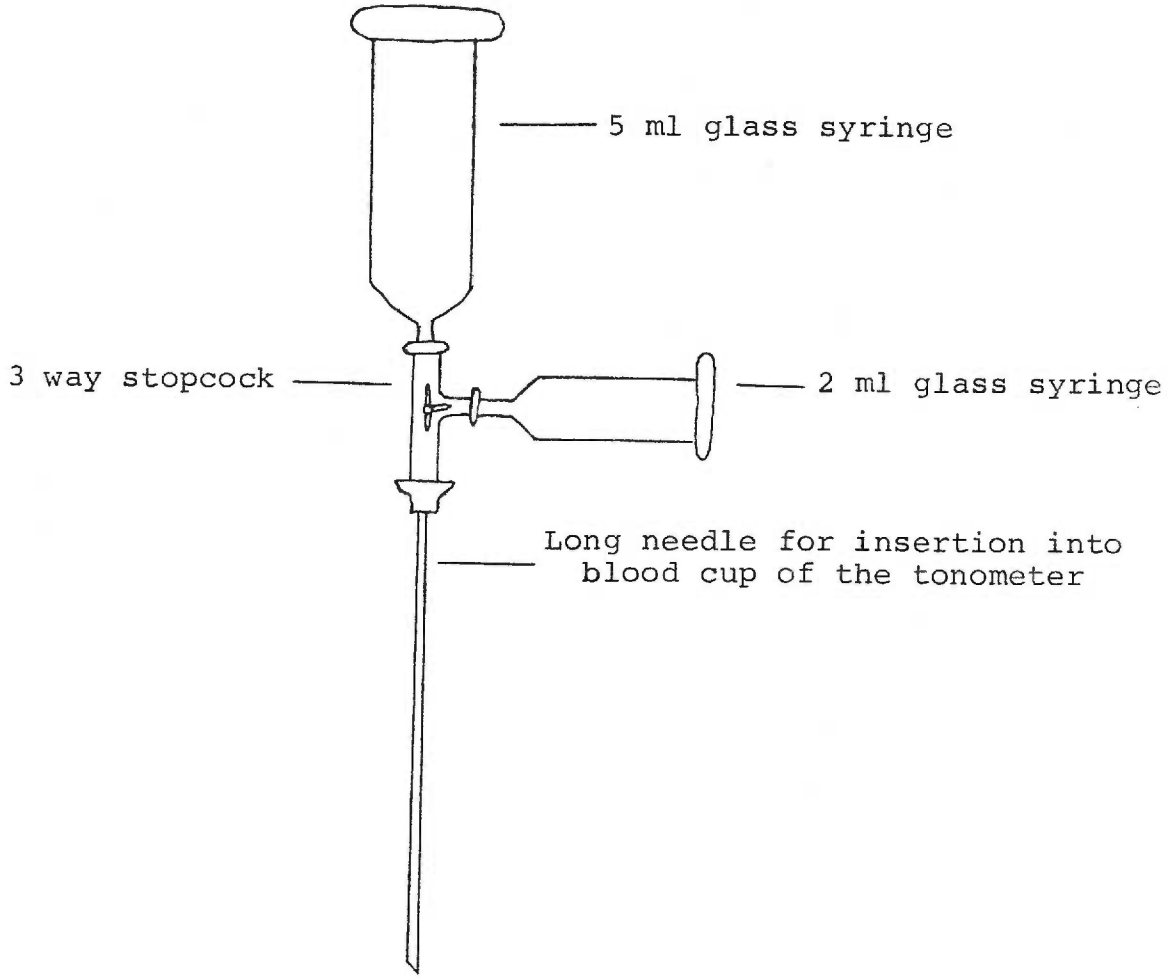


Figure 1. Syringe and stopcock assembly used to transfer samples

gentle rocking of the syringe for two to four minutes prior to analysis. At least 10 analyses were performed on each sample. The mean of the three closest readings for each gas value was calculated.

The same two ml glass syringe was used for all blood samples. After each sample was analyzed, this syringe was rinsed with water, dried with Q-tips and greased with stopcock grease to prepare for the next sample.

Treatment of the Heparin

Twenty-five multidose vials (10 ml each) of Panheprin^{R} (sodium heparin) from the same lot (23663 Af, expiration date 12-1-83) were purchased for use in this research. For each of the three gas mixtures four different experiments were conducted on four separate days. The heparin was treated in the following way:

Room temperature heparin no air injected into the vial	Refrigerated heparin no air injected into the vial
Room temperature heparin 2 ml of air injected into the vial	Refrigerated heparin 2 ml of air injected into the vial

The vials of room temperature heparin were stored on a cart in the laboratory room. Beginning the day of purchase, a group of heparin vials were stored in a refrigerator (average temperature 4°C) in the laboratory. The refrigerated heparin used in subsequent experiments was stored in the refrigerator for a minimum of 48 hours. On the day of the experiment testing the effects of injection of air into the multidose vial, two ml of air was injected into the multidose vial at the beginning of the day. The vial was left undisturbed on the counter or in the refrigerator for at least three hours to allow pressure equilibration within the vial. The volume of two ml was selected to exaggerate the effect of air injection into multidose vials of heparin.

In each experiment the top of the multidose vial was

removed with minimum of shaking using a pair of pliers. The heparin was immediately withdrawn through a 16 gauge needle into a 10 ml greased glass syringe with a three-way stopcock. All air was expelled as quickly as possible through the 16 gauge needle and the needle inserted into a rubber stopper. The air in the side port of the stopcock was expelled, and the side port filled with heparin before a metal cap was attached. This 10 ml syringe served as the main supply of heparin for the day. If the heparin was to be at room temperature, the syringe was allowed to remain on the counter during the day. If the heparin was to be refrigerated, the syringe was returned to the refrigerator where it remained except for brief intervals when the syringe was removed to transfer heparin into the 2 ml glass syringe.

The transfer of heparin from the 10 ml syringe into the 2 ml syringe was conducted in the same manner for all experiments. The stopcock was turned to allow heparin to flow only from the 10 ml syringe to the side port. A small amount of heparin was expelled to flush the dead space of the side port of the stopcock. The two ml syringe was then quickly attached to the side port and 0.2 ml to 0.3 ml of heparin transferred into the two ml syringe. Air bubbles and all excess heparin except that in the dead space was expelled. Again after flushing the side port of the stopcock with heparin, the 2 ml syringe was attached and a slightly larger volume than designated was transferred into the 2 ml syringe.

Transfer was performed as with the blood samples with equal pressure applied to the plungers of each of the two syringes. After the transfer of heparin was completed, the two ml syringe was removed and the side port of the stopcock flushed with heparin. The metal cap was attached to the side port and the control of the stopcock turned halfway between all openings to prevent contamination. A 26 gauge ½" needle was attached to the two ml syringe and the extra volume of heparin beyond the designated volume was ejected through the needle. The needle was inserted into a rubber stopper to prevent air entry.

In all experiments the heparin was transferred into the two ml syringe just before withdrawing the blood sample from the tonometer. The time lapse between the transfer of the heparin into the two ml glass syringe and the transfer of the blood sample into the 2 ml syringe with the heparin was less than five minutes.

Each day at the end of experiment the heparin remaining in the 10 ml glass syringe was analyzed. As with the blood samples, the mean of the three closest readings for each value was calculated.

Determination of the Dead Space Volume of the 2 ml Syringe

After all the data was collected, the volume of the dead space was measured. The 2 ml glass syringe was weighed on a Sartorius 2464 Analytical Balance. This weight was

found to be 11.1008 gm. Then the dead space of the syringe was filled with water and the syringe weighed again. The weight of the syringe with water was 11.1444 gm. Therefore, the weight of the water in the dead space was 0.0436 gm. The temperature of the water was 23.7°C. The density of water at 23.7°C is 0.9774 gm/mL (Weast & Selby, 1967). The calculated volume of the dead space then was 0.0437 ml. The dead space volume for the 2 ml syringe used in this study then was 0.04 ml.

Chapter III

RESULTS

The results of this research are discussed under the following categories: 1) general changes in the blood gas composition observed with the different methods of handling the heparin and with the addition of increasing volumes of heparin, 2) effect on blood gas composition when heparin fills only the dead space of the syringe, and 3) analysis of the heparin itself.

The raw data were analyzed in the following way. Two control samples without additional heparin were analyzed for each set of samples. The values obtained were then averaged and served as mean control values. The differences between mean control values and the values obtained from serial addition of heparin are reported in Tables 4 through 7.

A summary of the data organized according to method of handling the heparin and addition of increasing volumes of heparin appears in Appendix A.

General Changes

pH

There was no consistent pattern of change reported for pH (Figure 2). Changing the temperature of the heparin,

volume of heparin added to the blood sample or the pressure within the multidose vial did not produce a consistent pattern of change in the pH values. The differences from the mean control values ranged from -0.023 units to 0.026 units (Table 4).

pCO_2

In all experiments the pCO_2 values decreased in a linear or near linear pattern as the volume of heparin added to the blood sample increased (Figure 3 and Appendix A). Changing the temperature of the heparin or the pressure within the multidose vial did not affect this pattern.

The magnitude of change in pCO_2 was related to the fractional concentration of CO_2 of the gas mixture used to equilibrate the blood sample. The greater the fractional concentration of CO_2 in the equilibrating gas mixture, the larger the change observed (Table 5).

Increasing the pressure within the vial of room temperature heparin produced a greater change in pCO_2 values than that obtained with heparin at room temperature without air injection in all but two cases. There was no apparent pattern present between the samples using refrigerated heparin with and without air injection. When air was not injected into the multidose vial, there was no regular pattern of difference in the results between samples whether the heparin was refrigerated or at room temperature (Table 5).

pO_2

The change in pO_2 values was most closely related to the fractional concentration of O_2 in the equilibrating gas mixture (Figure 4). The pO_2 values obtained using the gas mixture of lowest O_2 concentration ($O_2$5%, $CO_2$10%, $N_2$85%) varied from -2.05 to 1.38 mmHg. When the fractional concentration of O_2 of the equilibrating gas mixture was increased, there was a greater change in the pO_2 values obtained. The largest changes were observed when equilibrating the blood sample with the gas mixture of highest O_2 concentration (Table 6).

When altering the temperature of the heparin or the pressure within the vial, there was no consistent change in pO_2 observed except with the gas mixture of highest O_2 concentration. In that case when 0.6 ml of heparin was added, there was an increase in differences from room temperature heparin without air injection to refrigerated heparin with 2cc of air injection (Table 6). Further, when refrigerated heparin with 2cc of air injected was used in volumes of 0.2ml or greater, the change in pO_2 was greater than with other methods of handling heparin.

There was no predictable pattern observed by increasing the volume of heparin except when equilibrating the blood sample with the gas mixture containing the highest O_2 concentration. With this gas mixture the changes in pO_2 generally increased as the volume of heparin in the blood sample increased.

Bicarbonate Concentration [HCO_3^-]

The bicarbonate concentration decreased when the volume of heparin was increased (Figure 5). The pattern of difference between mean control and volume of heparin was the same as that found for changes in pCO_2 (Table 7).

Effect of Heparin Filling the Dead Space of the Syringe

pH

When heparin filled only the dead space of the syringe, the change in pH for the three gas mixtures regardless of the method used to handle the heparin ranged from -0.004 to 0.004 units except in 3 of the 12 cases. The changes in these 3 samples were -0.008 units, -0.014 units and +0.026 units (Table 4).

pCO_2

The changes in pCO_2 after the addition of heparin in the dead space of the syringe ranged from 0 to 5.00 mmHg. Ten of the twelve samples changed less than 2.05 mmHg. The two largest changes were observed using refrigerated heparin with and without air injection into the vial (Table 5).

pO_2

The changes in pO_2 found when heparin filled only the dead space was less than 1.45 mmHg except in two instances. In these two cases the change was 4.2 mmHg and 4.5 mmHg (Table 6).

Bicarbonate Concentration [HCO_3^-]

The difference in [HCO_3^-] between mean control samples and samples with heparin filling only the dead space ranged from -0.4 to 2.0 mEq/L. No particular pattern emerged with different methods of handling heparin (Table 7, Figure 5).

Analysis of the Heparin Itself

The mean pH values of the heparin varied only from 6.390 to 6.543 units with the different methods of handling (Table 8). The pCO_2 of the heparin was 2.0 mmHg or less and remained unchanged regardless of the method used in handling the heparin. However, the mean pO_2 of heparin changed most when the vial of heparin was refrigerated. A mean increase of 50 mmHg was found when the heparin vials were refrigerated without air injection. Injecting 2 cc of air into the multidose vial of heparin at room temperature produced a mean increase in pO_2 of 10 mmHg. No increase in mean pO_2 was observed when air was injected into the refrigerated vial of heparin. However, there was more variability in the pO_2 values of these samples than with other samples (Table 8).

Table 4

pH Differences From Mean Control in Units

	Room Temperature Heparin With No Air Injection	Room Temperature Heparin With 2cc Air Injection	Refrigerated Heparin With No Air Injection	Refrigerated Heparin With 2 cc Air Injection
Air 97%, CO_2 3%				
$\bar{x}$ control - DS	-0.003	0.002	-0.004	0.026
$\bar{x}$ control - 0.2 ml	-0.005	-0.001	-0.014	0.016
$\bar{x}$ control - 0.4 ml	-0.008	-0.007	-0.014	0.016
$\bar{x}$ control - 0.6 ml	-0.010	-0.011	-0.006	0.006
O_2 10%, CO_2 5%, N_2 85%				
$\bar{x}$ control - DS	-0.003	0.004	0.002	-0.002
$\bar{x}$ control - 0.2 ml	-0.001	-0.023	-0.016	-0.014
$\bar{x}$ control - 0.4 ml	0.004	-0.017	-0.012	-0.004
$\bar{x}$ control - 0.6 ml	-0.004	-0.023	-0.022	-0.002
O_2 5%, CO_2 10%, N_2 85%				
$\bar{x}$ control - DS	-0.014	-0.008	-0.002	0.000
$\bar{x}$ control - 0.2 ml	-0.009	-0.010	-0.007	-0.008
$\bar{x}$ control - 0.4 ml	-0.012	-0.014	-0.002	-0.010
$\bar{x}$ control - 0.6 ml	-0.004	-0.006	0.007	-0.014

Table 5

pCO_2 Differences from Mean Control in mmHg

	Room Temperature Heparin With No Air Injection	Room Temperature Heparin With 2cc Air Injection	Refrigerated Heparin With No Air Injection	Refrigerated Heparin With 2 cc Air Injection
Air 97%, CO_2 3%				
$\bar{x}$ control - DS	0.5	0.0	0.4	1.2
$\bar{x}$ control - 0.2 ml	3.0	3.8	3.3	3.6
$\bar{x}$ control - 0.4 ml	4.8	5.1	5.6	5.8
$\bar{x}$ control - 0.6 ml	6.8	7.5	7.7	7.6
O_2 10%, CO_2 5%, N_2 85%				
$\bar{x}$ control - DS	1.2	2.0	0.3	0.2
$\bar{x}$ control - 0.2 ml	4.6	4.8	4.9	3.6
$\bar{x}$ control - 0.4 ml	9.2	9.8	8.6	8.6
$\bar{x}$ control - 0.6 ml	12.6	13.6	12.9	12.4
O_2 5%, CO_2 10%, N_2 85%				
$\bar{x}$ control - DS	1.3	2.0	5.0	3.3
$\bar{x}$ control - 0.2 ml	9.6	10.5	11.8	11.9
$\bar{x}$ control - 0.4 ml	18.4	18.3	18.2	19.2
$\bar{x}$ control - 0.6 ml	24.6	25.5	25.2	27.4

Table 6

pO_2 Differences from Mean Control in mmHg

	Room Temperature Heparin With No Air Injection	Room Temperature Heparin With 2cc Air Injection	Refrigerated Heparin With No Air Injection	Refrigerated Heparin With 2 cc Air Injection
Air 97%, CO_2 3%				
$\bar{x}$ control - DS	1.0	1.1	4.5	-1.0
$\bar{x}$ control - 0.2 ml	-1.4	-1.0	-0.7	-10.6
$\bar{x}$ control - 0.4 ml	-9.0	-12.1	-8.2	-22.1
$\bar{x}$ control - 0.6 ml	-11.3	-17.9	-21.8	-26.6
O_2 10%, CO_2 5%, N_2 85%				
$\bar{x}$ control - DS	1.4	4.2	1.4	-0.8
$\bar{x}$ control - 0.2 ml	-2.0	-1.3	-2.0	-3.6
$\bar{x}$ control - 0.4 ml	-1.5	-2.2	-3.4	-5.9
$\bar{x}$ control - 0.6 ml	-7.6	-0.5	-7.6	-5.9
O_2 5%, CO_2 10%, N_2 85%				
$\bar{x}$ control - DS	1.2	-0.8	-0.1	0.6
$\bar{x}$ control - 0.2 ml	-0.4	-1.1	1.2	-1.5
$\bar{x}$ control - 0.4 ml	1.4	-1.8	0.3	-0.9
$\bar{x}$ control - 0.6 ml	0.2	-2.0	-1.1	-0.4

Table 7

Bicarbonate Concentration [HCO_3^-]

Differences from Mean Control in mEq/L

	Room Temperature Heparin With No Air Injection	Room Temperature Heparin With 2cc Air Injection	Refrigerated Heparin With No Air Injection	Refrigerated Heparin With 2 cc Air Injection
Air 97%, CO_2 3%				
$\bar{x}$ control - DS	0.3	0.1	0.2	2.0
$\bar{x}$ control - 0.2 ml	2.3	2.8	2.3	3.5
$\bar{x}$ control - 0.4 ml	4.2	4.0	4.4	5.1
$\bar{x}$ control - 0.6 ml	5.7	5.9	6.4	6.4
O_2 10%, CO_2 5%, N_2 85%				
$\bar{x}$ control - DS	0.6	1.3	0.3	0.1
$\bar{x}$ control - 0.2 ml	2.7	1.8	2.1	1.6
$\bar{x}$ control - 0.4 ml	5.6	4.9	4.6	5.1
$\bar{x}$ control - 0.6 ml	7.4	7.0	6.8	7.4
O_2 5%, CO_2 10%, N_2 85%				
$\bar{x}$ control - DS	-0.4	0.3	1.8	1.3
$\bar{x}$ control - 0.2 ml	3.1	3.3	4.1	4.0
$\bar{x}$ control - 0.4 ml	6.2	6.1	7.0	6.7
$\bar{x}$ control - 0.6 ml	8.9	9.1	9.8	9.6

Table 8

Analysis of the Heparin

Room Temperature Heparin with No Air Injection

	pH	pCO_2	pO_2
	6.443	1.2	185.7
	6.481	2.0	189.9
	6.434	1.5	187.1
$\bar{x}$	6.453	1.5	187.6
±SD	0.025	0.4	2.1

Room Temperature Heparin with 2 cc of Air Injection

	pH	pCO_2	pO_2
	6.515	1.5	200.0
	6.392	1.5	195.3
	6.407	1.4	196.9
$\bar{x}$	6.438	1.4	197.4
±SD	0.067	0.1	2.4

Refrigerated Heparin with No Air Injection

	pH	pCO_2	pO_2
	6.424	1.4	237.1
	6.342	0.9	237.9
	6.403	1.7	238.8
$\bar{x}$	6.390	1.3	237.9
±SD	0.043	0.4	0.9

Refrigerated Heparin with 2 cc of Air Injection

	pH	pCO_2	pO_2
	6.573	1.7	232.9
	6.584	1.4	209.1
	6.471	1.5	269.3
$\bar{x}$	6.543	1.5	237.1
±SD	0.062	0.2	30.3

Figure 2

pH Versus Volume of Heparin Added to the Blood Sample

A represents the results obtained when the blood sample was equilibrated with the gas mixture: 3% CO_2 in air.

B represents the results obtained when the blood sample was equilibrated with the gas mixture: O_2 10%, CO_2 5%, N_2 85%.

C represents the results obtained when the blood sample was equilibrated with the gas mixture: O_2 5%, CO_2 10%, N_2 85%.

The mean control values are plotted on the zero point of the abscissa.

D.S. represents the dead space volume of the 2 ml glass syringe.

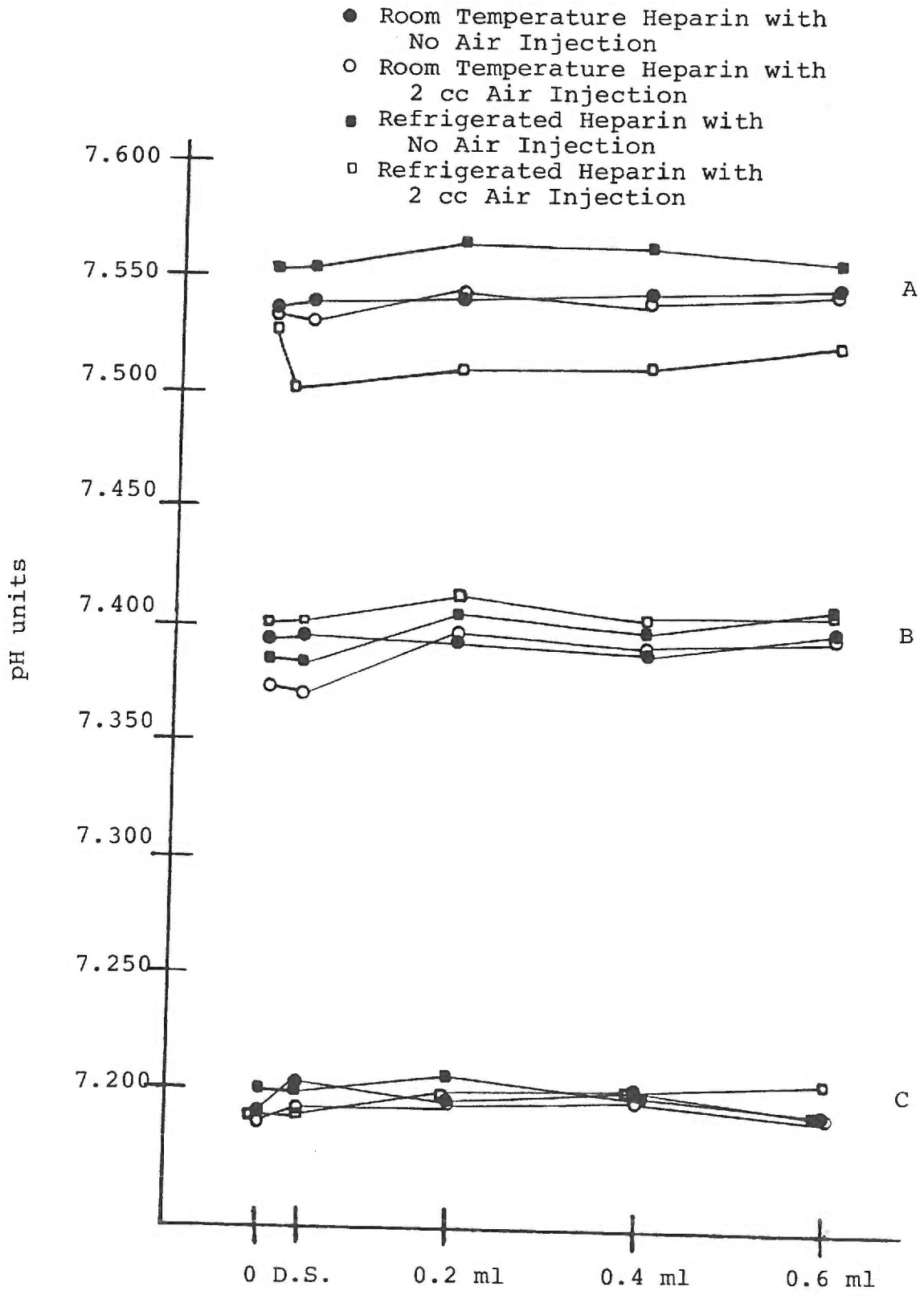

Room Temperature Heparin with No Air Injection
Room Temperature Heparin with 2 cc Air Injection
Refrigerated Heparin with No Air Injection
Refrigerated Heparin with 2 cc Air Injection
7.600
7.550
7.500
7.450
7.400
7.350
7.300
7.250
7.200
pH units
A
B
C
0 D.S.
0.2 ml
0.4 ml
0.6 ml
Volume of Heparin Added to the Blood Sample

Figure 3

pCO_2 Versus Volume of Heparin Added to the Blood Sample

A represents the results obtained when the blood sample was equilibrated with the gas mixture: 3% CO_2 in air.

B represents the results obtained when the blood sample was equilibrated with the gas mixture: O_2 10%, CO_2 5%, N_2 85%.

C represents the results obtained when the blood sample was equilibrated with the gas mixture: O_2 5%, CO_2 10%, N_2 85%.

The mean control values are plotted on the zero point of the absicissa.

D.S. represents the dead space volume of the 2 ml glass syringe.

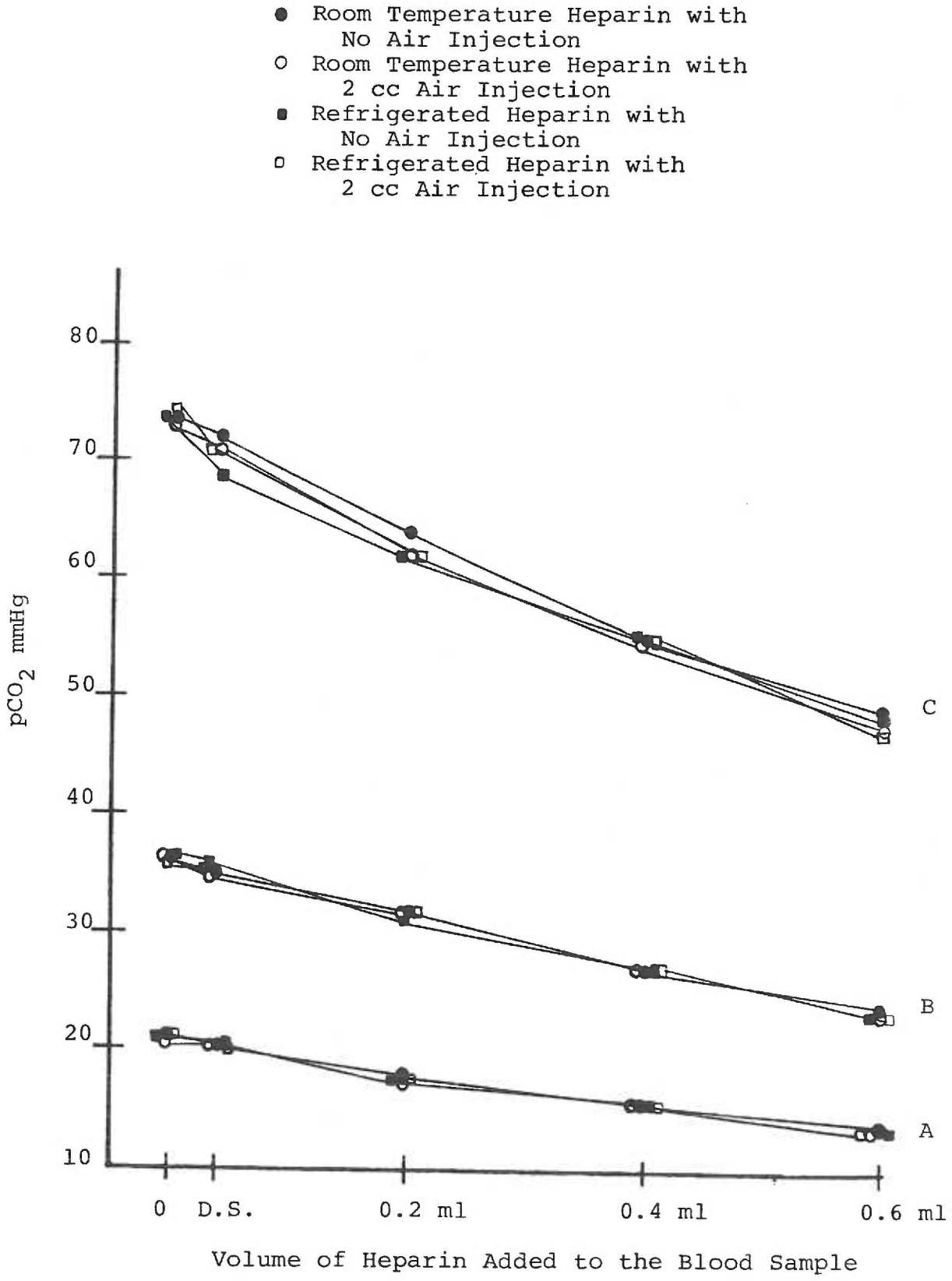

● Room Temperature Heparin with No Air Injection
○ Room Temperature Heparin with 2 cc Air Injection
■ Refrigerated Heparin with No Air Injection
□ Refrigerated Heparin with 2 cc Air Injection
80
70
60
50
40
30
20
10
pCO2 mmHg
C
B
A
0 D.S.
0.2 ml
0.4 ml
0.6 ml
Volume of Heparin Added to the Blood Sample

Figure 4

pO_2 Versus Volume of Heparin Added to the Blood Sample

A represents the results obtained when the blood sample was equilibrated with the gas mixture: 3% CO_2 in air.

B represents the results obtained when the blood sample was equilibrated with the gas mixture: O_2 10%, CO_2 5%, N_2 85%.

C represents the results obtained when the blood sample was equilibrated with the gas mixture: O_2 5%, CO_2 10%, N_2 85%.

The mean control values are plotted on the zero point of the abscissa.

D.S. represents the dead space volume of the 2 ml glass syringe.

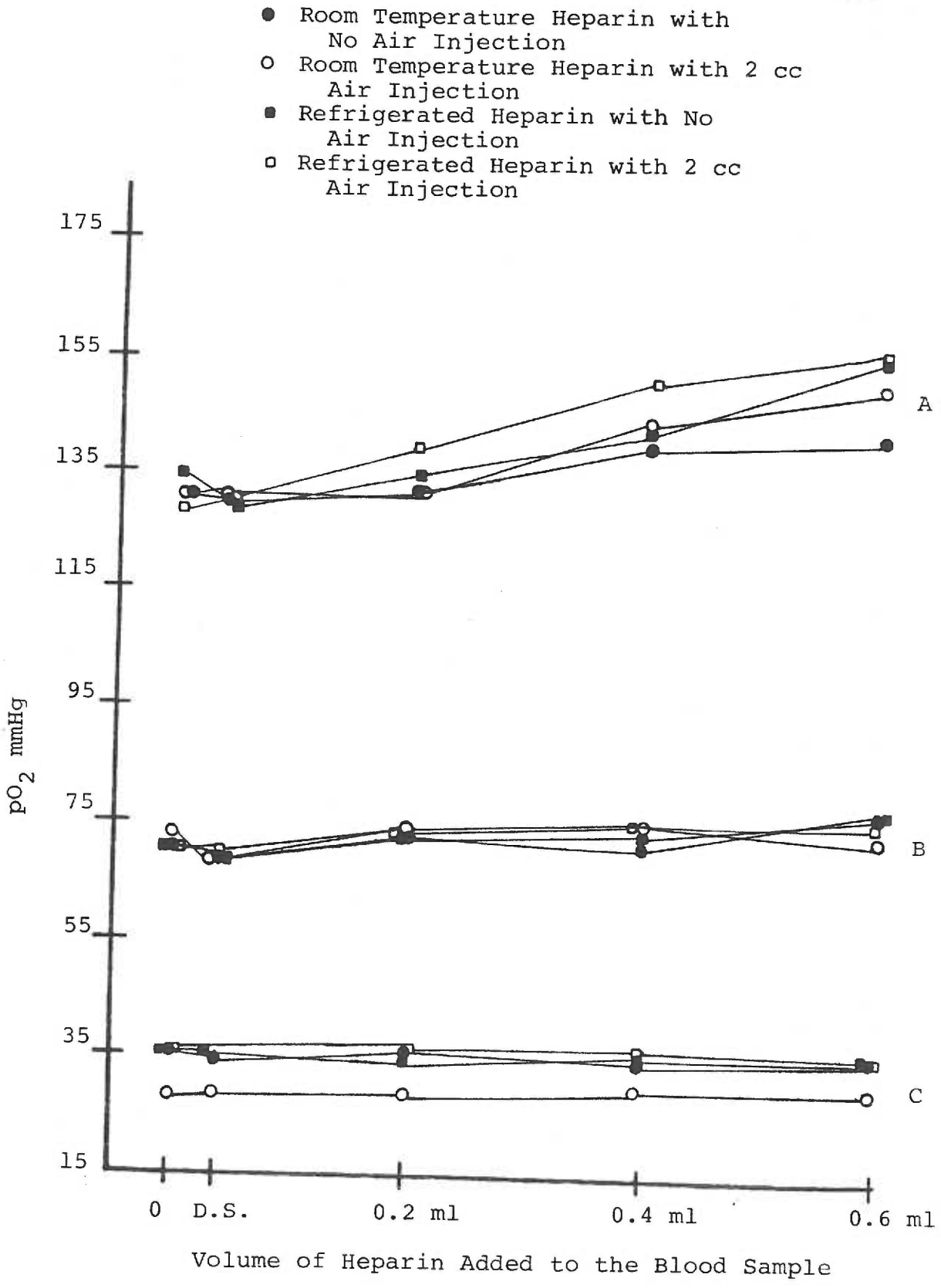

Room Temperature Heparin with No Air Injection
Room Temperature Heparin with 2 cc Air Injection
Refrigerated Heparin with No Air Injection
Refrigerated Heparin with 2 cc Air Injection
175
155
135
115
95
75
55
35
15
pO_2 mmHg
A
B
C
0
D.S.
0.2 ml
0.4 ml
0.6 ml
Volume of Heparin Added to the Blood Sample

Figure 5

Bicarbonate Concentration Versus Volume of Heparin Added to the Blood Sample

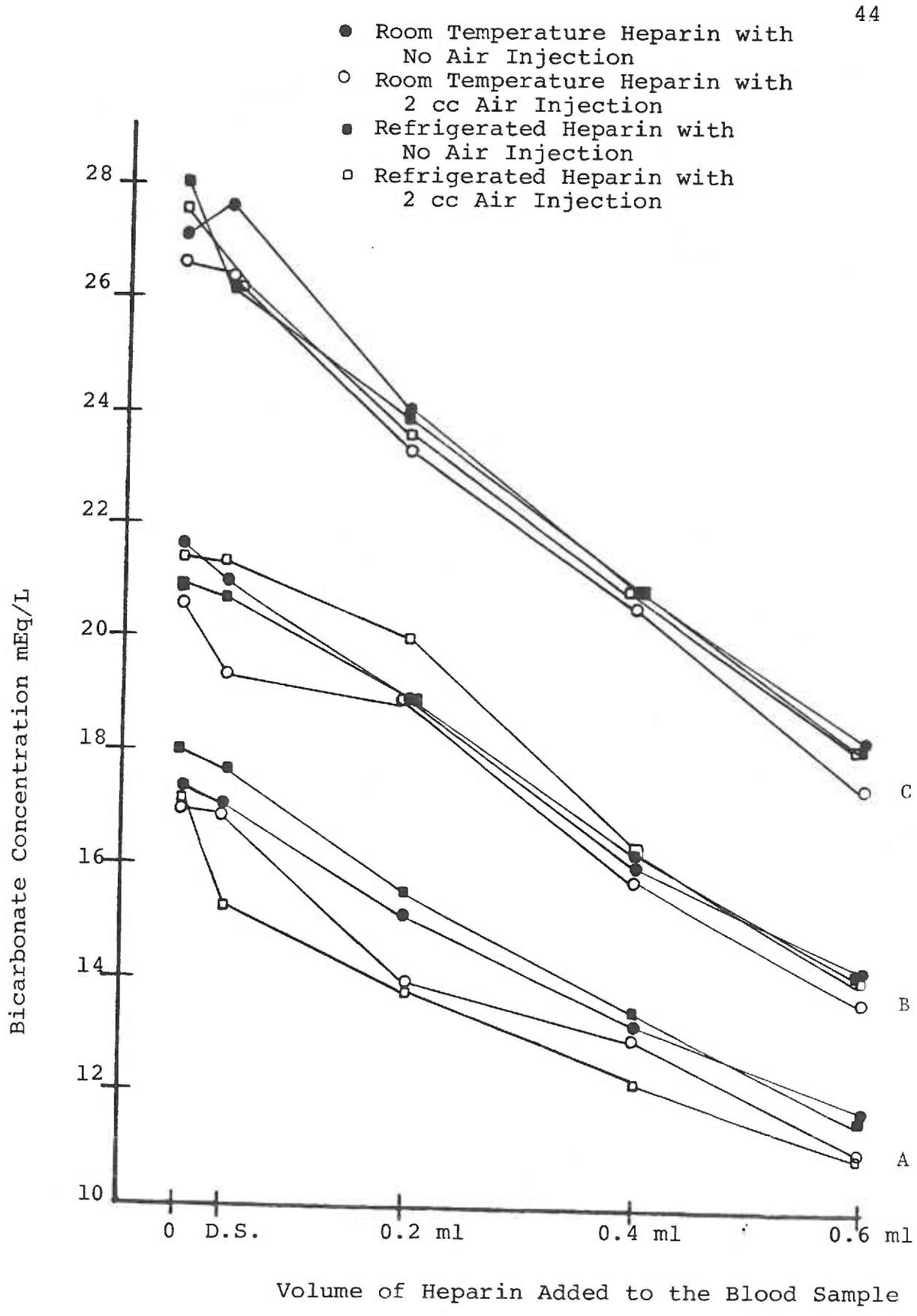
A represents the results obtained when the blood sample was equilibrated with the gas mixture: 3% CO_2 in air.

B represents the results obtained when the blood sample was equilibrated with the gas mixture: O_2 10%, CO_2 5%, N_2 85%.

C represents the results obained when the blood sample was equilibrated with the gas mixture: O_2 5%, CO_2 10%, N_2 85%.

The mean control values are plotted on the zero point of the abscissa.

D.S. represents the dead space volume of the 2 ml glass syringe.


Room Temperature Heparin with No Air Injection
Room Temperature Heparin with 2 cc Air Injection
Refrigerated Heparin with No Air Injection
Refrigerated Heparin with 2 cc Air Injection
Bicarbonate Concentration mEq/L
28
26
24
22
20
18
16
14
12
10
C
B
A
0
D.S.
0.2 ml
0.4 ml
0.6 ml
Volume of Heparin Added to the Blood Sample

Chapter IV

DISCUSSION

The differences between the mean control values and the values obtained by different methods of handling heparin and by increasing the volume of heparin in the blood sample (Tables 4 through 7) were evaluated according to two standards: 1) instrumentation significance, and 2) clinical significance. The instrumentation significance refers to the limits established by the manufacturer of the blood gas analyzer that represent the acceptable variability for equivalent samples. Outside these limits the samples are considered to be different. The limits for this blood gas analyzer are:

1) pH ± 0.005 units
2) pCO_2 ± 0.50 mmHg and
3) pO_2 ± 1.00 mmHg.

It must be remembered that although two samples may be classified as different, the difference may or may not be clinically significant. The standard for clinical significance is not a rigid set of limits as with instrumentation significance. Each clinical situation will determine what limits are acceptable. Also changes in blood gas composition may be called significant by one observer and not significant by another. There are circumstances where a change in blood gas composition

can easily be classified as clinically insignificant or clinically significant. Yet, there is also a range of changes where, depending on the clinical situation and the observers making the judgment, a change in blood gas composition may be of questionable clinical significance. Statistical analysis for significance does not solve the dilemma either. Often values may be statistically significant but clinically fall into a broad, acceptable range and vice versa. The values may have no statistical significance but may have profound clinical implications.

Therefore, for the purpose of this study and based on the observation of this investigator, the following guidelines have been selected to evaluate the changes in values obtained in this research. Changes will be classified as clinically significant when:

1) pH changes by 0.050 units or more,

2) pCO_2 changes by 5 mmHg or more,

3) pO_2 changes by 5 mmHg or more, and

4) $[HCO_3^-]$ changes by 3 mEq/L or more.

There will, of course, be situations where changes of less magnitude may be called significant, however, most informed clinicians would agree that changes of the magnitude listed above would be considered significant clinically. If a particular procedure produces a change of sufficient magnitude from control value such that there is no question of clinical significance, then the obvious conclusion is that the pro-

cedure should not be performed in the clinical setting.

General Changes Including Effects of Heparin in the Dead Space of the Syringe

pH

Thirty of the 48 cases, or 62.5% of the samples, showed a change in pH greater than ± 0.005 units. This signifies that in the majority of the samples the different methods of handling heparin or the addition of heparin to the blood sample produced a change in pH from the mean control value. None of the differences was greater than ± 0.026 units, which is below the limit of change or 0.050 units chosen to define clinical significance. Therefore, although the pH did change from the mean control value in the majority of samples, there were no cases of clinically significant changes in pH. This confirms the results reported by Bech-Jansen and Beck, 1972; Cissik, et al., 1977; Hansen and Simmons, 1977; and Scheinhorn, 1978.

pCO_2

Only 4 of the 48 samples had a change of less than 0.5 mmHg, thus 44 met the criteria of a significant change by the standards of instrumentation. The change in pCO_2 observed by altering the temperature of the heparin and/or the pressure within the vial were not clinically significant for any given gas mixture (Table 5 and Figure 3).

When assessing the effect of changing the volume of heparin

added to the blood sample, it is apparent from Table 5 that the addition of 0.2 ml produces clinically significant changes when using the 10% CO_2 gas mixture. The frequency of clinically significant changes increased when the volume of heparin was increased. It should be noted that the changes in pCO_2 of blood samples with the addition of heparin to the dead space alone were clinically insignificant except in one case (Table 5).

These results of a reduction in pCO_2 with the addition of increasing volume of heparin to the sample are consistent with the results reported by Cissik, et al., 1977; Hamilton, et al., 1978; Scheinhorn, 1978; and Siggaard-Andersen, 1961.

pO_2

None of the changes in pO_2 obtained using the 5% O_2 gas mixture were clinically significant. Eight of the 12 changes in pO_2 were below the criteria for instrumentation significance. In addition the changes in pO_2 obtained with the addition of heparin to the dead space alone were not clinically significant for any gas mixture or any method of handling heparin.

The largest changes in pO_2 were observed when equilibrating the blood sample with 3% CO_2 in air mixture. The change in pO_2 using this gas mixture became clinically significant with the addition of 0.2 ml of refrigerated heparin with 2cc of air inspection. Changes were also clinically significant when volumes of heparin greater than 0.2ml were added to the

blood sample regardless of the method of handling the heparin (Table 6).

There was a consistent pattern of change when using the different methods of handling heparin for the gas mixture of highest O_2 concentration. With the addition of 0.6 ml of heparin the differences from mean control increased from room temperature heparin without air injection to refrigerated heparin with 2cc of air injection (Table 6). Also the difference from mean control was clinically significant when using 0.2 ml or 0.4 ml of refrigerated heparin with 2cc air injection as compared to other methods of handling heparin. It is postulated that the reason for the greater changes in pO_2 observed when using the gas mixture of highest O_2 concentration is due to saturation of the hemoglobin molecule. When the blood sample was equilibrated with the 5% O_2 gas mixture, the hemoglobin molecule was not saturated and could combine with most of the dissolved O_2 added by the heparin. When the O_2 concentration of the gas mixture was increased, the hemoglobin molecule became nearly saturated and could not accommodate the increase in dissolved O_2. Therefore, the additional oxygen from the heparin remained dissolved in the plasma and the change in pO_2 was greater.

Bicarbonate Concentration, $[HCO_3^-]$

There is no instrumentation significance for bicarbonate concentration because bicarbonate concentration is a calculated,

not measured, value. Nineteen of the 48 samples had clinically insignificant changes. None of the samples containing heparin in the dead space alone had clinically significant changes. Changes became clinically significant with the addition of 0.2 ml of heparin. All of the differences obtained using volumes of heparin greater than 0.2 ml were clinically significant. The change in bicarbonate concentration produced by altering either the temperature and/or the pressure within the vial were not clinically significant for any given gas mixture (Table 7, Figure 5). The value of bicarbaonate concentration is essential in the evaluation of metabolic components of acid base status of the patient, and therefore, change, whether real or artifact, influence therapy.

It is of interest that bicarbonate concentration changed directly with change in pCO_2. This can be evaluated in terms of the Hendersen-Hasselbalch equation (Davenport, 1974):

$$pH = pKa + \log \frac{[HCO_3^-]}{S \cdot pCO_2} \qquad (1)$$

S = solubility coefficient for O_2 = $0.0301 \frac{mM\ CO_2}{L\ mmHg\ pCO_2}$.

pKa at 37° = 6.1

Therefore, $[HCO_3^-] = 10^{(pH-pKa)} \cdot S \cdot pCO_2$ (2)

Since pH was found to be approximately constant in this research, and pKa and S are constant, it follows that:

$$[HCO_3^-] = K \cdot pCO_2 \qquad (3)$$

where K is a constant. Therefore any change in pCO_2 will have

a direct effect on $[HCO_3^-]$.

Analysis of the Heparin Itself

There is a marked difference in the values obtained from the analysis of heparin compared to the normal values for blood. Heparin has a lower pH, a much lower pCO_2 and a greater pO_2 than the normal range of pO_2 for arterial blood (Table 8). Yet the pH of the blood sample showed only minor changes with the addition of heparin. It is probable that the buffers of the blood neutralized the more acidic heparin, while the dilution did not alter pH significantly (Equation 1). With the addition of heparin the pCO_2 of the blood sample decreased via dilution. For the pO_2 the addition of heparin had an additive effect once the hemoglobin molecule became nearly saturated. The increased pO_2 from the heparin produced increased pO_2 values for the blood sample as well.

Chapter V

SUMMARY AND CONCLUSIONS

Altering the temperature of the heparin and/or the pressure within the multidose vial of heparin does not produce a consistent pattern of change in pH, pCO_2, or pO_2 of the blood sample, nor a clinically significant change except for pO_2 when using the gas mixture of highest O_2 concentration. The greatest effect of heparin observed in this research occurred with the addition of heparin to the blood sample. The difference from mean control became clinically significant for pO_2, pCO_2 and bicarbonate concentration with the addition of 0.2ml of heparin.

The addition of heparin to the dead space of the syringe produced clinically insignificant changes for pH, pO_2 and bicarbonate concentration and only one instance of clinical significance for pCO_2. Therefore under the conditions of this study heparin filling only the dead space of the syringe will not affect the results of blood gas analysis. On the basis of this study the following recommendations are made for the preparation of the syringe prior to blood gas analysis:

1) Only the dead space volume should be filled with heparin;

2) Heparin should not be refrigerated nor maintained under increased pressure within the vial for any period of time.

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APPENDIX A

Table A-1

Summary of Data Using Room Temperature Heparin with No Air Injected Into the Multidose Vial of Heparin

	pH units	pCO_2 mmHg	pO_2 mmHg	$[HCO_3^-]$ mEq/L
Air 97%, CO_2 3%				
Control Begin	7.532	21.4	131.7	17.4
End	7.544	21.1	132.4	17.6
$\bar{x}$ Control	7.538	21.2	132.0	17.5
± SD	0.008	0.2	0.5	0.1
DS	7.541	20.7	131.0	17.2
0.2 ml	7.543	18.2	133.5	15.2
0.4 ml	7.546	15.9	141.0	13.3
0.6 ml	7.548	14.0	143.3	11.8
Heparin	6.434	1.5	187.1	0.1
O_2 10%, CO_2 5%, N_2 85%				
Control Begin	7.391	36.6	71.1	21.5
End	7.398	36.5	71.4	21.8
$\bar{x}$ Control	7.395	36.6	71.2	21.7
± SD	0.005	0.1	0.2	0.2
DS	7.398	35.3	69.8	21.1
0.2 ml	7.396	31.9	73.2	19.0
0.4 ml	7.391	27.3	72.7	16.1
0.6 ml	7.399	23.9	78.8	14.3
Heparin	6.443	1.2	185.7	0.1
O_2 5%, CO_2 5%, N_2 85%				
Begin	7.190	73.7	36.4	27.3
Control End	7.189	73.7	35.6	27.2
$\bar{x}$ Control	7.190	73.7	36.0	27.2
± SD	0.001	0.0	0.6	0.1
DS	7.204	72.4	34.8	27.7
0.2 ml	7.198	64.0	36.3	24.1
0.4 ml	7.202	55.3	34.6	21.0
0.6 ml	7.193	49.1	35.8	18.3
Heparin	6.481	2.0	189.9	0.1

The control samples (the first and last blood samples analyzed--"Begin" and "End") had no additional heparin. DS (dead space), 0.2 ml, 0.4 ml, 0.6 ml refer to the volume of heparin added to the blood sample. "Heparin" refers to the analysis of the heparin alone.

Table A-2

Summary of Data Using Room Temperature Heparin with 2 cc Air Injected Into the Multidose Vial of Heparin

	pH units	pCO_2 mmHg	pO_2 mmHg	$[HCO_3^-]$ mEq/L
Air 97%, CO_2 3 %				
Control Begin	7.538	20.6	131.7	17.0
End	7.533	20.8	134.8	17.0
$\bar{x}$ Control	7.535	20.7	133.3	17.0
± SD	0.004	0.1	2.2	0.0
DS	7.533	20.7	132.1	16.9
0.2 ml	7.533	20.7	132.1	16.9
0.4 ml	7.542	15.6	145.4	13.0
0.6 ml	7.546	13.2	151.2	11.1
Heparin	6.407	1.4	196.9	0.1
O_2 10%, CO_2 5%, N_2 85%				
Control Begin	7.358	37.1	74.0	20.2
End	7.392	36.0	74.1	21.2
$\bar{x}$ Control	7.375	36.5	74.0	20.7
± SD	0.024	0.8	0.0	0.6
DS	7.371	34.6	69.8	19.4
0.2 ml	7.398	31.7	75.4	19.0
0.4 ml	7.392	26.8	76.2	15.8
0.6 ml	7.398	22.9	74.5	13.7
Heparin	6.515	1.5	200.0	0.1
O_2 5%, CO_2 10%, N_2 85%				
Control Begin	7.186	73.3	28.3	26.9
End	7.184	72.5	27.7	26.5
$\bar{x}$ Control	7.185	72.9	28.0	26.7
± SD	0.001	0.6	0.5	0.3
DS	7.193	70.9	28.8	26.4
0.2 ml	7.195	62.4	29.1	23.4
0.4 ml	7.199	54.6	29.8	20.6
0.6 ml	7.191	47.4	30.0	17.6
Heparin	6.392	1.5	195.3	0.1

The control samples (the first and last blood samples analyzed--"Begin" and "End") had no additional heparin. DS (dead space), 0.2 ml, 0.4 ml, 0.6 ml refer to the volume of heparin added to the blood sample. "Heparin" refers to the analysis of the heparin alone.

Table A-3

Summary of Data Using Refrigerated Heparin With

No Air Injected Into the Multidose Vial of Heparin

	pH units	pCO_2 mmHg	pO_2 mmHg	$[HCO_3^-]$ mEq/L
Air 97%, CO_2 3%				
Control Begin	7.545	21.0	132.5	17.6
End	7.558	21.3	137.2	18.4
$\bar{x}$ Control	7.552	21.1	134.9	18.0
$\pm$ SD	0.009	0.2	3.3	0.5
DS	7.556	20.7	130.4	17.8
0.2 ml	7.566	17.8	135.6	15.7
0.4 ml	7.565	15.5	143.1	13.6
0.6 ml	7.558	13.4	156.7	11.6
Heparin	7.403	1.7	238.8	1.0
O_2 10%, CO_2 5%, N_2 85%				
Control Begin	7.365	36.3	70.8	20.1
End	7.408	35.9	70.9	22.0
$\bar{x}$ Control	7.386	36.1	70.8	21.0
$\pm$ SD	0.030	0.3	0.1	1.3
DS	7.384	35.8	69.4	20.7
0.2 ml	7.405	31.2	72.8	19.0
0.4 ml	7.398	27.5	74.3	16.4
0.6 ml	7.408	23.2	78.5	14.2
Heparin	6.424	1.4	237.1	0.1
O_2 5%, CO_2 10%, N_2 85%				
Control Begin	7.204	73.8	36.1	28.2
End	7.196	73.8	34.8	27.7
$\bar{x}$ Control	7.200	73.8	35.4	28.0
$\pm$ SD	0.006	0.0	1.0	0.4
DS	7.202	68.8	35.5	26.2
0.2 ml	7.207	62.0	34.2	23.9
0.4 ml	7.198	55.6	35.1	21.0
0.6 ml	7.193	48.6	36.5	18.1
Heparin	6.342	0.9	237.9	0.0

The control samples (the first and last blood samples analyzed--"Begin" and "End") had no additional heparin. DS (dead space), 0.2 ml, 0.4 ml, 0.6 ml refer to the volume of heparin added to the blood sample. "Heparin" refers to the analysis of the heparin alone.

Table A-4

Summary of Data Using Refrigerated Heparin With 2 cc Air Injected Into the Multidose Vial of Heparin

	pH units	pCO_2 mmHg	pO_2 mmHg	$[HCO_3^-]$ mEq/L
Air 97%, CO_2 3%				
Control Begin	7.542	21.8	128.6	18.2
End	7.515	20.9	131.7	16.4
$\bar{x}$ Control	7.528	21.4	130.2	17.3
$\pm$ SD	0.019	0.6	2.2	1.3
DS	7.502	20.1	131.2	15.3
0.2 ml	7.512	17.7	140.8	13.8
0.4 ml	7.512	15.6	152.3	12.2
0.6 ml	7.523	13.7	156.8	10.9
Heparin	6.471	1.6	269.3	0.1
O_2 10, CO_2 5%, N_2 85%				
Control Begin	7.402	35.9	72.3	21.6
End	7.399	35.8	68.1	21.4
$\bar{x}$ Control	7.400	35.8	70.2	21.5
$\pm$ SD	0.002	0.0	3.0	0.1
DS	7.402	35.6	71.0	21.5
0.2 ml	7.414	32.2	73.8	20.0
0.4 ml	7.404	27.2	76.1	16.5
0.6 ml	7.403	23.4	76.1	14.1
Heparin	6.574	1.7	232.9	0.1
O_2 5%, CO_2 10%, N_2 85%				
Control Begin	7.191	75.4	35.5	28.0
End	7.192	73.3	36.2	27.3
$\bar{x}$ Control	7.191	74.4	35.8	27.6
$\pm$ SD	0.001	1.5	0.4	0.5
DS	7.191	71.1	35.2	26.4
0.2 ml	7.199	62.5	37.4	23.6
0.4 ml	7.201	55.2	36.8	21.0
0.6 ml	7.206	47.0	36.3	18.1
Heparin	6.584	1.4	209.1	0.1

The control samples (the first and last blood samples analyzed--"Begin" and "End") had no additional heparin. DS (dead space), 0.2 ml, 0.4 ml, 0.6 ml refer to the volume of heparin added to the blood sample. "Heparin" refers to the analysis of the heparin alone.

AN ABSTRACT OF THE THESIS OF

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For the MASTER OF NURSING

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Title: THE EFFECTS OF HEPARIN ON THE RESULTS OF BLOOD GAS ANALYSIS

Approved: [redacted]
Jack L. Keyes, Ph.D., Thesis Advisor

Blood samples equilibrated with three different gas mixtures were examined for the influence of the addition of serial volumes of heparin handled by four different methods. The different methods of handling the heparin included changing the temperature and/or pressure within the multidose vial of heparin. Heparin was added in volumes equal to dead space, 0.2 ml, 0.4 ml, 0.6 ml to the blood sample to make a total sample volume of 2 ml. Only a slight, insignificant effect on pH was noted. The effect on pCO_2, pO_2 and bicarbonate concentration became significant beginning with the addition of 0.2 ml of heparin. The pCO_2 and bicarbonate concentration decreased in a linear or near linear pattern as the volume of heparin increased. The pO_2 increased with increased volume of heparin when the hemoglobin in the sample was nearly saturated. There was no significant effect from changing the temperature and/or pressure within the vial except for pO_2.