THE EFFECTS OF ANTICOAGULANTS ON THE VALUES OBTAINED DURING ARTERIAL BLOOD-GAS ANALYSIS

by

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CHAPTER I

INTRODUCTION

Arterial blood-gas analysis is the determination of the pH, pCO_2 and pO_2 of arterial blood. It is one of the laboratory tests most frequently performed on blood obtained from critically ill patients. With present techniques an arterial blood sample is drawn anaerobically from a patient into a syringe containing an anticoagulant. The sample in the syringe is then placed on ice to slow in vitro cell metabolism which can alter the pH of the blood. A blood-gas analyzer is used to determine the blood-gas composition, i.e., the pCO_2, pO_2 and pH of the sample. The pCO_2 and pH values are then used to determine the plasma bicarbonate concentration either from a nomogram or from the Henderson-Hasselbalch equation (Appendix A).

Data obtained from blood-gas analysis have become vital in assessing the critically ill patient. The pCO_2, pO_2 and pH of arterial blood provide basic information about a patient's cardiopulmonary status and may be used to help determine whether or not a patient will receive oxygen therapy or will need mechanical ventilation. Blood-gas composition and the calculated plasma bicarbonate concentration are also used to evaluate a patient's acid-base status. Serial blood-gas

analyses may indicate whether or not a patient is able to compensate adequately for acid-base imbalances without medical intervention.

Various problems may occur during the process of arterial blood-gas analysis. These include sampling errors, storage and transportation errors, analysis errors, and errors in the reporting and recording of results. Techniques used for obtaining and analyzing arterial blood samples may alter the blood-gas composition of the samples. Methods which result in large alterations may cause institution of inappropriate medical treatment including changes in medications, oxygen therapy and/or mechanical ventilation of the patient. The purpose of this study is to determine how one aspect of arterial blood sampling--the kind of anticoagulant used--affects measured blood-gas values.

Review of the Literature

With present techniques of blood-gas analysis some type of anticoagulant must be used. Sodium heparin (or heparin) is most commonly used for this purpose. However, some researchers have found heparin alters blood gas values. Also, heparin may not be readily available in some parts of the world (Goodwin & Schreiber, 1979). The following review of the literature is undertaken to determine if previous studies indicated how other commonly used anticoagulants--trisodium citrate, potassium oxalate and ethylene-diamine tetraacetate

(EDTA)--affect measured blood-gas values. Also, the effect of sodium heparin on measured blood-gas parameters is reviewed. For the purpose of clarity the effect of each anticoagulant will be discussed separately, although in two studies anticoagulants were compared.

Heparin

Heparin is the anticoagulant most frequently studied. In 1935 Yoshimura examined the effects of heparin on the pH of blood. He used a glass electrode technique for pH measurement. He compared samples of rabbit blood with a known concentration of heparin to samples without any anticoagulant. Two experiments were done with a 0.5% concentration of heparin, using one part of this heparin solution to 9 parts blood. In two other experiments a 0.1% concentration of heparin was used with the same proportion of heparin solution to blood (1:9). Samples without anticoagulant were also drawn from the same animal and analyzed immediately. Yoshimura found heparin decreased the pH by an average of 0.005 units in the 4 samples studied as compared with the blood samples without heparin. He concluded that a pH difference of 0.01-0.02 units was within the scope of experimental error. Therefore, a pH difference of 0.005 units would not be significant.

It is difficult to determine the actual potency of the heparin used, since at the time Yoshimura conducted this study heparin was not measured in standard units. Heparin is a biological product and potency may vary in preparations.

Presently in the United States heparin is measured in U.S.P. units. A U.S.P. unit of heparin is defined as the minimum quantity that will prevent 1.0 ml of citrated sheep plasma from clotting for one hour after the addition of 0.2 ml of a 1:100 $CaCl_2$ solution (Levine, 1975).

In four experiments in 1961 Siggaard-Andersen evaluated the effect of heparin on the acid-base status of blood in vitro. A thermostated capillary glass electrode was used for pH measurements and the pCO_2 was calculated from the actual pH and from the pH of the blood equilibrated with a known CO_2 tension. Powdered heparin was used for all experiments.

In this investigation 0.01 mg/ml of heparin solution was added initally to each ml of blood to prevent coagulation. Varying additional amounts of heparin were then added to provide concentrations of 2, 4, and 10 mg heparin per ml of blood. Siggaard-Andersen calculated for every mg of heparin per ml of blood, pH decreased by 0.003 units, and pCO_2 increased by 0.1 mmHg. He did not indicate if this was within the range of experimental error with his technique. Also, heparin concentration was not listed in U.S.P. units.

Subsequent to the use of a new technique for arterial blood sampling, Bech-Jansen and Beck (1972) studied the influence of heparin on blood pH. Because smaller syringes were being used, smaller blood samples were taken, resulting in a larger ratio of heparin to blood than in larger syringes. Since blood-gases are drawn under anaerobic conditions heparin is used to

replace the air which would normally be present in the lumen of the needle and hub of the syringe. Even if excess heparin is expressed from the syringe, this so-called dead space will contain a finite volume of heparin. There is a larger ratio of dead space volume to total syringe volume in smaller syringes.

Bech-Jansen and Beck wished to determine what effect these higher concentrations of heparin would have on pH determinations. They used a 20 ml glass syringe to obtain 20 ml of blood anaerobically from an anesthetized patient. This blood was mixed with 250 units of heparin which was determined to be the minimum amount needed to prevent coagulation. A standard heparin solution of 5,000 I.U./ml was used for one study. The heparin was diluted with saline to 1,000 I.U./ml for a second study and 100 I.U./ml for a third. These solutions were added to the dead space of 1 cc glass syringes. Then 0.1 ml increments of blood were added to each syringe in amounts of 0.1 to 1.0 ml. The authors studied blood samples from 15 patients in this manner. Analysis revealed that when the sample of blood in the syringe was 0.6 ml or greater the accuracy of the pH measurement was affected very little, regardless of the heparin concentration. When the sample of blood was less than 0.6 ml the pH decreased with all three concentrations of heparin. The most concentrated heparin solution (5,000 I.U./ml) resulted in the greatest decrease in pH.

Bradley (1972) also reported the effects of heparin.

He used small syringes and was interested in errors of measurement of blood pCO_2 due to heparin dilution. In this study blood with an amount of heparin necessary to prevent coagulation was used but the ratio of heparin to blood was not indicated. The blood was then equilibrated with a gas mixture of known pCO_2 (37 mmHg) and pO_2 (71 mmHg). Subsequent dilutions of the blood were made with physiological saline and the samples were analyzed. Because the amount of heparin in each syringe did not change, the author was actually measuring the results of dilution with physiological saline. The pH and pO_2 did not change significantly. The pCO_2 decreased significantly with increasing saline dilution. With saline dilutions as small as 4% of the total volume the pCO_2 decreased by approximately 10%.

Hanson and Simmons (1977) reported the effects of heparin on values of pCO_2, pH and bicarbonate in arterial blood. They first determined the dead space of various sizes of syringes. They also calculated the amount of heparin necessary to fill a three-way stopcock, since some techniques for blood sampling may involve the use of a stopcock. The authors found a potential dead space that ranged from 0.07 to 0.26 ml.

Next, a 20 ml syringe containing 0.2 ml of heparin was filled with fresh venous blood. Then six samples of 3 ml each were drawn from this larger sample into 3 ml plastic syringes containing measured amounts of heparin. The remaining blood in the 20 ml syringe was used as a control. All syringes

were labeled and given in random order to a technician for analysis. The procedure was repeated on four occasions.

The authors state they found no significant change in the pH of the samples. They did find that the pCO_2 of the samples declined according to the percentage of heparin in the sample. They calculated a 1% decline in pCO_2 for every 1% increase in heparin volume.

Hanson and Simmons also surveyed syringe and blood sample sizes received in their clinical laboratory during one day. They found that most syringes were not filled to capacity. They postulated that if dead space of these syringes had been filled in the normal fashion, 17% of the arterial pCO_2 determinations would have been in error by 17% of more due to dilution with heparin. Since the bicarbonate values are calculated from pH and pCO_2 the bicarbonate values would also be affected by the sampling error.

Cissik, Salustro, Patton, and Louden (1977) also studied the effects of heparin on the results obtained from arterial blood-gas analysis. They began their study by an analysis of the pH, pO_2, and pCO_2 values of the heparin solution. The heparin used was found to have a pO_2 of about 186 mmHg which is about 90 to 100 mmHg higher than the pO_2 of normal arterial blood.

They then used eleven different volume ratios of heparin (U.S.P. 1000 u./1 cc) to blood (ranging from 0.05 cc heparin/ 1.0 cc blood to 1.0 cc heparin/1.0 cc blood). They found no

significant effect on pH. They did find that beginning with a concentration of 0.2 cc heparin to 1.0 cc of blood the pCO_2 was decreased and the pO_2 was increased. The authors found a 16.9% decrease in pCO_2 value with a ratio of 0.2 cc heparin to 1.0 cc of blood. With increasing amounts of heparin the pCO_2 was decreased further. There was a 43.4% decrease in the pCO_2 from control pCO_2 with a ratio of 1.0 cc of heparin to 1.0 cc of blood.

The measured pO_2 increased by 7.8% (over control pO_2) with 0.2 cc heparin to 1.0 cc of blood. The increase in pO_2 when the ratio was 1.0 cc of heparin to 1.0 cc of blood was reported to be 75.6% over control pO_2.

Following the study by Cissik, et al. (1977), Hamilton, Crockett, and Alpers (1978) evaluated the effects of heparin on pH, pCO_2, and pO_2. In order to provide a source of blood with constant blood-gas characteristics they used a technique previously reported by Bradley (1972). An I.L. 237 tonometer was used to provide blood with known blood-gas values.

Prior to blood analysis, the heparin solution was analyzed. The pO_2 of the heparin was found to be 159.3 ± 3.6 mmHg. This value is within the range of the pO_2 of dry air at sea level and is greater than the approximate pO_2 that would be expected in any solution equilibrated with air at a low altitude. This pO_2 of heparin is lower than the value reported by Cissik, et al. (1977) and higher than the mean value of pO_2 normally found in arterial blood.

Hamilton, et al. obtained 30 ml of venous blood for sampling and initially added 100 units of heparin. Then 6 ml portions of this blood were tonometered to equilibration with a known gas mixture. A measured amount of heparin was placed in a 5 ml syringe and 2 ml of blood was then drawn into it anaerobically from the tonometered sample. Each 6 ml sample of tonometered blood was used for two samples with measured amounts of heparin in this manner. The remainder of the blood was used for 2 control samples. This process was repeated so that 0.1, 0.2, 0.3, 0.4, 0.5 and 1.0 ml of heparin were used.

Hamilton, et al. found a decrease in pH when heparin constituted 10% or more of the heparin/blood mixture. However, they believed this change in pH would not be clinically significant (result in a change in medical treatment) until the heparin constituted 25% of the sample. The relationship between heparin concentration and pH change was found to be non-linear and thought to be caused by "complex buffering action of the blood."

The pCO_2 was found to decrease as the amount of heparin in the syringe increased. The authors attributed this to a dilutional effect due to the mixing of two liquids with different pCO_2 values. The heparin used was found to have a pCO_2 (mmHg) of 5.3 ± 0.1 (n = 10). Control blood had a pCO_2 (mmHg) of 43.4 ± 0.6 (n = 40).

A significant increase of the pO_2 was found with concentrations of heparin greater than 15% of the heparin/blood

mixture. Hamilton, et al. found the error in pO_2 produced by the addition of heparin was difficult to predict. They attributed this difficulty to "the complexity of the oxyhaemoglobin dissociation curve and its dependence on pH, salt concentration, etc."

The most recently reported study of the effects of heparin on blood-gas values is by Goodwin and Schreiber (1979). They used heparin of various concentrations (1000 I.U./ml, 5000 I.U./ml, and 25,000 I.U./ml). They filled the dead space of 5 ml glass syringes or the dead space plus 0.5 ml with the various concentrations of heparin. They then collected arterial blood samples from four patients filling the syringes with blood to the 2 ml mark. Blood was analyzed on an IL431 blood-gas analyzer.

They found a measurable decrease in pH with increasing concentrations of heparin. The greatest concentration (25,000 I.U./ml heparin filling dead space plus 0.5 ml) resulted in a clinically significant decrease in pH. No control pH values were reported but minimal dilution and heparin concentration resulted in a pH of 7.44 units while maximal dilution and heparin concentration produced a pH of 7.21.

The authors reported that pCO_2 was decreased least with the highest concentration of heparin (25,000 I.U./ml). This is in contrast to the findings of others. Goodwin and Schreiber (1979) believed this difference might be due to variations in the pH and solubility of CO_2 in the diluent of

the heparin as well as possible direct effects on the CO_2 electrode.

Results of the effect of heparin on pO_2 were not reported but were said to be within the level of accuracy of the analyzer used.

Other Anticoagulants

Anticoagulants other than heparin have been studied much less frequently. The report by Yoshimura (1935) and the recent study by Goodwin and Schreiber (1979) are the only two that were found.

Sodium Citrate. Yoshimura (1935) used sodium citrate in concentrations to blood in the ratio of 1 part citrate to 9 parts blood. He found the pH values decreased an average of 0.012 pH units for the 3.5% concentration, 0.021 for the 5% concentration, and 0.088 pH units for the 10% concentration.

Goodwin and Schreiber (1979) reported results using 3.89% sodium citrate and methods previously described for heparin. They reported pH and pO_2 results were within the accuracy of the blood-gas analyzer used when only the dead space of the syringe was filled with sodium citrate. However, pH was decreased significantly when an additional 0.5 ml of citrate was used. The pCO_2 was decreased by 8-10% with the syringe filled to dead space plus 0.5 ml of citrate.

Potassium Oxalate. Yoshimura (1935) used potassium oxalate in concentrations of 2%, 5%, and 10% added to blood

in the proportion of 1 part oxalate to 9 parts blood. He found the 2% solution decreased the pH 0.001 units on the average and the 5% solution decreased the pH an average of 0.01 units. In contrast a 10% solution caused the pH to rise by 0.039 units. Because the pH of the oxalate itself was found to be almost neutral according to the author (pH 6.88) he thought this change was not due to buffering by the oxalate but to a change in the blood itself.

Goodwin and Schreiber (1979) used Heller-Paul oxalate which consists of 1.2% ammonium oxalate and 0.8% potassium oxalate. They reported that Heller-Paul oxalate gave results comparable to those obtained with sodium citrate. The pH decreased less with excess oxalate. The pCO_2 decreased by 8-10% over control values with the syringe filled to dead space plus 0.5 ml of oxalate.

EDTA. Only Goodwin and Schreiber (1979) have reported the use of EDTA. They used EDTA with two samples but this resulted in such alterations in the values obtained that this anticoagulant was not studied further.

Conclusion

In summary, most authors have found that heparin causes a decrease in pH, pCO_2 and an increase in pO_2, but the actual clinical significance of these changes is poorly defined in most instances. Clinically significant errors are those that would result in an inaccurate assessment of a patient's medical

condition and/or inappropriate medical treatment.

Yoshimura (1935) found that sodium citrate decreased the pH. Potassium oxalate was found to increase the pH in high concentrations.

Goodwin and Schreiber (1979) found the anticoagulants trisodium citrate and Heller-Paul oxalate caused a decrease in pH and a decrease in pCO_2 but no significant change in pO_2. They only reported results on four samples.

Goodwin and Schreiber discarded the use of EDTA after only two trials without apparent inquiry into the cause of the distorted blood-gas values.

In this study the anticoagulants heparin, trisodium citrate, Heller-Paul oxalate, and EDTA were used in conjunction with blood tonometered to known blood-gas composition. The experimental as well as clinical significance of the values obtained were then evaluated.

Implications for Nursing

Nurses in critical care units have become the health professionals most directly involved in patient assessment. Frequently the nurse determines if laboratory tests should be performed, obtains appropriate samples for analysis, and determines if the values obtained from the analysis are sufficiently deviant from normal to warrant nursing intervention and/or notifying the physician.

One of the laboratory tests most often obtained and initially evaluated by the critical care nurse is arterial

blood-gas analysis. If the patient is being mechanically ventilated or if the patient has symptoms of respiratory failure, the nurse often must decide when changes in the patient's condition indicate arterial blood should be drawn. The nurse is often responsible for sampling arterial blood, either from an indwelling arterial catheter or an arterial puncture. Also, the nurse is often the first health professional directly involved in patient care to see the results of blood-gas analysis.

Because critical care nurses are often directly involved in sampling arterial blood and because the blood-gas values may determine treatment for the patient, it is important for nurses to be familiar with the techniques which result in the most accurate values obtainable from blood-gas analysis.

CHAPTER II

METHODS

Four anticoagulants were studied in three different concentrations (Table 1). These included heparin, trisodium citrate, Heller-Paul oxalate, and an EDTA mixture. Additional studies were done using tetrasodium EDTA and disodium EDTA.

For each series of tests 20 ml of blood was drawn from a healthy dog in a 20 ml plastic syringe. Since all blood analyses must be done on uncoagulated blood, the dead space of the syringe was filled with heparin (1000 USP units/ml). The blood was equilibrated with 3 different gas mixtures.

Procedure

The initial 20 ml sample was immediately placed on ice to slow in vitro metabolism. Approximately 6.5 ml of this blood was then transferred to a Dynex tonometer and equilibrated with a gas mixture of known pCO_2 and pO_2. (See Table 2 for the three gas mixtures that were used.) After equilibration of one hour the blood in the tonometer was withdrawn anaerobically into a 10 ml glass syringe. One milliliter aliquots of this blood were then transferred anaerobically from the syringe into five 2 ml glass syringes. (For details of the transfer technique see Appendix B.) Three of these 2 ml syringes contained anticoagulant of a specific concentration filling the dead space. The other two

syringes contained no anticoagulant and were used as control samples. All glass syringes were lubricated with stopcock grease to decrease the possibility of air leaking into the samples. The five samples thus obtained were immediately placed on ice. The blood samples were then analyzed in a random fashion using a Radiometer BMS3 Mark II blood-gas analyzer. The analyzer was calibrated each day prior to use according to the technique described in the Radiometer blood-gas analyzer Instruction Manual. The pCO_2 and pO_2 electrode calibrations were rechecked before every sample. The pH calibration was rechecked after every set of five samples.

While the first five samples were being analyzed a second 6.5 ml of blood was placed in the tonometer, allowed to equilibrate for one hour with a second gas mixture, and then transferred into syringes as described above. This second set of syringes contained the same anticoagulant in the same concentrations as the first set of syringes. While the second set of blood samples was being analyzed 6.5 ml of blood was allowed to equilibrate with a third gas mixture and the techniques described above were used to obtain a third set of five samples. The four anticoagulants in three concentrations were studied in this manner.

Since the EDTA mixture was found to result in pronounced changes in pH, tetrasodium EDTA and disodium EDTA were studied separately using the same techniques.

Dead Space Measurement

The dead space was measured on four 20 ml plastic syringes and eight 2 ml glass syringes which were used in the experiments. This was done so that the ratio of anticoagulant to blood could be determined.

For all dead space measurements a dye dilution technique was used. The dead space of 20 ml plastic syringes was filled with a concentrated potassium chromate solution. Care was taken so that no air bubbles remained in the dead space of the syringe. The solution from the dead space was then transferred to a volumetric flask and diluted to 250 ml. A spectrophotometer was used to determine the concentration of this 20 ml solution. The original dead space volume was then calculated. (See Appendix C for technique and the equation used.)

The eight 2 ml glass syringes were lubricated with stopcock grease. The same procedure was followed using a concentrated potassium chromate solution. The potassium chromate from the dead space of the 2 ml syringe was diluted in a 25 ml volumetric flask. Potassium chromate concentrations were determined and dead space volumes were calculated using the same methods as those for the 20 ml syringes.

Anticoagulant Solutions

Heparin. Heparin is readily available in solutions of various concentrations. Solutions of 1,000 USP units/ml; 10,000 USP units/ml, and 20,000 USP units/ml were chosen for the purposes of this study. Although the higher concentrations

of heparin were in excess of the amount necessary to prevent coagulation, it is possible these concentrations might inadvertently be used in a clinical situation for blood-gas analysis. Also, it was hoped these higher concentrations would magnify any changes in the results obtained from blood-gas analysis.

Trisodium citrate. Goodwin and Schreiber (1979) reported they used a 3.86% solution of trisodium citrate in their study. Raphael (1976, p. 1077) and Davidsohn and Henry (1974, p. 103) both indicate a 3.8% solution of trisodium citrate can be used in the ratio of one part citrate solution to nine parts blood to prevent coagulation. This would result in 4.2 mg of trisodium citrate per ml of blood. In order to approximate this concentration solutions of 7.72%, 15.44%, and 30.88% were prepared. This resulted in concentrations of trisodium citrate per ml of blood as follows: 2.2 mg citrate/ml of blood; 4.6 mg citrate/ml of blood; and 9.3 mg citrate/ml of blood. Only the lowest concentration of anticoagulant (7.72%) would result in less than the recommended concentration of citrate in blood. These anticoagulants were prepared at the same time, refrigerated until use, and used within 5 days of preparation.

Heller-Paul Oxalate. Heller-Paul Oxalate is an anticoagulant frequently used for blood analysis. The ammonium salt in this mixture will cause swelling of red blood cells when used alone while the potassium salt leads to shrinkage.

When these salts are used together in appropriate concentrations there is no change in erythrocyte volume.

Raphael (1976, p. 1077) recommends the following mixture:

Ammonium oxalate	1.2g
Potassium oxalate	0.8g
Distilled water	100ml

This mixture is usually dispensed into tubes in the proportion of 0.1 ml for each ml of blood. In order to provide for this ratio in the present studies it would be necessary to prepare the anticoagulant solution at least three times the recommended concentration. However, solutions greater than four times recommended concentration would not dissolve. Therefore, solutions were used of the recommended concentration, twice the recommended concentration, and only 4 times the recommended concentration. These solutions were prepared at the same time, refrigerated, and used as described for trisodium citrate.

EDTA. Raphael (1976, p. 1077) and Teitz (1976, p. 50) both recommend using 1 to 2 mg of EDTA per ml of blood. They indicate that the disodium or dipotassium salts are both used, the latter being more readily soluble. Goodwin and Schreiber (1979) did not indicate the type of EDTA they used. They reported that the values using EDTA "were so distorted from the norm" that the solution was used with only 2 samples and discarded. For the purpose of this study a mixture of half disodium EDTA and half tetrasodium EDTA was found to have a pH approximately the same as the normal pH of blood. It

was thought that this mixture might result in less distortion of pH values than those observed by Goodwin and Schreiber (1979). This EDTA mixture was prepared in 2%, 4% and 8% solutions. This resulted in concentrations of anticoagulant in blood of 0.6 mg per ml, 1.2 mg per ml, and 2.4 mg per ml. Thus, only the lowest concentration of anticoagulant (2%) was not within the recommended concentration.

Subsequent studies were done using the disodium EDTA and tetrasodium EDTA separately. Each anticoagulant was prepared in 2%, 4%, and 8% solutions resulting in the same anticoagulant to blood ratio as with the EDTA mixture.

All EDTA solutions were prepared and used in the same manner as the other anticoagulants previously discussed.

Coagulation Studies

All blood used in this study contained an anticoagulant (heparin) prior to being equilibrated with a known gas mixture. Therefore, it was not known if the other anticoagulants used in this study would prevent coagulation when used alone in the given concentrations. Coagulation studies as described in Appendix D were done using all the anticoagulants described above except heparin. Heparin was not studied because the amount of heparin used to initially prevent coagulation of the blood was much less than the subsequent amounts used for the study.

pH Measurements

In order to determine if the pH of the anticoagulants could be responsible for pH changes in the blood samples, pH measurements were obtained on all concentrations of anticoagulants using a Beckman pH electrode (39501) and meter (model 4500).

CHAPTER III

RESULTS

Dead Space Measurements

Results of dead space measurements are shown in Table 3. The mean dead space volume for the four 20 ml syringes was 0.214 ml (± 0.027 ml). The mean dead space volume for the eight 2 ml syringes was 0.03 ml (± 0.003 ml).

Coagulation Studies

Results of coagulation studies are shown in Table 4. The use of the lowest concentration of Heller-Paul oxalate, disodium EDTA, Tetrasodium EDTA, and the EDTA mixture all resulted in blood clotting in less than thirty minutes. The remaining concentrations of anticoagulants were adequate to prevent coagulation for at least 2 hours.

pH Measurement of Anticoagulants

Results of pH measurement of all anticoagulants used in this study are shown in Table 5. The pH ranged from quite acid (disodium EDTA) to quite alkaline (tetrasodium EDTA).

Results of Blood-Gas Analysis

The values (pH, pCO_2 and pO_2) obtained from blood-gas

analysis were from two control samples and three samples with additional anticoagulant of known concentration. Each set of five samples was from a larger sample placed in the tonometer and equilibrated with a known gas mixture. Since each of these larger samples was placed in the tonometer at different times and since three different gas mixtures were used, no statistical comparison could be made among sets of samples. The difference between the mean of two control samples and the mean of three samples with additional anticoagulant were analyzed using student's *t* test. While the number of samples analyzed was quite small, the precise measurements obtained with use of a blood-gas analyzer make this a valid statistical comparison.

Heparin

Heparin was the anticoagulant which least affected the results obtained from blood-gas analysis. The pH of some samples was less than that of controls while with other samples the pH was greater than controls (Figure 1). None of the pH changes were greater than 0.02 units different from control values (Table 6). Statistical analysis indicated only 4 of the 9 mean pH values differed significantly from controls.

The pCO_2 of the blood decreased when heparin was used as the anticoagulant (Table 7, Figure 2). The greatest change was evident with blood samples of the highest pCO_2. Statistical analysis indicated 6 out of 9 mean pCO_2 values differed significantly from controls. The mean decrease in pCO_2 values

with the most concentrated heparin was only 2 torr (Figure 2).

Two graphs are included (Figure 3, Figure 4) which show the changes in pO_2 values for all anticoagulants. The first graph indicates large standard deviations for changes with some anticoagulants. These large deviations are due to the fact that blood samples with high pO_2 values had a greater change from control samples than those samples with a lower pO_2. These greater changes may be the result of larger values initially. (For example, a 5% decrease of a pO_2 of 40 torr would result in a pO_2 of 38 torr while a 5% decrease of a pO_2 of 140 torr would result in a pO_2 of 133 torr). Also, the gas mixture with the high pO_2 resulted in pO_2 control values of 122 to 153 torr. These pO_2 values are higher than would normally be observed in a patient in a clinical setting. Therefore a second graph (Figure 4) is included which shows changes when samples with high pO_2 values are excluded. As can be seen from the second graph, changes are generally in the same direction but not as pronounced. In the case of heparin, pO_2 was decreased in most instances, but only 3 of 9 values differed statistically from controls (Table 8).

Heparin decreased the bicarbonate [HCO_3^-] concentration as did all the anticoagulants studied. However, as shown in Figure 5, heparin decreased the [HCO_3^-] the least. Statistically, less than half these decrements were significant (Table 9).

Trisodium citrate

Trisodium citrate consistently decreased the pH values of blood samples (Table 6, Figure 1). The higher the concentration of citrate the greater the decrease in pH. The mean decrement was 0.08 pH units when the most concentrated citrate was used (30.88%). Only 2 of the 9 mean measurements were not statistically different from controls. These two measurements occurred when the lowest concentration of citrate (7.72%) was used.

The pCO_2 changes with citrate varied (Figure 2). The lowest concentrations of citrate tended to decrease the pCO_2 while the higher concentrations were associated with increased pCO_2. None of these changes were greater than 2.8 torr and only 3 changes were found to be statistically significant (Table 7).

As previously discussed with heparin, there was a wide range of differences in pO_2 values with the use of citrate when all samples were considered (Figure 3). With the highest concentration of citrate (30.88%) and the highest pO_2 the decrease was 14.1 torr (Table 8). When high pO_2 samples were not considered the citrate still consistently decreased pO_2. Also, seven of these decreases were statistically significant.

The bicarbonate concentration was reduced with the use of citrate. The greatest reductions were found with the highest concentrations of citrate. In only one case the change was

not statistically significant (Table 9).

Heller-Paul Oxalate

The changes in the results obtained from blood-gas analysis using Heller-Paul oxalate as the anticoagulant were similar to those obtained with heparin. From Figure 1 it is evident that the oxalate decreased the pH values more than heparin but the decrease was less than for the other two anticoagulants. For the most part the changes in pH were not statistically significant (Table 6).

The pCO_2 values decreased with the use of oxalate in all instances. Most of these decrements were also statistically significant (Table 7). The greatest changes were observed with the highest pCO_2 values. As can be seen in Figure 2, when mean changes were considered the highest concentrations of oxalate decreased the pCO_2 the least.

In all cases oxalate decreased the pO_2 of the samples (Figures 3 and 4). The greatest decrease (12 torr) occurred at the highest pO_2 and the greatest concentration of oxalate. Five of the nine means were statistically different from control values (Table 8).

As with the use of other anticoagulants, bicarbonate concentration was decreased with the use of oxalate (Table 9). The largest decrease was 1.3 mEq/L which occurred with the most concentrated oxalate solution. Most of the $[HCO_3^-]$ decrements were statistically significant.

EDTA

The EDTA used for this study altered the values obtained during blood-gas analysis more than the three other anticoagulants. The pH was decreased as much as 0.08 units with the highest EDTA concentration (Table 6). All decreases in pH were statistically significant.

In contrast to other anticoagulants the use of EDTA resulted in increases in pCO_2 in every instance (Table 7). In general, the greatest increase in pCO_2 occurred with the use of the 8% EDTA solution. The change in pCO_2 was not statistically significant in only one instance.

The EDTA mixture was the anticoagulant which least affected the pO_2 (Table 8, Figures 3 and 4). In most cases EDTA increased the pO_2 but only one increase was statistically significant.

The $[HCO_3^-]$ was decreased with the use of EDTA. Seven of the nine decrements were statistically significant (Table 9).

The EDTA mixture changed the pH, pCO_2, and $[HCO_3^-]$ significantly but the pH of the solution of anticoagulant itself was close to the normal pH of the blood (pH 7.35). Due to these findings additional data was obtained using two different forms of EDTA as anticoagulants with blood equilibrated with gas mixture a (Table 2). Disodium EDTA and tetrasodium EDTA solutions were used in 2%, 4%, and 8% concentrations. The acid solution, disodium EDTA (see Table 5) decreased the pH while alkaline tetrasodium EDTA (Table 5) increased the pH (Table 10).

The pCO_2 was increased when disodium EDTA was used. Tetrasodium EDTA decreased the pCO_2 but only one of these decrements was statistically significant (Table 11).

Although the use of EDTA mixture did not change pO_2 consistently, disodium EDTA use resulted in an increased pO_2 while tetrasodium EDTA resulted in a decreased pO_2 (Table 12).

The use of disodium EDTA was associated with a decreased $[HCO_3^-]$ and these changes were statistically significant. The $[HCO_3^-]$ did not change when tetrasodium EDTA was used.

CHAPTER IV

DISCUSSION

The following discussion will focus on the significant differences between control and experimental blood-gas values that were observed in this study with the use of four anticoagulants and some possible mechanisms that might have brought about these differences. Then, the clinical implications of the use of these anticoagulants will be discussed.

Heparin

Heparin is an acidic mucopolysaccharide which is present in most tissues of the body in concentrations less than that required to prevent blood clotting. It is found in highest concentrations in the liver and lungs. Heparin acts as an anticoagulant by combining with antithrombin. The antithrombin-heparin complex then neutralizes thrombin which is essential for the clotting process. Heparin also inhibits other clotting factors (Meyers, Jawetz & Goldfien, 1980, p. 176).

The amount of heparin used in these studies exceeded the amount necessary to prevent coagulation. Filling the syringe dead space with heparin containing 1000 USP units/ml and then adding 1 ml of blood resulted in an anticoagulant concentration of approximately 30 units of heparin per ml of

blood. This is three times the concentration recommended by Raphael (1976, p. 1078). The heparin solutions of 10,000 units/ml and 20,000 units/ml resulted in heparin concentrations of 300 and 600 units per ml of blood respectively. Therefore, changes observed were magnified from those which would normally be seen in a clinical situation if the suggested concentration of heparin in blood were used.

Because the same volume of heparin was used in all cases (dead space volume of the syringe) changes observed in blood-gas composition with increasing concentrations of heparin can be attributed to the action of heparin itself and not to a dilution of the blood.

The pH changes in blood samples containing heparin were not consistent (Table 6). Therefore, one cannot conclude that the use of heparin results in either an increase or decrease in pH values. Although much of the literature reviewed suggests heparin decreases the pH of the blood, only two studies (Hamilton, et al., 1978 and Goodwin & Schrieber, 1979) stated that this change was significant. In the Hamilton study (1978) the authors stated "it is not until heparin concentration reaches approximately 25% that the pH fall could be clinically important." The authors varied the volume of heparin used, not the concentration, so it is difficult to compare their findings with the present study.

Goodwin and Schreiber found a measurable decrease in pH with increasing concentrations of heparin. However, they used

0.5 ml of anticoagulant in half of the samples. This is a much greater volume of anticoagulant than the amount used in the present study and much greater than should be used clinically. Also, mean values were reported from four patients but controls were not clearly identified and no standard deviations nor other estimates of variance were given making interpretation of the data very difficult. From the results reported in the present study it can be concluded that when heparin is used in the recommended concentration the pH of the blood is not affected.

The pCO_2 values were decreased with the use of heparin (Table 7, Figure 2). Most of the other studies previously cited also reported that heparin appeared to decrease the pCO_2. However, since large volumes of heparin were used in many of these other studies, this change can probably be attributed in part to dilution of blood samples. In the present study slightly greater changes in pCO_2 were observed with more concentrated heparin solutions. Therefore, it is possible that heparin itself directly affects the pCO_2. This change is minimal and the cause remains obscure.

The use of heparin was frequently associated with decreases in pO_2 from control values but most of these changes were not statistically significant. This finding is consistent with those from most other studies. The reason for the occasionally significant decrease in pO_2 is not apparent. These changes do not correlate with significant changes in pH or pCO_2.

All bicarbonate concentrations were calculated from

the Henderson-Hasselbalch equation (Appendix A). Decreases in pCO_2 and pH account for the decreases in $[HCO_3^-]$. Less than half the decrements were statistically significant (Table 9). Therefore, it can be concluded from the present study that use of heparin in appropriate concentrations does not result in crucial changes in $[HCO_3^-]$.

Trisodium Citrate

Trisodium citrate acts as an anticoagulant by binding calcium which is necessary for the clotting mechanism to occur. The amounts of trisodium citrate used in this study were adequate to prevent coagulation in the concentrations used. Very concentrated solutions were used due to the small volume of anticoagulant solution filling the dead space.

The pH of the trisodium citrate solutions used in this study were alkaline (pH 9.04-9.31). However, the use of citrate was associated with a decrease in the pH of blood samples. This is consistent with findings by Yoshimura (1935) and Schrieber (1979). Neither author speculated on causes of this change. It is possible that citrate which is not involved in the reaction with calcium diffuses into erythrocytes. Inside the red cells citrate might cause metabolic changes which would account for some of the differences observed in blood-gas values with the use of citrate.

Within the erythrocytes the citrate may act as an inhibitor of phosphofructokinase. This is the case when citrate is

produced by the mitochondria of other types of cells and the citrate enters the cytosol (Lehninger, 1976, p. 537). When phosphofructokinase is inhibited in the glycolytic pathway in erythrocytes, glucose-6-phosphate then enters the pentose pathway. In this pathway 6-phosphogluconate is decarboxylated to form a pentose-5-phosphate (Erslev & Gabuzda, 1975, p. 83). The resulting increased CO_2 concentration within the erythrocytes would then equilibrate with plasma (Figure 6). The increased $[CO_2]$ in the erythrocytes would also react with water to form increased carbonic acid (H_2CO_3) which in turn dissociates into hydrogen ion and $[HCO_3^-]$. This increased hydrogen ion concentration would cause a decrease in pH within the erythrocytes and in the plasma. One might then predict that $[HCO_3^-]$ of the erythrocytes and plasma would increase. However, citrate also causes an increased rate of glycolysis via the pentose phosphate pathway. The end product of this anaerobic metabolism is lactic acid. The $[HCO_3^-]$ in the red blood cells would buffer some of the H^+ from the lactic acid. The net effect would be a reduced $[HCO_3^-]$ in the red blood cells and also in the plasma. This mechanism might account for decreases in pH, and $[HCO_3^-]$ observed with the use of citrate.

The use of citrate was not related to an increase in pCO_2 until the most concentrated citrate solution was used. The less concentrated citrate solutions (7.72%, 15.44%) might not have resulted in enough excess citrate entering the erythrocytes to produce measureable changes in the plasma pCO_2.

The use of citrate was related to a decrease in pO_2. The greatest decrease was seen with the use of the most concentrated citrate solution (Table 8). This is not consistent with the mechanism described above. Within the erythrocytes an increase in $[H^+]$ and an increase in pCO_2 causes hemoglobin to give up oxygen (Bohr effect). This oxygen equilibrating with plasma would tend to increase the pO_2. Since the change in pO_2 is opposite to that predicted other metabolic processes in the blood are probably affected with the use of trisodium citrate solutions.

Heller-Paul Oxalate

Oxalates act as anticoagulants by precipitating calcium so that it is not available for the clotting mechanism (Raphael, 1976, p. 1077). The standard Heller-Paul oxalate mixture was the least concentrated oxalate solution used in this study. This lowest concentration of the mixture was not adequate to prevent coagulation with the blood and anticoagulant (dead space) volumes used in this study (Table 4). Therefore, it is more appropriate to consider changes in blood-gas values with the use of the more concentrated oxalate solutions.

As can be seen from Figure 1, the pH changes with the use of oxalate are comparable to those with the use of heparin. Considering the more concentrated oxalate solutions, half of the changes are statistically significant. The acidity of the more concentrated oxalate solutions (Table 5) might account

for these observed changes.

A decrease in pCO_2 was observed with the use of oxalate (Figure 2). However, the decrement was less with higher concentrations of oxalate. While many of these changes were statistically significant they resulted in an average pCO_2 decrement of less than two torr. This change is minimal and the cause is not apparent.

The use of oxalate was associated with decreases in pO_2, especially with the most concentrated oxalate mixture. The significant decreases in pO_2 correlate with significant decrements in pCO_2. However, the decrements in pO_2 averaged less than 6 torr even when the gas mixtures with high pO_2 were considered (Figure 3). Again, the cause of this change is not known.

The bicarbonate concentration decreased an average of 1.0 mEq/L or less with the use of the oxalate mixture. This decrease is consistent with decreases in pH and pCO_2. In some instances previously described the pH of the oxalate mixture may have been sufficiently low to decrease the blood pH. This decreased pH would decrease plasma $[HCO_3^-]$ by buffering. In other instances the cause of this decrease in $[HCO_3^-]$ is not known.

EDTA

Ethylene diamine tetraacetic acid acts as an anticoagulant by chelating the calcium ion so that it cannot act in the clotting mechanism (Raphael, 1976, p. 1077). The EDTA mixture of lowest concentration used in this study was not

adequate to prevent coagulation of blood (Table 4). Therefore, the use of this anticoagulant should be evaluated in terms of the more concentrated solutions.

The EDTA mixture that was used in this study was chosen because it had a pH that was close to the normal pH of arterial blood. It was hoped that this would eliminate the changes in pH associated with the use of EDTA as reported by Goodwin and Schreiber (1979). However, use of the EDTA mixture still resulted in average decreases in pH of 0.05 units. It may be that when the EDTA solution was added to blood the molecules of EDTA dissociated releasing H^+ ions. Since such a concentrated EDTA solution was used a sufficient number of H^+ ions might have been released to decrease the pH (Figure 7).

The pCO_2 increased with the use of the EDTA mixture. If the EDTA released H^+ ions in plasma as postulated above then plasma $[HCO_3^-]$would be consumed in the buffering reaction. The net result would be a decreased plasma $[HCO_3^-]$ and an increased pCO_2. This, in fact, was the case. The $[HCO_3^-]$ decreased with the use of the EDTA mixture and the pCO_2 increased (Tables 7 and 9).

The mixture of EDTA used in this study did not significantly alter the pO_2. From Figure 7 it would appear that pO_2 should increase but this was not the case.

Further EDTA Studies

Each type of EDTA was studied separately to determine its

separate actions. It is significant that the mixture solutions were prepared of equal weights of each kind of EDTA. The molecular weight of disodium EDTA is 372 while the molecular weight of tetrasodium EDTA is 416. Therefore, the mixture solutions contained more disodium than tetrasodium EDTA molecules. This may account for the fact that the EDTA mixture reacted more like the disodium EDTA.

The use of disodium EDTA which is acidic was associated with changes in blood-gas values in the same direction but of a greater magnitude than the changes observed with the EDTA mixture (Tables 10, 11, and 13). In addition, pO_2 was significantly increased over control values (Table 12). All of these changes can be accounted for by the mechanisms illustrated in Figure 7 and previously described.

The use of tetrasodium EDTA was associated with changes in the blood-gas values that were opposite from those seen with the use of disodium EDTA (Tables 10, 11 and 12). Figure 8 shows the mechanisms that would account for all the observed changes of decreased pCO_2 and pO_2 and increased pH. However, from the model it would be expected that bicarbonate concentration would have increased but this was not the case (Table 13). Since tetrasodium EDTA solution was not as concentrated as the disodium EDTA it is possible that a more concentrated tetrasodium solution is necessary to exhibit measurable changes in $[HCO_3^-]$.

Clinical Implications

Values obtained from blood-gas analysis are used with several other parameters to assess a patient's cardiopulmonary function or acid-base status. Ideally, blood-gas analysis should result in values as close as possible to the true in vivo composition of the patient's blood at the site from which the sample was obtained. Medical and nursing judgements are made taking into consideration the patient's appearance, vital signs, past medical history, as well as all other pertinent laboratory tests. It is doubtful that minor changes in blood-gas values alone would result in changes in medical treatment. However, textbooks or manuals frequently give limiting blood-gas values to consider when evaluating the need for treatment. The following examples are from the Manual of Medical Therapeutics (Freitag & Miller, 1980):

> In an acutely ill patient with an arterial blood pH of less than 7.2, $NaHCO_3$ should be used parenterally. (p. 39)
>
> Therapy is critical when HCO_3 falls below 15 mEq/L. (p. 39)
>
> Generally, patients receiving high concentrations of inspired oxygen who have a persistently high respiratory rate (>35/min.), a PaO_2 less than 50 mmHg, or acidemia (pH < 7.3) usually require intubation and mechanical ventilation. (p. 150)

Such guidelines, when taken out of context, might mean that some changes in blood-gas values due to the use of anticoagulants as shown in this study would result in inappropriate medical treatment.

Heparin

In general heparin is the most reliable anticoagulant for use with blood-gas analysis. As previously discussed the concentrations used in this study were in excess of concentrations and amounts necessary to prevent coagulation. Considering the experiments with heparin 1000 units/ml, the pH was increased an average of 0.003 units; the mean pCO_2 decrease was 1 torr; the mean pO_2 decrease was 3.8 torr; and the $[HCO_3^-]$ was increased an average of 0.5 mEq/L. It is highly unlikely that changes in blood-gas values of this magnitude would result in changes in a patient's medical treatment. When minimal concentrations of heparin are used it is even less likely that changes in blood-gas values will have clinical significance.

Contrary to other parts of the world (Goodwin & Schreiber, 1979) heparin is readily available and inexpensive in the United States. Heparin 1000 USP units/ml is available in multiple dose 10 ml vials at a cost of 65¢. Even if blood-gases were being drawn hourly from a patient and excessive amounts of heparin were being withdrawn and discarded, heparin use would constitute a very small portion of patient expenses.

A further advantage to the use of heparin is that it is nontoxic. Should a small amount of sterile heparin be inadvertently injected into a patient's artery during blood sampling the heparin would be rapidly degradated and the low concentration of heparin would have no noticeable effect on blood coagulation.

Trisodium Citrate

The solutions of trisodium citrate used in this study would not be appropriate for use with blood samples for blood-gas analysis. The lowest concentrations of citrate resulted in a mean decrease of 0.016 pH units, a mean decrease in pCO_2 of 1.6 torr, an average pO_2 decrease of 7.8 torr and an average $[HCO_3^-]$ decrease of 1.2 mEq/L. Changes of this magnitude could cause misdiagnosis of a patient's condition and perhaps lead to inappropriate intervention especially if the patient had abnormal blood-gas values initially.

In some cases significant changes occurred in pH and $[HCO_3^-]$ without concomitant changes in pCO_2 or pO_2. These changes might lead to an inappropriate diagnosis of a metabolic acidosis. Furthermore, many of the changes in blood-gas values became more apparent with more concentrated citrate solutions. Thus, if an excessive concentration or volume of citrate were inadvertently used in a clinical setting, the changes in blood gas values would be even greater.

Goodwin and Schreiber (1979) reported that "citrate would appear to offer (a) reasonable, cheap, and readily available" alternative to the use of heparin. However, they used a 3.86% trisodium citrate solution and they reported the pH of this solution as 7.65. The authors do not state if this was a commercially prepared solution. This might account for the discrepancy between the pH of the citrate solution Goodwin and Schreiber used and the pH of the citrate

solutions in the present study. The different properties of the citrate solution Goodwin and Schreiber used might account in part for their evaluation. The authors did report that when "an excessive volume of citrate . . . (was) used, the pH was excessively low." The authors give no evidence or arguments in support of their evaluation that citrate is a reasonable alternative to heparin. Certainly the results of the present study do not support their conclusions.

Heller-Paul Oxalate

The Heller-Paul oxalate compared favorably to heparin as an anticoagulant for use in blood-gas analysis. As can be seen from Figures 1-5 changes in blood-gas values are generally in the same direction and of the same magnitude as compared with heparin. Clinically, these differences would probably not result in a change in medical treatment. However, the solution is not presently available and must be prepared chemically. Also, the solution is toxic (Davidsohn & Henry, 1974, p. 103) and the inadvertent injection of the anticoagulant into a patient's bloodstream might cause local or systemic reactions.

EDTA

The EDTA mixture used in this study is not appropriate for use in blood-gas analysis. When concentration 2 (Table 1) was used the mean decrease in pH was 0.073 units, the pCO_2 increased an average of 4.4 torr, and the mean decrease in $[HCO_3^-]$ was 1.6 mEq/L. These differences have obvious clinical

significance. The use of the mixture might be considered if only the pO_2 of the blood sample were required since the mixture affected the pO_2 the least of any of the anticoagulants studied. However, a patient should be evaluated with the use of all blood-gas parameters. Therefore, the mixture probably has no value in a clinical setting.

CHAPTER V

SUMMARY AND CONCLUSIONS

Throughout this study there were inconsistent changes brought about with the use of different anticoagulants. Several possible explanations are presented which may account for these inconsistencies.

Some of the changes observed may have been due to a dilutional effect. Since control syringes did not contain any solution in the dead space the volume of dead space might account for some of the decrements seen. However, this volume was small (about 3% of the total sample) and could not account for increasing changes seen with increasing concentrations of anticoagulant.

Hemolysis or crenation of red blood cells may have occurred due to the acidity, alkalinity or concentration of the anticoagulants used. Although this was not observed in any of the blood samples it is possible that damage could have occurred to enough erythrocytes to change the properties of the blood. If this type of reaction were occurring in some of the samples it would be a further reason for not using some of these anticoagulants.

When the dead space of syringes was filled with anticoagulant, care was taken to be consistent in the volume used. However, preliminary studies indicated that dead space volume

varied from 0.025 to 0.037 ml. These differences in volume might account for some of the inconsistencies observed.

This study has demonstrated that heparin produces minimal changes in blood-gas values when used in the recommended concentration. Since it is also nontoxic, inexpensive, and readily available in the United States there seems little need to promote the use of other anticoagulants for blood-gas analysis. In parts of the world where shortages of heparin have occurred the occasional use of Heller-Paul oxalate might be justified. However, care needs to be taken so that an excess amount of anticoagulant is not used and that none of the anticoagulant is injected into the patient.

Some mechanisms have been postulated for the differences in blood-gas values with the use of trisodium citrate and EDTA. These mechanisms do not totally account for the observed differences and other metabolic changes undoubtedly occurred. It is not within the scope of this study to explore all the possible metabolic disturbances that might have taken place with the erythrocytes and plasma. This study has demonstrated that trisodium citrate and EDTA are not appropriate alternatives to the use of heparin for sampling of blood for blood-gas analysis.

Suggested Future Studies

While heparin has been used extensively for arterial blood sampling there is little information regarding the use of Heller-Paul oxalate clinically. Studies need to be done

obtaining simultaneous arterial blood samples from patients. One syringe would contain heparin filling the dead space while the other would contain Heller-Paul oxalate. These samples could be analyzed immediately and the results compared. Several samples thus obtained would provide more information on the feasibility of using Heller-Paul oxalate when heparin is unavailable.

Further studies also need to be done evaluating variations in technique with the use of heparin. Refrigeration of heparin or injection of air into multiple dose heparin vials are two factors that might alter the effect of heparin on the values obtained during artieral blood-gas analysis.

Finally, further studies are needed to evaluate other sampling and storage techniques which may contribute to changes in the arterial blood sample before analysis. Ideally, technologists may some day devise a system that will monitor in vivo blood-gas composition. Until that time, care of critically ill patients depends upon blood sampling techniques which provide samples with blood-gas composition as close to the in vivo values as possible.

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Table 1

Anticoagulant Solutions Used in This Study

Concentration of Anticoagulant	Sodium Heparin	Trisodium Citrate	Heller-Paul Oxalate	EDTA Mixture	Disodium EDTA	Tetrasodium EDTA
1	1000 USP units/ml (Abbott Laboratories)	7.72g/ 100ml H_2O	Ammonium Oxalate 1.2g Potassium Oxalate 0.8g H_2O 100 ml	Disodium EDTA 1g Tetrasodium EDTA 1g H_2O 100ml	2g/ 100ml H_2O	2g/ 100ml H_2O
2	10,000 USP units/ml (Organon)	15.44g/ 100ml H_2O	Ammonium Oxalate 2.4g Potassium Oxalate 1.6g H_2O 100 ml	Disodium EDTA 2g Tetrasodium EDTA 2g H_2O 100ml	4g/ 100ml H_2O	4g/ 100ml H_2O
3	20,000 USP units/ml (Organon)	30.88g/ 100ml H_2O	Ammonium Oxalate 4.8g Potassium Oxalate 3.2g H_2O 100 ml	Disodium EDTA 4g Tetrasodium EDTA 4g H_2O 100ml	8g/ 100ml H_2O	8g/ 100ml H_2O

Table 2

Gas Mixtures Used in This Study

	Oxygen	Carbon Dioxide	Nitrogen
a	5%	10%	85%
b	10%	5%	85%
c	20%	3%	77%

Table 3

Dead Space Measurement of Syringes

2ml Syringes		20ml Syringes	
	Volume ml		Volume ml
	0.032		0.223
	0.032		0.196
	0.026		0.205
	0.031		0.185
	0.030		
	0.030		
	0.037		
	0.025		
$\bar{x}$	0.030	$\bar{x}$	0.214
SD	±0.003	SD	±0.027

Table 4

Results of Blood Coagulation Studies

Concentration of Anticoagulant	Heller-Paul Oxalate	Trisodium Citrate	Disodium EDTA	Tetrasodium EDTA	Mixed EDTA
1	both tubes clotted in 20 min.	ϴ	one tube clotted in 10 min. one tube clotted in 30 min.	one tube clotted in 20 min. one tube clotted in 30 min.	both tubes clotted in 5 min.
2	ϴ	ϴ	ϴ	ϴ	ϴ
3	ϴ	ϴ	ϴ	ϴ	ϴ

ϴ indicates no evidence of clotting was observed in 2 hours

Table 5

The pH of Anticoagulant Solutions Used in This Study

Concentration of Anticoagulant	Heparin	Heller-Paul Oxalate	Trisodium Citrate	EDTA Mixture	Disodium EDTA	Tetrasodium EDTA
1	6.872	6.894	9.040	7.340	4.503	10.822
2	6.975	6.886	9.125	7.307	4.465	10.911
3	7.122	6.803	9.308	7.283	4.453	10.944

Table 6

Effect of Anticoagulant Type and Concentration on pH

Concentration of Anticoagulant		Heparin		Trisodium Citrate		Heller-Paul Oxalate		EDTA Mixture	
		Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
1	a	7.128 ±0.007	7.134 ±0.011	7.176 ±0.002	7.160 ±0.006	7.189 ±0.003	7.191 ±0.001	7.192 ±0.002	7.146* ±0.011
	b	7.333[d]	7.320 ±0.008	7.354 ±0.001	7.339* ±0.010	7.322 ±0.006	7.324 ±0.003	7.339 ±0.008	7.291* ±0.002
	c	7.419 ±0.006	7.435* ±0.002	7.431 ±0.001	7.429 ±0.006	7.458 ±0.005	7.448 ±0.005	7.456 ±0.004	7.399* ±0.007
2	a	7.159 ±0.003	7.167* ±0.001	7.197 ±0.004	7.155* ±0.001	7.169 ±0.001	7.169 ±0.001	7.175 ±0.004	7.102* ±0.005
	b	7.354 ±0.003	7.363* ±0.001	7.335 ±0.001	7.307* ±0.009	7.326 ±0.004	7.332 ±0.006	7.360 ±0.006	7.294* ±0.002
	c	7.421 ±0.014	7.430 ±0.006	7.483 ±0.000	7.453* ±0.012	7.445 ±0.003	7.437* ±0.002	7.462 ±0.004	7.383* ±0.004
3	a	7.171 ±0.009	7.180 ±0.004	7.178 ±0.007	7.106* ±0.008	7.177 ±0.002	7.172* ±0.002	7.191 ±0.012	7.110* ±0.001
	b	7.332 ±0.001	7.329 ±0.003	7.351 ±0.001	7.260* ±0.007	7.340 ±0.002	7.330 ±0.011	7.321 ±0.003	7.245* ±0.002
	c	7.459 ±0.002	7.447* ±0.001	7.450 ±0.004	7.357* ±0.005	7.442 ±0.003	7.413* ±0.004	7.455 ±0.001	7.378* ±0.002

Note: Values shown are pH mean ±1 SD
Concentrations of anticoagulants are described in Table 1
Control samples contained 10 units heparin/ml blood
a,b,c Indicates gas mixtures as shown in Table 2
d Indicates only one control sample
* $p < .05$ where p is the probability that the values tested are from the same population.

Table 7

Effect of Anticoagulant Type and Concentration on pCO_2

Concentration of Anticoagulant		Heparin		Trisodium Citrate		Heller-Paul Oxalate		EDTA Mixture	
		Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
1	a	73.8 ±0.3	71.6* ±0.4	73.0 ±2.0	70.2 ±0.4	73.4 ±0.4	70.2* ±0.3	74.6 ±0.4	78.5* ±0.8
	b	38.6[d]	38.9 ±0.8	38.2 ±0.1	37.1* ±0.5	39.3 ±0.4	37.7* ±0.4	39.5 ±0.3	41.4 ±0.6
	c	24.6 ±0.1	23.4* ±0.2	25.4 ±1.2	24.2 ±0.0	23.9 ±0.0	23.1* ±0.2	24.4 ±0.0	25.9* ±0.2
2	a	76.6 ±0.0	73.3* ±0.2	71.9 ±0.6	70.8 ±0.4	71.0 ±1.2	69.7 ±0.5	73.6 ±0.7	80.5* ±0.2
	b	39.2 ±0.8	36.4* ±0.4	39.8 ±0.1	38.9 ±0.4	40.1 ±0.5	37.7* ±0.2	39.1 ±0.5	42.9* ±1.0
	c	25.3 ±0.6	23.8* ±0.2	24.5 ±0.0	24.2 ±0.2	24.5 ±0.3	23.9 ±0.2	25.0 ±0.1	27.6* ±0.0
3	a	77.8 ±2.7	73.2 ±1.2	73.7 ±2.0	74.8 ±0.2	73.2 ±0.9	70.8* ±0.6	76.9 ±2.2	83.7* ±0.8
	b	38.3 ±0.7	37.5 ±0.1	38.1 ±0.2	39.4* ±0.1	38.2 ±0.2	37.7* ±0.1	38.6 ±0.1	42.8* ±0.1
	c	24.8 ±0.2	23.8* ±0.3	24.6 ±0.1	26.0* ±0.3	24.8 ±0.0	24.4* ±0.1	23.6 ±0.1	26.7* ±0.4

Note: Values shown are pCO_2 (torr.) mean ± 1 SD.
Concentrations of anticoagulants are described in Table 1.
Control samples contained 10 units heparin/ml blood.
a,b,c Indicates gas mixtures as shown in Table 2.
d Indicates only one control sample.
* $p < .05$ where p is the probability that the values tested are from the same population.

Table 8

Effect of Anticoagulant Type and Concentration on pO_2

Concentration of Anticoagulant		Heparin		Trisodium Citrate		Heller-Paul Oxalate		EDTA Mixture	
		Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
1	a	38.0 ±0.7	37.7 ±1.1	38.1 ±0.1	36.3* ±0.4	38.0 ±0.7	36.9 ±1.3	39.6 ±0.0	39.5 ±0.3
	b	77.2[d]	78.5 ±1.9	75.8 ±1.1	72.4* ±0.7	79.2 ±0.7	75.1* ±0.6	81.5 ±1.6	82.2 ±0.4
	c	141.3 ±2.1	129.4* ±2.7	153.0 ±2.0	134.8* ±1.9	129.1 ±4.4	123.3 ±1.7	133.6 ±1.1	137.0 ±1.8
2	a	37.2 ±0.7	36.9 ±1.4	37.3 ±0.1	34.4* ±0.4	38.1 ±1.2	37.0 ±0.2	37.5 ±0.9	38.4 ±0.1
	b	75.7 ±3.0	71.9 ±1.1	77.6 ±1.4	77.1 ±0.4	78.5 ±1.0	72.7* ±1.4	77.8 ±1.4	80.3 ±1.4
	c	139.7[d]	136.7 ±2.9	122.3 ±0.3	121.4 ±3.9	124.6 ±2.4	123.5 ±0.8	132.7 ±3.0	132.6 ±2.5
3	a	37.8 ±1.0	34.8* ±0.4	37.6 ±0.1	33.3* ±0.2	35.5 ±0.2	33.9* ±0.4	37.8 ±0.1	38.9 ±0.7
	b	76.9 ±0.2	73.5* ±0.3	76.1 ±0.2	70.1* ±1.3	74.4 ±0.5	72.2* ±0.8	79.5 ±0.0	81.1* ±0.7
	c	138.5 ±0.9	130.2 ±4.8	128.8 ±2.1	114.7* ±3.9	132.9 ±4.5	120.9* ±1.6	127.3[d]	130.4 ±0.2

Note: Values shown are pO_2 (torr.) mean ± 1 SD.
Concentrations of anticoagulants are described in Table 1.
Control samples contained 10 units heparin/ml blood.
a,b,c Indicates gas mixtures as shown in Table 2.
d Indicates only one control sample
*p < .05 where p is the probability that the values tested are from the same populatio

Table 9

Effect of Anticoagulant Type and Concentration on $[HCO_3^-]$

Concentration of Anticoagulant		Heparin		Trisodium Citrate		Heller-Paul Oxalate		EDTA Mixture	
		Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
1	a	23.7 ±0.4	23.3 ±0.7	26.1 ±0.6	24.3* ±0.3	27.1 ±0.1	26.0* ±0.1	27.7 ±0.4	26.3 ±0.6
	b	19.9[d]	19.8 ±0.3	20.6 ±0.1	19.3* ±0.5	19.7 ±0.0	19.0* ±0.2	20.6 ±0.2	19.2* ±0.3
	c	15.5 ±0.2	15.3 ±0.2	16.4 ±0.7	15.6 ±0.3	16.4 ±0.2	15.5* ±0.2	16.6 ±0.2	15.5* ±0.3
2	a	26.4 ±0.2	25.8* ±0.1	27.1 ±0.0	24.2* ±0.1	25.1 ±0.3	24.6 ±0.2	26.3 ±0.0	24.3* ±0.2
	b	21.1 ±0.3	20.1* ±0.2	20.6 ±0.1	18.9* ±0.3	20.3 ±0.0	19.4* ±0.3	21.4 ±0.0	20.2 ±0.5
	c	15.9 ±0.1	15.3* ±0.2	17.8 ±0.0	16.4* ±0.4	16.4 ±0.1	15.6* ±0.1	17.4 ±0.2	15.9* ±0.1
3	a	27.6 ±0.4	26.5 ±0.4	26.5 ±0.2	22.8* ±0.4	26.3 ±0.3	25.1* ±0.2	28.5 ±0.0	25.8* ±0.3
	b	19.6 ±0.4	19.1 ±0.2	20.4 ±0.0	17.2* ±0.2	20.0 ±0.0	19.4 ±0.4	19.4 ±0.1	18.0* ±0.1
	c	17.1 ±0.2	15.9* ±0.3	16.7 ±0.1	14.7* ±0.1	16.4 ±0.1	15.1* ±0.2	16.0 ±0.1	15.2* ±0.2

Note: Values shown are $[HCO_3^-]$ (mEq/L) mean ± 1 SD.
Concentrations of anticoagulants are described in Table 1.
Control samples contained 10 units heparin/ml blood.
a,b,c Indicates gas mixtures as shown in Table 2.
d Indicates only one control sample.
*$p < .05$ where p is the probability that the values tested are from the same population.

Table 10

Effect of Three EDTA Solutions on pH

Concentration of Anticoagulant	Mixed EDTA		Disodium EDTA		Tetrasodium EDTA	
	Control	Experimental	Control	Experimental	Control	Experimental
1	7.192 ±0.002	7.146* ±0.011	7.179 ±0.008	7.078* ±0.024	7.192 ±0.002	7.198 ±0.003
2	7.175 ±0.004	7.102* ±0.005	7.187 ±0.002	7.076* ±0.009	7.200 ±0.005	7.241* ±0.016
3	7.191 ±0.012	7.110* ±0.001	7.189 ±0.006	6.905* ±0.013	7.181 ±0.007	7.274* ±0.026

Note: pH mean ±1 SD
Concentrations of anticoagulants described in Table 1
Control samples contained 10 units Heparin/ml blood
* $p < .05$

Table 11

Effect of Three EDTA Solutions on pCO_2

Concentration of Anticoagulant	Mixed EDTA		Disodium EDTA		Tetrasodium EDTA	
	Control	Experimental	Control	Experimental	Control	Experimental
1	74.6 ±0.4	78.5* ±0.8	73.5 ±0.7	82.6* ±3.6	72.2 ±0.8	69.5 ±1.1
2	73.6 ±0.7	80.5* ±0.2	73.1 ±0.2	85.4* ±1.6	70.2 ±2.3	63.7 ±3.3
3	76.9 ±2.2	83.7* ±0.8	73.2 ±0.4	108.1* ±7.9	72.9 ±1.0	59.3* ±4.5

Note: pCO_2 (torr.) mean ±1SD
Concentrations of anticoagulants described in Table 1
Control samples contained 10 units Heparin/ml blood
* $p < .05$

Table 12

Effect of Three EDTA Solutions on pO_2

Concentration of Anticoagulant	Mixed EDTA		Disodium EDTA		Tetrasodium EDTA	
	Control	Experimental	Control	Experimental	Control	Experimental
1	39.6 ±0.0	39.2 ±0.4	39.1 ±0.0	42.7* ±1.1	37.6 ±0.4	36.5* ±0.2
2	37.5 ±0.9	38.4 ±0.1	38.4 ±0.9	44.9* ±1.7	38.8 ±0.5	35.1* ±0.9
3	37.8 ±0.1	38.5 ±0.6	37.4 ±0.4	49.8* ±0.2	38.4 ±0.2	32.0* ±1.3

Note: pO_2 (torr.) mean ±1 SD
Concentrations of anticoagulants described in Table 1
Control samples contained 10 units Heparin/ml blood
* $p < .05$

Table 13

Effect of Three EDTA Solutions on HCO_3^-

Concentration of Anticoagulant	Mixed EDTA		Disodium EDTA		Tetrasodium EDTA	
	Control	Experimental	Control	Experimental	Control	Experimental
1	27.7 ±0.4	26.3 ±0.6	26.5 ±0.3	23.6* ±0.7	26.8 ±0.4	26.2 ±0.2
2	26.3 ±0.0	24.3* ±0.2	26.9 ±0.2	24.3* ±0.7	26.6 ±0.6	26.7 ±0.5
3	28.5 ±0.0	25.8* ±0.3	27.2 ±0.2	20.7* ±1.0	26.4 ±0.0	26.6 ±0.9

Note: $[HCO_3^-]$ (mEq/L) mean ±1 SD
Concentrations of anticoagulants described in Table 1
Control samples contained 10 units Heparin/ml blood
*$p < .05$

Figure 1. Difference between control and experimental values of pH using different anticoagulants. Mean values ± 1 SD are shown. The numbers on the abcissa indicate concentrations of anticoagulants as presented in Table 1.

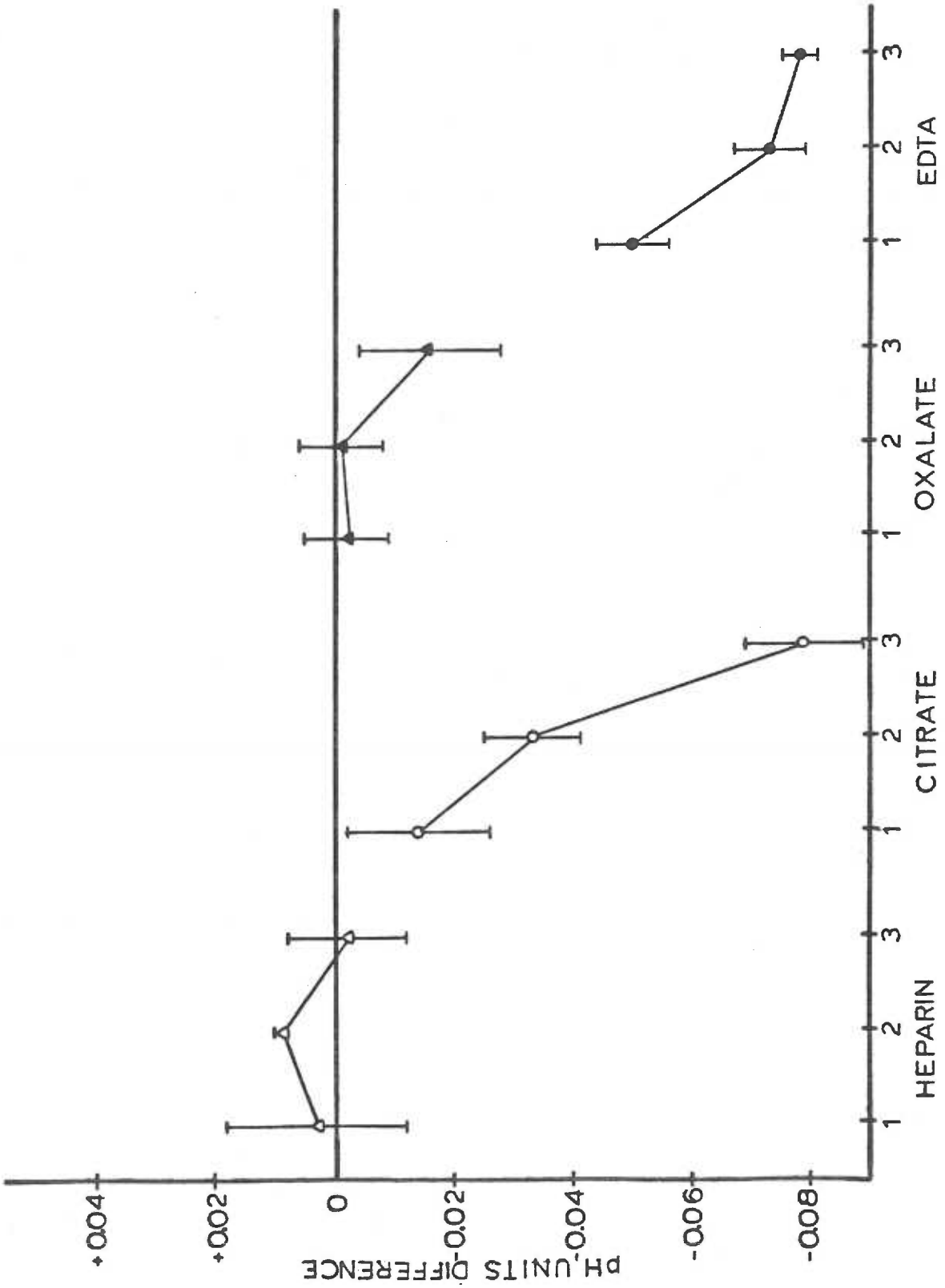

+0.04
+0.02
0
-0.02
-0.04
-0.06
-0.08
pH UNITS DIFFERENCE
1
2
3
HEPARIN
CITRATE
OXALATE
EDTA

Figure 2. Difference between control and experimental values of pCO_2 (torr) using different anticoagulants. Mean values ± 1 SD are shown. The numbers on the abcissa indicate concentrations of anticoagulants as presented in Table 1.

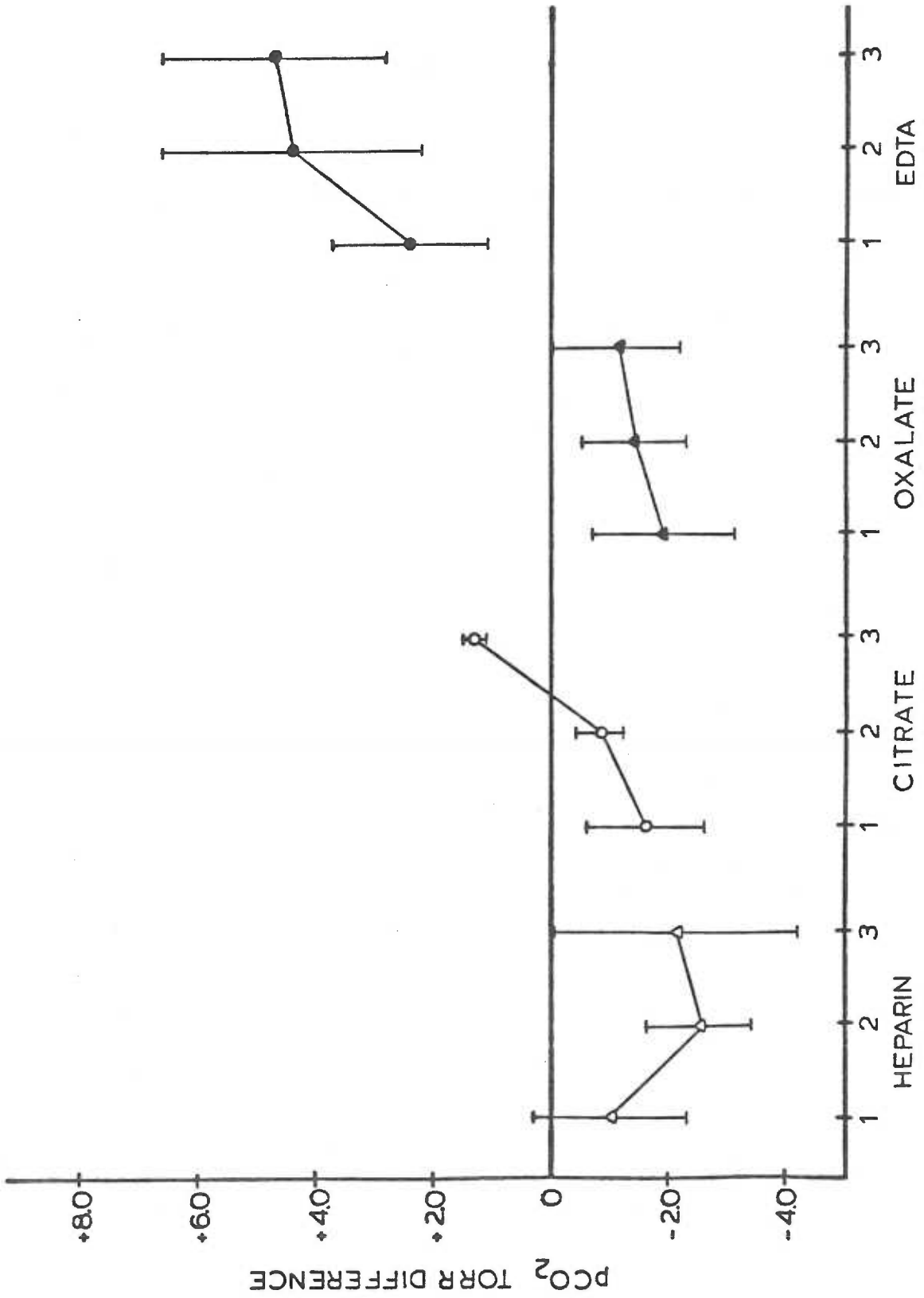

+8.0
+6.0
+4.0
+2.0
0
-2.0
-4.0
pCO_2 TORR DIFFERENCE
1
2
3
HEPARIN
CITRATE
OXALATE
EDTA

Figure 3. Difference between control and experimental values of pO_2 (torr) using different anticoagulants. Mean values ± 1 SD are shown. The numbers on the abcissa indicate concentrations of anticoagulants as presented in Table 1.

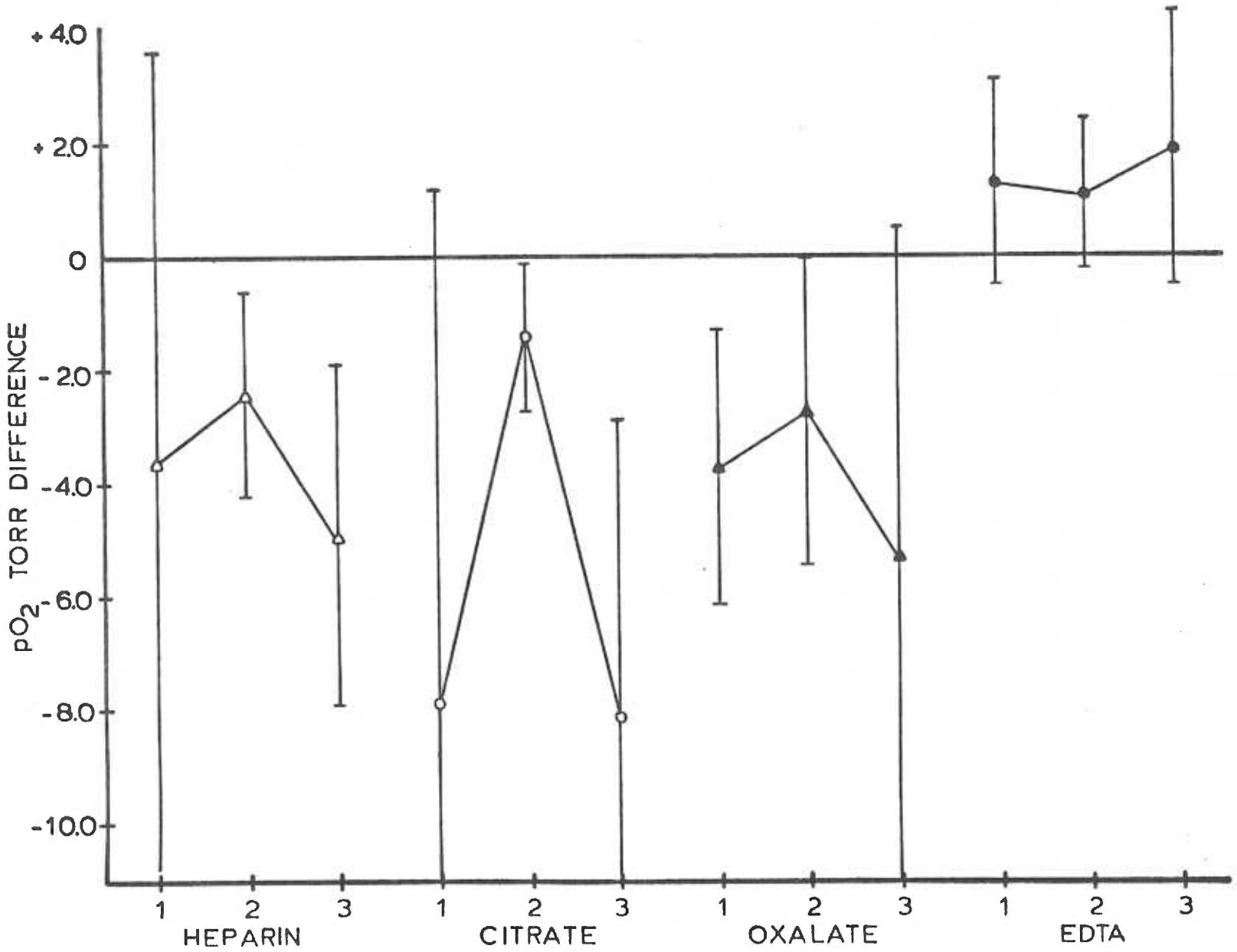

+4.0
+2.0
0
-2.0
-4.0
-6.0
-8.0
-10.0
pO2 TORR DIFFERENCE
1
2
3
HEPARIN
CITRATE
OXALATE
EDTA

Figure 4. Differences between control and experimental values of pO_2 (torr) using different anticoagulants. Results from the gas mixture with a high pO_2 are not included. Mean values ± 1 SD are shown. The numbers on the abcissa indicate concentrations of anticoagulants as presented in Table 1.

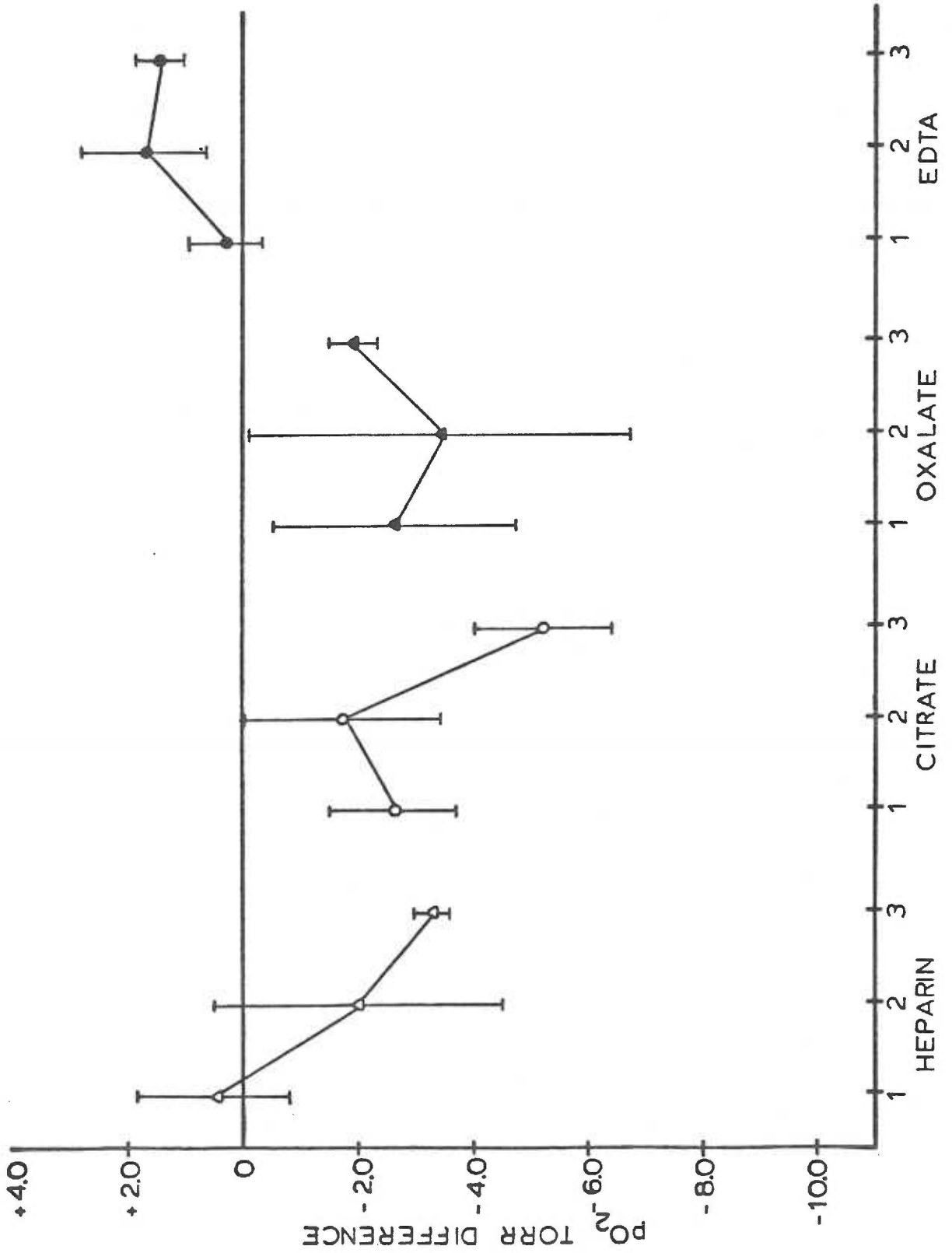

+4.0
+2.0
0
-2.0
-4.0
-6.0
-8.0
-10.0
pO_2 TORR DIFFERENCE
1
2
3
HEPARIN
CITRATE
OXALATE
EDTA

Figure 5. Difference between control and experimental values of $[HCO_3^-]$ (mEq/L) using different anticoagulants. Mean values ± 1 SD are shown. The numbers on the abcissa indicate concentrations of anticoagulant as presented in Table 1.

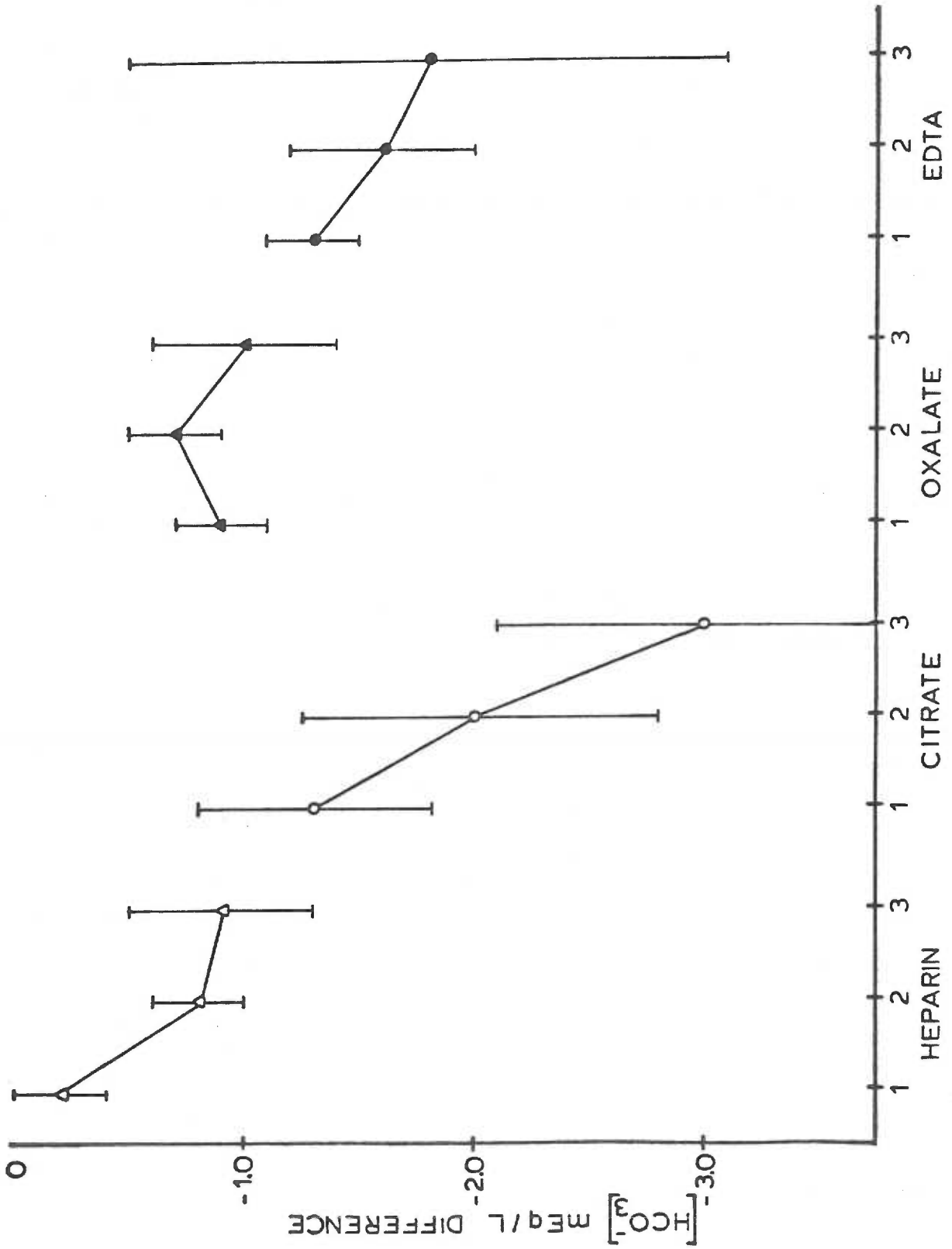
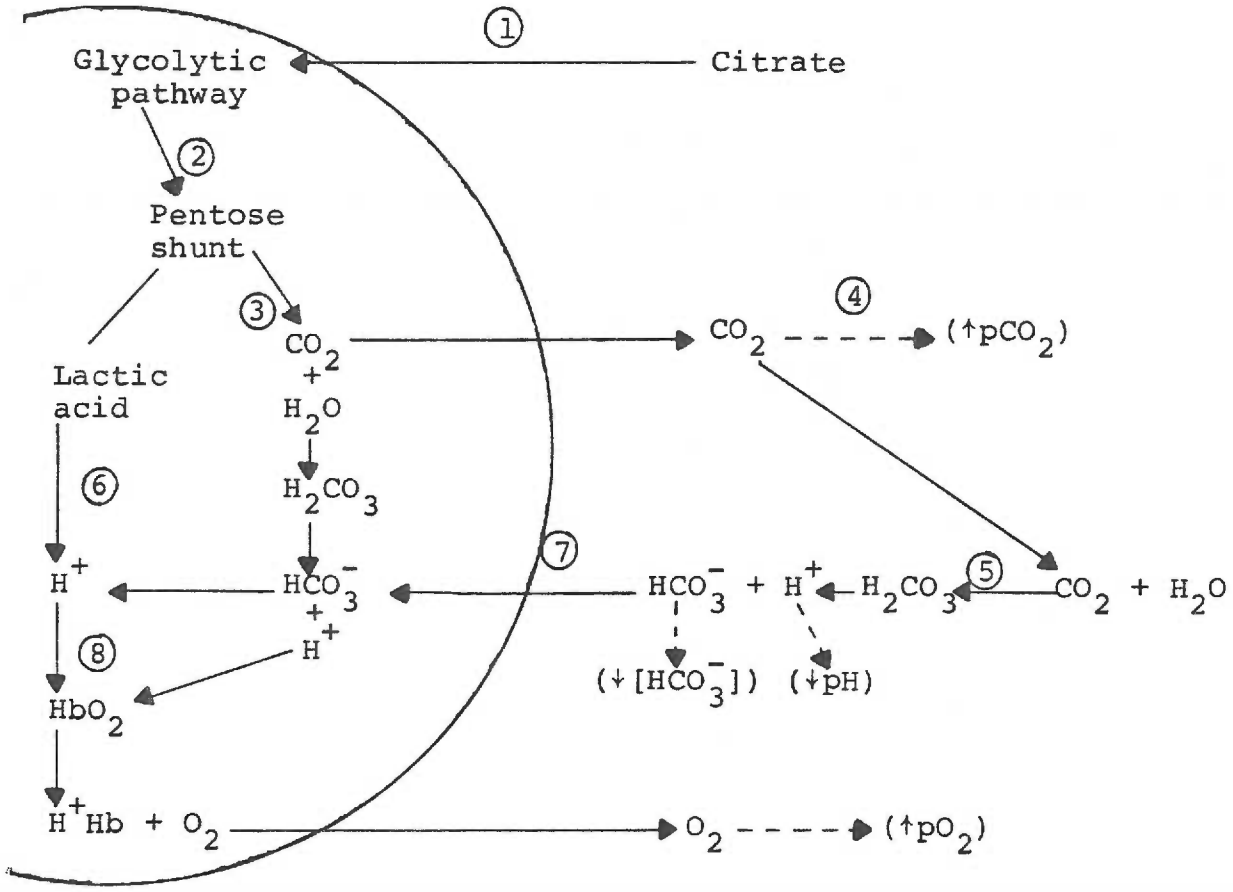

0
-1.0
-2.0
-3.0
$[HCO_3^-]$ meq/L DIFFERENCE
HEPARIN
CITRATE
OXALATE
EDTA
1
2
3

Figure 6

Actions of Trisodium Citrate



Enthrocyte Plasma

The mechanisms of the glycolytic pathway are not delineated. Reactions are shown as unidirectional although they are equilibrium reactions. Figures in parenthesis indicate direction of change.

1) Citrate enters the erythrocyte and inhibits phosphofructokinase.

2) Glucose-6-phosphate enters the pentose shunt which results in increased CO_2 within the erythrocyte (3).

4) The intracellular CO_2 equilibrates with plasma CO_2 resulting in increased pCO_2.

5) The increased CO_2 in plasma reacts with water producing increased H^+ which decreases the plasma pH.

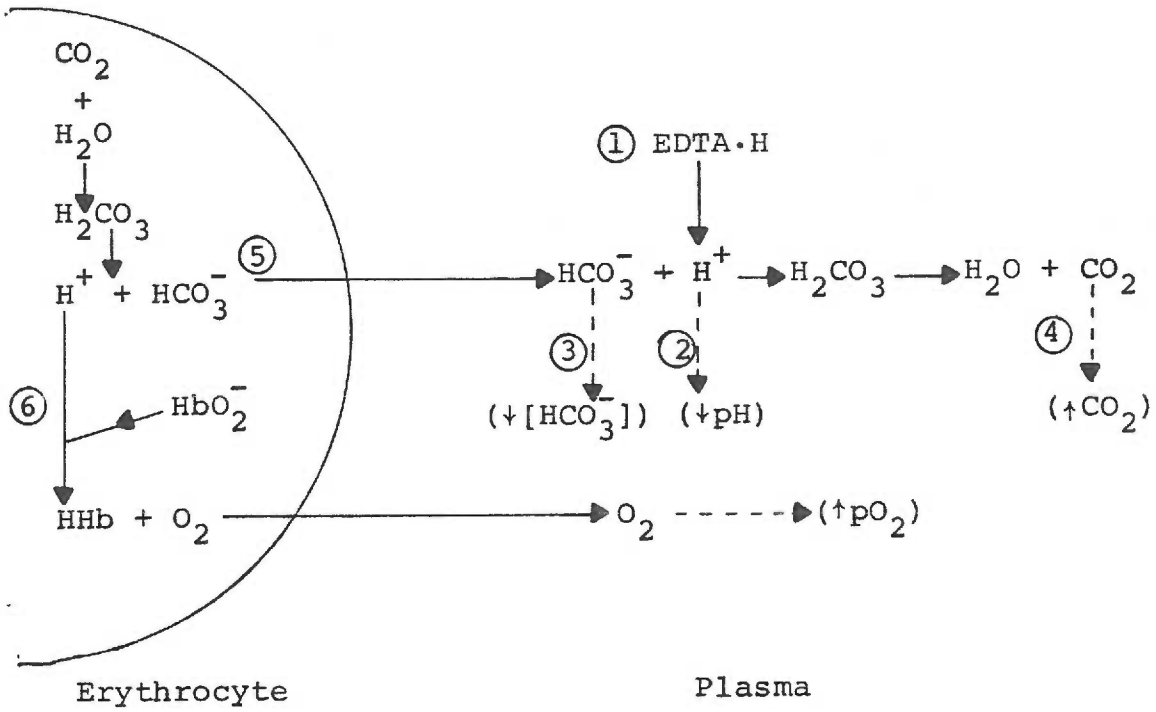
6) Increased metabolism via the pentose shunt results in increased lactic acid production which further increases H^+ and decreases the pH of the erythrocyte.

7) The intracellular HCO_3^- buffers some of the H^+ from lactic acid and plasma HCO_3^- equilibrates with intracellular HCO_3^- lowering the plasma $[HCO_3^-]$.

8) The increased intracellular H^+ causes hemoglobin to give up O_2 which equilibrates with plasma resulting in increased pO_2.

Figure 7

Actions of Disodium EDTA and EDTA Mixture



Reactions are shown as unidirectional although they are equilibrium reactions. Figures in parenthesis indicate direction of change.

1) When acid EDTA is aded to the blood it disassociates.

2) Increasing H^+ results in a decrease in plasma pH.

3) Plasma HCO_3^- reacts to buffer some of the excess H^+. Therefore, plasma [HCO_3^-] decreases.

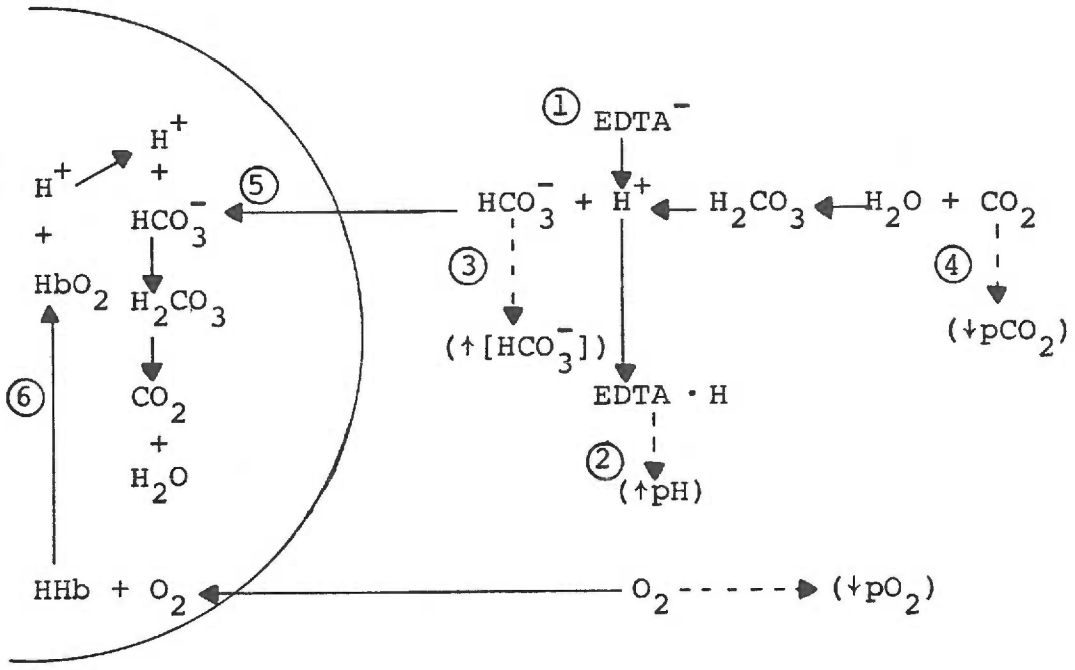
4) The plasma HCO_3^- and H^+ form H_2CO_3 which in turn produces H_2O and CO_2 resulting in an increased plasma pCO_2.

5) Intracellular bicarbonate diffuses out of the RBC replacing some of the HCO_3^- which was lost by buffering.

6) The loss of intracellular HCO_3^- results in increased intracellular H^+ causing hemoglobin to give up O_2. The O_2 equilibrates with plasma resulting in an increased pO_2.

Figure 8

Actions of Tetrasodium EDTA



Erythrocyte Plasma

Reactions are shown as unidirectional although they are equilibrium reactions. Figures in parenthesis indicate direction of change.

1) When alkaline EDTA is added to the blood it buffers H^+.

2) Decreasing H^+ produces an increase in plasma pH.

3) Buffering of H^+ results in an increase in plasma $[HCO_3^-]$.

4) As plasma H^+ is buffered, CO_2 and H_2O combine to produce H_2CO_3 which in turn produces H^+ and HCO_3^-. Thus plasma pCO_2 decreases.

5) Some plasma bicarbonate diffuses into the RBC combining with H^+.

6) The loss of intracellular H^+ results in an increase in intracellular pH. This results in hemoglobin having an increased affinity for O_2. As intracellular O_2 combines with hemoglobin, plasma O_2 equilibrates with intracellular O_2. The plasma pO_2 decreases.

APPENDIX A

HENDERSON-HASSELBALCH EQUATION

APPENDIX A

Bicarbonate concentrations were calculated from the Henderson-Hasselbalch equation:

$$pH = pK'a + \frac{\log [HCO_3^-]}{s \cdot pCO_2}$$

$$\text{Therefore } [HCO_3^-] = [10^{(pH - pK'a)}] \cdot [s \cdot pCO_2]$$

where $pK'a = 6.1$ at 37°C and

$s = 0.0301$ mM CO_2/L · mmHg pCO_2 at 37°C

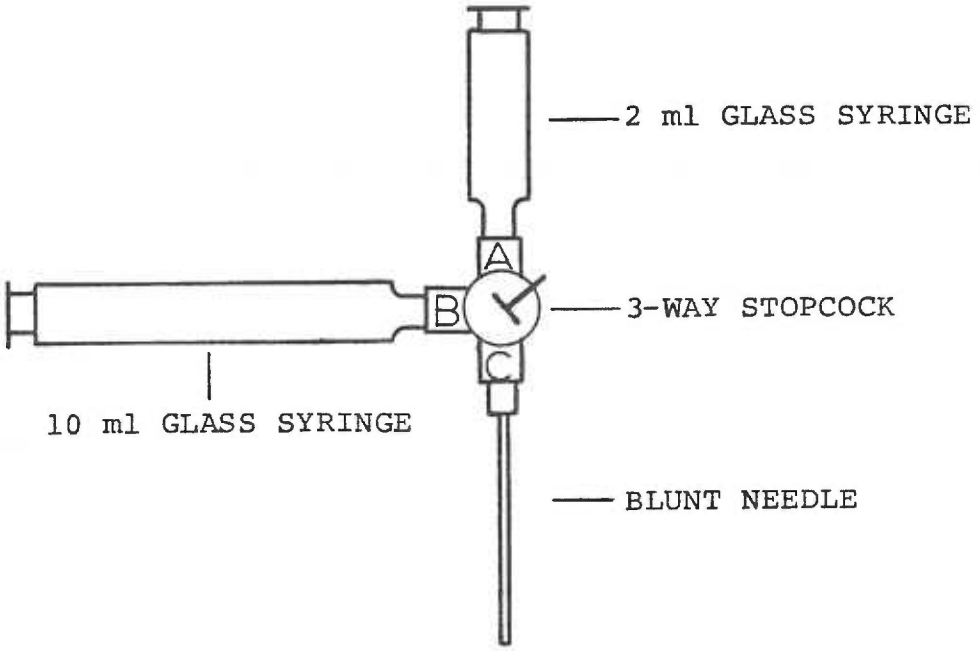
(Davenport, 1974)

APPENDIX B

TRANSFER TECHNIQUE

APPENDIX B

TRANSFER TECHNIQUE



All blood was equilibrated for 1 hr. in a Dynex tonometer. The blood was then removed from the tonometer well using the following procedure:

1. Five 2 ml glass syringes were prepared in the following manner:
   a. All syringes were lubircated with stopcock grease to prevent air leakage.
   b. Syringes were labeled no. 1-5.
   c. Syringes no. 1 and no. 5 were left empty.
   d. Syringes nos. 2, 3, and 4 were filled to dead space with an anticoagulant of known concentration.
2. Syringe no. 1 was connected to the apparatus as shown above.

3. The stopcock was turned so that the 2 ml glass syringe port was off (port A).
4. The blunt needle of the apparatus was placed in the tonometer well.
5. The 10 ml glass syringe was purged three times with the gas mixture in the tonometer well using the technique described in the Dynex manual.
6. All of the blood in the tonometer well was aspirated into the 10 ml glass syringe.
7. The stopcock was turned so that port B was off.
8. The 2 ml glass syringe was purged 3 times with the gas mixture in the tonometer using the technique described in the Dynex manual.
9. All ports on the stopcock were closed.
10. The apparatus was removed from the tonometer well.
11. The stopcock was turned so that port C was off.
12. Approximately 1 ml of blood was transferred from the 10 ml syringe to the 2 ml syringe (syringe no. 1) via the stopcock.
13. Syringe no. 1 was removed from the apparatus.
14. A 26 ga. needle was placed on the syringe.
15. Any gas bubbles and a small amount of blood were expressed from the syringe.
16. A cork was put on the 26 ga. needle and the syringe was placed on ice.
17. A few drops of blood were expressed from port A.

18. Syringe no. 2 was attached to port A and filled with exactly 1 ml of blood from the 10 ml syringe.
19. Syringe no. 2 was removed as in steps 13-16.
20. Syringe nos. 3 and 4 were filled as in steps 17-19.
21. The stopcock was turned so that port B was off.
22. Syringe no. 5 was attached to port A.
23. The apparatus was placed in the tonometer well.
24. Syringe no. 5 was filled as described for syringe no. 1 in steps 8-16.

APPENDIX C

TECHNIQUE FOR MEASUREMENT OF DEAD SPACE VOLUME OF SYRINGES

APPENDIX C

TECHNIQUE FOR MEASUREMENT OF DEAD SPACE VOLUME OF SYRINGES

1) An 0.05 N solution of KOH was prepared in a volume adequate for the purposes of this study (3L).
2) A stock standard of alkaline chromate solution was made by dissolving 2.5000g $KCrO_4$ in 100.0 ml 0.05N KOH.
3) 4 ml of the stock standard solution was diluted with 50 ml of 0.05N KOH.
4) The resulting $KCrO_4$ solution (200 mg%) was used to prepare the following standards:

working standard:	2 mg%	4 mg%	6 mg%
ml of $KCrO_4$ (200 mg%):	1 ml	2 ml	3 ml
ml of KOH (0.05N):	99 ml	98 ml	97 ml

5) A Beckman spectrophotometer (Model 25) was used to measure the absorbance of the standards of known concentration (2 mg%, 4 mg%, 6 mg%) at a wave length of 370 nM.
6) A graph was prepared plotting the relationship of the known concentration of $KCrO_4$ to absorbance.
7) The dead space of four 20 ml plastic syringes was filled with the stock standard alkaline chromate solution (2500 mg%). Care was taken so that no air bubbles remained in the dead space.
8) The $KCrO_4$ solution from the dead space of each syringe was

transferred quantitatively to a separate volumetric flask and diluted to 250 ml with 0.05N KOH. The syringes were flushed several times so that all the $KCrO_4$ solution from the dead spaces was transferred to the flasks.

9) The spectrophotometer was used to measure the absorbance of the $KCrO_4$ solutions from the four volumetric flasks.

10) The concentrations of the four solutions were determined by the use of the graph described in Step 6.

11) The dead space volume of the four 20 ml syringes was calculated as follows:

$$\text{Concentration of } KCrO_4 \text{ (from graph)} = \frac{\text{x amt in mg}}{250 \text{ ml}}$$

$$x = \text{mg } KCrO_4 \text{ from dead space}$$

$$x \text{ mg } KCrO_4 \text{ from dead space} \div \frac{25 \text{ mg } KCrO_4}{\text{ml}} \text{ (concentration of original solution in dead space)}$$

$$= \text{ml of dead space}$$

12) Eight 2 ml glass syringes were lubricated with stopcock grease. The dead spaces of these 8 syringes were filled in the same manner as the 20 ml syringes.

13) The solution in the dead space of the 2 ml syringes was transferred to eight 25 ml volumetric flasks and diluted to 25 ml with 0.05 N KOH.

14) The $KCrO_4$ concentrations were determined by use of the spectrophotometer as previously described.

15) The dead space volume of the eight 2 ml glass syringes was calculated as follows:

$$\text{Concentration of } KCrO_4 = \frac{\text{x amt in mg}}{25 \text{ ml}}$$

$$x = \text{mg } KCrO_4 \text{ from dead space}$$

$$\text{x mg } KCrO_4 \text{ from dead space} = \frac{25 \text{ mg } KCrO_4}{\text{ml}} \quad \begin{matrix}\text{(concentration} \\ \text{of original} \\ \text{solution in} \\ \text{dead space)}\end{matrix} = \begin{matrix}\text{ml of} \\ \text{dead} \\ \text{space}\end{matrix}$$

Results of dead space volume measurements are shown in Table 3.

APPENDIX D

COAGULATION STUDIES

APPENDIX D

Coagulation Studies

1. Oxford pipettes were used to transfer 0.025 ml of each concentration of anticoagulant to 5 ml test tubes. Each concentration was done in duplicate.

2. Fresh whole blood was obtained and 1 ml of blood was added to each tube with anticoagulant.

3. The test tubes were individually agitated once every ten minutes for one-half hour and then once every 30 minutes for one and one-half hours. The samples were observed for any evidence of clot formation.

4. If no clotting occurred in 2 hours the anticoagulant was presumed to be adequate for use in blood gas analysis.

AN ABSTRACT OF THE THESIS OF

SALLY H. MORTON

For the MASTER OF NURSING

Date of Receiving this Degree: June 12, 1981

Title: THE EFFECTS OF ANTICOAGULANTS ON THE VALUES OBTAINED DURING ARTERIAL BLOOD-GAS ANALYSIS

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

Four anticoagulants in three concentrations were studied to determine how their use with blood samples effects blood-gas values. Sodium heparin, trisodium citrate, Heller-Paul oxalate and an EDTA mixture were the four anticoagulants used. Fresh dog blood was obtained, placed in a tonometer, and equilibrated with one of three gas mixtures. This blood was then withdrawn into control syringes or syringes containing one of the anticoagulants. The pH, pCO_2 and pO_2 of the samples were then measured and the $[HCO_3^-]$ was calculated.

The result of this study indicated that heparin is the most reliable anticoagulant for use with blood-gas analysis. Heller-Paul oxalate compared favorably to heparin. The use of trisodium citrate solutions resulted in statistically

significant decreases in all blood-gas values. The EDTA mixture produced decreases in pH and increases in pCO_2. The EDTA mixture affected the pO_2 the least of any of the anticoagulants studied. Physiological mechanisms are presented to explain, in part, the changes in blood-gas values observed with the use of trisodium citrate and EDTA.