1965

Stress and its effect on the coagulability of blood

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Submitted in Partial Fulfillment for the Degree of
Doctor of Medicine

College of Medicine, University of Nebraska

February 1, 1965

Omaha, Nebraska

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INTRODUCTION

In this paper, I intend to review the theory and observations that ionic fatty acid levels in human plasma increase during periods of stress and that, further, the increased level of ionic fatty acids increases the coagulability of the blood. This hypercoagulability may result in thromboembolism. I will present the results of my study that substantiate these observations and suggest possible ways for reducing the danger of thromboembolism.

Nature of the Problem. - Stress has been defined by Hans Selye (1) as a non-specific tension in living matter which manifests itself in tangible morphologic changes. This stress manifestation occurs in three separate stages. The first is the alarm reaction which is the initial mobilization of the body's defenses. This is described as being a catabolic state in which growth functions and the normal balance of the interieur millieu are sacrificed temporarily to meet the challenge of the immediate stress situation.

After this first stage, the course may proceed in either of two directions depending upon the duration and severity of the stress. If the stress is short lived or mild the body resumes an anabolic state which includes growth and repair functions. This is termed the stage of resistance or adaptation. The endocrine glands quit secreting hormones which bring about emergency responses. Hemodilution and other adjustment mechanisms become active.

The third or exhaustion stage may occur either due to prolonged, severe stress, the body never having entered the stage of resistance, or after long term stress which has exhausted the body adjustment mechanisms and has returned the body to an acute distress situation. The stage of exhaustion is functionally similar to the alarm reaction with catabolism predominating.

The stress inducer, or stressor, may take many forms. These fall into two main categories, physic and physical. Psychic stressors, notably those of fear and anxiety produce manifestations similar to those induced by physical stress. (2)

Present State of Problem. - An example of psychic stress is a study done with rats in which escape and repeated attempts at capture were used to produce a fear situation. (3) After actual capture the rats' blood was evaluated for levels of glucose and ionic fatty acids. Frequently handled rats were used as a control group. The ionic fatty acids rose to a peak by 3 minutes and began to fall off after this time in the stressed rats, whereas a constant level was maintained in the control group. Blood glucose rose to a peak of 130 mgm. percent after six minutes and returned to normal by twelve minutes. Again the control group maintained a constant level.

Psychological stress situations in humans reveal an identical phenomenon as regards ionic fatty acids. In one series using blood donors note was made of ionic fatty acid levels before and after the donation of blood. (4) The ionic fatty acid level after

donation rose an average of 15 percent and as high as 70 percent. Total lipids, on the other hand, showed a net decrease of 10 percent.

Cardon and Gordon (5) demonstrated a rapid increase in plasma ionic fatty acid after a fear reaction in humans also.

Physical stress may be produced in many ways. Violent physical exertion such as running, stair climbing, or strenous athletic pursuits, body trauma, loss of blood, burns, and poisons are a few of the many forms in which physical stress may present itself.

Exercise as a form of physical stress has been the subject of several studies. Those using short periods of exercise found a decrease of ionic fatty acids in the plasma. (6, 7) This was thought to be due to an increased uptake and oxidation by the exercising tissues which is augmented by increased blood flow through those tissues. Additional evidence supporting the blood flow hypothesis is a linear relationship between heart rate and ionic fatty acid decrease. (8)

More prolonged periods of exercise have been found to be associated with increased ionic fatty acid levels. It is thought that ionic fatty acid mobilization rises to meet the need of the exercising tissue. (8)

A small but indicative study was done, in 1960, by Davis and Settles (4) in which subjects were exercised by having them run up and down stairs for five minutes. This showed a slight decrease

in ionic fatty acids but also it showed an increase of serum lipase activity. This would suggest an actual increase in the release of ionic fatty acids and thus tend to substantiate the idea that increased utilization of ionic fatty acids is taking place.

Trauma to the human body is a subject of much interest in medicine. This applies not only to accidental trauma but also, and perhaps more particularly, to trauma caused by operative or manipulative procedures used in the treatment of patients.

A study simulating accidental trauma was done on rats by producing a bilateral femoral fracture and then testing the blood for consequential changes. (9) A finding of increased electrophoretic mobility of B-lipo protein was noted. This change has been found to be due to a rise in ionic fatty acids in the plasma. (10) It also can be reproduced in vitro (11) by the addition of ionic fatty acid (specifically sodium oleate) to the plasma in the sample.

In a study using twelve surgical patients, preoperative and postoperative levels of ionic fatty acids were determined. (12) These people were kept on a constant diet before and after operation. Only cholecystectomies and herniorrhaphies were used. Ionic fatty acid levels were found to be higher on the first postoperative day than on the first preoperative day. Glycerides and phospholipids were found to decrease.

James B. Shields, under the direction of Dr. H. L. Davis, completed a study during the summer of 1963, using a series of 27

patients who were candidates for various types of operations. The samples of blood tested for each patient were drawn before the evening meal prior to the operation, immediately prior to the operation, immediately following the operation, four hours following operation, and before breakfast the morning following operation.

The thirteen patients of the control group were operated upon and were given Hartman's solution during the operation. The results of the ionic fatty acid determinations on these individuals revealed a significant rise in the ionic fatty acid levels from the evening prior to the operation to the immediately preoperative sample. The peak levels variably occurred at the immediately postoperative and the four hour postoperative periods.

This study also shows a marked inverse correlation between ionic fatty acids and Lee-White two tube clot times and recalcified clot times. In addition to this, a lowered fibrinogen level is found associated with the elevated ionic fatty acids and lowered coagulation time.

Many studies have been done showing correlations between ionic fatty acids and increased tendency toward blood coagulation.

An in vitro study was done in which the time of onset of ionic fatty acid effect on coagulation was determined. (12, 13) After the first ten seconds the recalcified clot time was depressed 10 percent, by thirty seconds it was depressed 25 percent, and by 1 minute a 40 percent depression had occurred. After this point there was no further effect.

In this same study, the effects of the various individual fatty acids were investigated. The sodium stearate, palmitate, and oleate were found to show a pro-coagulant effect, but the shorter chain fatty acids and the unsaturated acids did not give this effect to any marked degree.

Another study was done in vitro in which varying concentrations of ionic fatty acids were used and the effects thereof determined. (14) A standard clot time value of 4 1/2 minutes was used. Sodium laurate gave a minimum clot time of 4 1/4 minutes, sodium stearate, 2 3/4 minutes, and sodium oleate, 2 1/2 minutes, all at 3.3 mEq/L concentration. Concentrations above or below this value yielded less depression. Some effect was noted with a concentration as low as 30 mEq/L. Oleate and stearate had no effect on prothrombin times, thromboplastin degeneration test, or Stypven time. Sodium laurate does lower Stypven time.

In a study done with dietary intake of miniature pigs (15) it was found that recalcification times were shortened with either tallow or safflower oil combined with cholesterol but that the safflower oil gave less hypercoagulable effect both in fasting and post-feeding states. The Stypven time, which is a rough index of lipemia, (16) is shorter in the tallow fed pigs, indicating greater lipemia with this than with safflower oil. The plasma turbidity was also made greater by the tallow as opposed to safflower oil.

Two separate studies were done using the in vitro thrombus producing mechanism of Chandler. (17) This apparatus consists of

a circular channel of plastic tubing which is filled somewhat over one half full of blood. The system is then closed and started to revolve as a wheel in the vertical plane at twelve revolutions per minute. Not only does this simulate the travel of the blood through a vessel, but it gives rise to a "thrombus" which is morphologically a faithful replica of an actual intravascular thrombus. The end point is reached when the flow resistance of the blood becomes so great as to cause the column of blood to be carried with the rotation of the tubing. The specific criteria is the depression of the trailing end of the column to 102 degrees.

Both studies dealt with the various lengths of chains in the fatty acids and their effects on thrombus formation time and length of thrombus two minutes after the end point was reached. A concentration of fatty acid of .320 mM/L was used. The blood usually has a concentration of .2 to 20 mM/L. Sodium palmitate (C 16) through the saturated series to sodium ceratate decreased thrombin formation by more than fifty percent. (18) The saline control thrombus formation time was 8.3 minutes. The stearic acid gave the greatest decrease with 2.9 minutes, arachidic with 2.74 minutes, and palmitic, 4.77 minutes progressing to caproic with 7.81 minutes. The unsaturated acids proved not to show a procoagulant effect. Oleate gave a thrombus formation time of 8.32 minutes, linoleate, 8.14 minutes, linolenate 8.42 minutes, and arachidonate, 8.00 minutes.

Increased thrombus length was also associated with increased chain length and saturation. (18) The series from palmitate (C-16) through ceratate (C-26) all give values of over 2 cm. with the longest being 4.8 cm. produced by bebenate (C-22).

A theory regarding the mechanism was put forth by Conner. (19) Bentonite, kaolin, and fine glass are known Hageman factor stimulators. After any of these materials were used on anti-coagulated blood, thereby exhausting the Hageman factor, sodium stearate would no longer exert a pro-coagulant effect on the blood. Thus it was postulated the fatty acids also are Hageman factor inhibitors.

Another theory of mechanism discussed in connection with the Conner (18) study is that of micelle formation. (2) These are negatively charged micelles. Short chain fatty acids and unsaturated fatty acids do not form these micelles (21) as readily as do the larger chain saturated acids, thus perhaps explaining their lack of effect upon the coagulation of blood.

A theory advanced by Dr. Herbert L. Davis (22) deals with the colloidal stability of fibrinogen. Fibrinogen itself has a negative charge and is hydrated. As with other colloidal systems, the fibrinogen is dependent upon this charge and hydration to remain a sol as opposed to becoming a gel. Therefore, materials adding to the charge and/or hydration of the fibrinogen may act as anti-coagulants. Anticoagulants such as heparin and heparinoids fit this criteria insofar as they add charge to the fibrinogen. Coagulants

detract from the charge and/or hydration of the fibrinogen. Cationic materials decrease the natural negative charge of the fibrinogen thereby destabilizing it. Such is the activity of calcium, magnesium, and hydrogen ions as well as other materials such as local anesthetics, styptics, histamine, and protamine. Other coagulants work by dehydration of the fibrinogen molecule. Alcohols, surfactant materials, anesthetics, radiopaque materials, and lipids fall under this category. Lipids must be finely dispersed to have this effect, however. Cholesterol and glycerides must be dissolved in solvents before they are effective. Phospholipids and ionic fatty acids are soluble enough in themselves to be effective.

The ionic fatty acids or soaps are effective in very low concentrations at pH 7.4. The sodium or hydrophilic ends of the acids attach themselves to the hydrophilic sites of the fibrogen molecule. The hydrophobic lipid pole of the ionic fatty acid is then left exposed to the solution making fibrinogen in effect hydrophobic. The long chain saturated fatty acids are highly hydrophilic so these are the most effective of the coagulants. Stearate and palmitate are the most effective of these. The double bonds of unsaturated acids are hydrophilic making them less effective as coagulants. Laurate with its saturated twelve carbon chain had approximately the same effect as does oleate with its 18 carbon chain and one double bond.

Having thus far established a relation between stress and increased ionic fatty acid levels, and in turn a correlation between elevated ionic fatty acid levels and increased clotting tendency, we may then carry the reasoning a step further and state that there is a direct correlation between the types of stress discussed and increased tendency for blood coagulation. The evidence presented would suggest that the ionic fatty acid levels are in some part the mechanism responsible. The following considerations help to substantiate this view.

Several studies have been done indicating that reduction in ionic fatty acid response to stress situations may be accomplished by carbohydrate sparing activity. This was also shown to reduce the correlation between stress and increased tendency toward coagulation. By combining these two facts, one might conclude that ionic fatty acids do bear some direct relationship to blood coagulation.

The study by Mayes, (3) already mentioned in connection with psychologic stress causing a rise in ionic fatty acids, shows that the ionic fatty acids rise before glucose levels do in response to stress. The ionic fatty acid levels peak at three minutes and begin to fall as the glucose levels rise 130 mgm. percent to their peak at six minutes. Conversely, as the glucose begins to recede to normal levels reached at twelve minutes, the ionic fatty acids rise significantly again to a sustained elevated level.

It was found in another study that the feeding of a mixed meal would cause a significant decrease in ionic fatty acids. (23)

In an average subject, the ionic fatty acids fell from 850 microequivalents per liter to 400 microequivalents per liter. In obese individuals the average decrease was from 1240 microequivalents per liter to 790 microequivalents per liter. Fasting for ten to twelve hours caused a rise in ionic fatty acids. The average fasting level after six hours was 1240 microequivalents per liter. A fatty meal yields no increase in ionic fatty acid levels over the six hour fasting state. Pure glucose, 50 gm. orally or intravenously, produced the most marked decrease in ionic fatty acids decreasing from 850 to 290 microequivalents per liter in 1 1/2 hours. This glucose had less than one-half the caloric value of the fatty meal, and yet it had a considerable fat sparing action; whereas, the fatty meal caused no change in either direction. Insulin injection (0.1 units/kg. of body weight) caused a drop of ionic fatty acid from 850 to 438 microequivalents per liter.

In a study in which epinephrine rather than a stressful situation was used to elevate the ionic fatty acid levels, glucose administration was again shown to prevent the rise of ionic fatty acids. (19, 24) Insulin administration also produced this effect as indicated previously but in this study the evidence was carried a step further. This effect could occur without having a hyperglycemia first, suggesting that glucose utilization, not just the amount present in the plasma, has the real effect on ionic fatty acid levels. This tends to substantiate the metabolic concept that if metabolic demands are not satisfied by carbohydrate calories, ionic

fatty acids are mobilized from adipose tissue and are oxidized into usable caloric form.

A study in which alloxan produced diabetic rats were used illustrates further the effect glucose administration has upon fatty acid metabolism. (25, 30) The fatty acids were labelled with carbon^{14}. The carbon^{14} content of the expired air and that of liver were measured. It was found that with insulin administration, the amount of radioactivity in the expired air decreased which suggests decreased oxidation of fatty acids. The insulin treated rats had less radioactive acetoacetate in their liver than untreated rats which again shows decreased breakdown of fatty acids when glucose utilization is greater. To show that insulin itself has no chemical properties which would cause decreased fatty acid utilization, an *in vitro* study was done showing that carbon^{14} labelled octanoate oxidation was not changed by insulin addition. As a conclusion of this study it was stated that insulin spares fatty acid oxidation by increasing glucose utilization in the liver. This same effect was observed when carbohydrates were added to intact rats. (26)

Carbohydrates used in relation to stress due to surgical procedures seem to show a marked effect on the fatty acid levels and on the tendency toward coagulation.

In a study done by Wadstrom, (27) 27.5 gm. of glucose were administered over two hours on the day following operation. An

immediate drop in ionic fatty acids was observed. No effect was noted on triglycerides or phospholipids.

The study done by James Shields,(28) the control group of which was aforementioned in connection with the correlations between stress, ionic fatty acid elevations, and increased clotting tendency, also included a group of thirteen patients who were given 5 percent dextrose in lactated Ringer's solution. One and six tenths liters of fluid was given over a four hour period beginning at the start of the operation. The same five samples were taken for both groups. These were, as you recall, one, before supper the evening before operation; two, immediately before operation and fluid administration; three, immediately following operation; four, four hours following operation; and five, the following morning morning before breakfast.

Compared to the control group, the first samples from the group to receive dextrose had nearly the same ionic fatty acid and clot time values. The second samples,likewise,were of similar values between the two groups. This is as it should be,however, since no fluid had yet been given to either group.

The third sample from the group receiving dextrose showed, in general, lower ionic fatty acid levels and higher clot times than did the control group.

Another value which showed a correlation with ionic fatty acid levels and clot times was the fibrinogen level. Whereas the values fell somewhat in both groups following the operation, the

fall was greater in the group not receiving dextrose. Therefore, a fall in the level of fibrinogen in the plasma was correlated with a higher ionic fatty acid value and a low coagulation time.

To eliminate the possibility of lowered ionic fatty acid level with dextrose administration being a function of dilution, an isovolumetric amount of lactated Ringer's solution was given the control group.

The fourth sample showed an increase in ionic fatty acids over the third sample with the group receiving dextrose. This may be explained by the fact that the dextrose administration had been discontinued by this time, thus no longer sparing fatty acid oxidation. As a correlate, the clot times went down slightly. The control group, on the other hand, showed either a constant or slightly reduced ionic fatty acid value from its already higher values. The fibrinogen and clot time values stayed the same or rose slightly in the control group between the third and fourth samples.

The fifth sample, which reflects a state of recuperation from the acute phase of stress showed all the values to be back to the initial value or to be tending in that direction.

In summary, the group which received carbohydrate showed less rise in ionic fatty acids during the time of dextrose administration and correspondingly higher clot times and fibrinogen values than the control group. A rise in ionic fatty acids, fall in clot times, and a fall in fibrinogen which occurred after discontinuance of the

dextrose solution further establishes the effect of the carbohydrate as opposed to individual variation.

Serum lipase activity and total lipid levels were determined but neither showed any consistent relationship to any other value.

Purpose and Scope of the Investigation. - The project that I conducted was in concept the same as Shield's study. Certain refinements and modifications were made in the hope of obtaining more conclusive results.

The main change made was in the administration of the carbohydrate. I used a dose of 0.5 gm. of carbohydrate per kg. of body weight in the present study instead of using the same dosage for each patients as in the previous study. I chose this dosage because it has been shown to be a maximal level of carbohydrate utilization. (29) A 10 percent solution of invert sugar in a balanced electrolyte solution was used. This had two advantages. The fructose portion of the invert sugar does not require insulin for its initial utilization, thus its uptake is more assured. Secondly, the 10 percent solution allows larger amounts of carbohydrate administration without an excessive volume of fluid being used.

Lipase determinations were not done because the test was found to be unreliable. The values obtained were not reproducible.

I found that ionic fatty acids increase with stress accompanied by and presumably causing increased coagulability of the blood. I also found that these effects could be inhibited by administration of invert sugar.

Methods and Materials

The procedures used in obtaining data will be discussed in detail at this time and only in passing later.

Patients. - Subjects for the project were chosen at random to eliminate bias. No special consideration was given to age, sex, physique, disorder, type of operation, state of nutrition or anesthetic agent except the cases where in our study might interfere with the intentions of the surgeon or anesthesiologist.

It was necessary to bypass individuals with extremely limited peripheral venous exposure which would make difficulty in obtaining five separate blood samples and would decrease the reliability of our coagulation and recalcification times by contamination of the samples with tissue thromboplastins.

Fluid administration. - The object of this series was to determine the fatty acid sparing effect of intravenous carbohydrate administration. The amount of carbohydrates to be given was determined by the weight of the patient. Patients can utilize effectively no more than 0.5 gm. of glucose per kg. of body weight per hour. (29) This then was the amount administered in these tests to obtain a maximal sparing effect.

In using Plasma-lyte with Travert 10 percent[1] (invert sugar) a suitable amount of carbohydrate could be administered without excessive volume of fluids. A liter of Plasma-lyte with Travert 10 percent contains 100 gm. of carbohydrate. Thus 5 ml. contains

0.5 gm. of CHO. I gave 5 ml. of solution or 0.5 gm. of CHO per kg. of body weight per hour. Patients in the control group were given a volume of Plasma-lyte[2] equal to that given those in the experimental group.

Each sample was drawn with a 20 gauge needle from an upper extremity. Usually the venapuncture was made in the antecubital area but occasionally was done in the lateral aspect of the wrist or on the dorsum of the hand.

Two 10 ml. syringes were used, the first containing 1 ml. of 3.8 percent sodium citrate solution.[3] This syringe was filled with blood to the 10 ml. mark and then disconnected from the needle, inverted for mixing, and then placed aside. The second syringe, rinsed with sterile saline, was then applied and more blood drawn up to an estimated 13-14 ml. Timing of clot time was started with the second withdrawal.

The second (saline rinsed) syringe was emptied immediately into three test tubes, the first two being small, saline rinsed tubes for clot times, each receiving approximately 1 ml. of blood, and the third tube being a clean, dry tube receiving approximately 12 ml. of blood.

The syringe containing the citrated blood was then emptied into a fourth clean, dry tube marked plasma.

All samples were refrigerated as soon as possible. The non-citrated blood in the large test tube designated "serum" was

allowed to clot and retract. Retraction was more complete if the clot had been jarred loose from the walls of the test tube. The citrated blood in the test tube designated "plasma" was well mixed and also refrigerated.

All tubes were centrifuged after a few hours and the supernatant serums and plasma pipetted off into appropriately labelled tubes. The residues from the "plasma" tubes were poured out onto a flat surface and checked for coagulated material. If found, note of it was made.

The serum was saved for ionic fatty acid determination and total lipid determination. The citrated plasma was used for recalcification times and fibrinogen determinations.

Special studies. - The blood specimens were collected on a schedule designed to illustrate a definite relation between preoperative, operative, and recovery periods.

First sample: The first sample was collected just prior to the evening meal on the evening before the operation was to be done. This sample was thus of a relatively fasting state and was taken before the acute anxiety which precedes an operative procedure occurred. Varying reactions to the venapuncture may have introduced a variable into the values obtained from this sample due to the inconstant degree of psychological stress involved.

Second sample: The second blood sample was drawn immediately before operation. At this time the patients had already received their

preoperative medications and were somewhat sedated. Their intravenous fluids had not yet been started and they were in a fasting state, having not been fed since the preceding evening. This sample reflected the condition of immediately preoperative anxiety.

Third sample: The third blood sample was collected in the recovery room immediately following the operation. The interval between this sample and the second or immediately preoperative sample varied with the duration of the operative procedure. This sample did bear a rather constant relationship to the termination of physical stress incurred by the operation. The experimental fluids were still running at this time in most cases.

Fourth sample: The fourth blood sample was collected at four hours after the operation. The patients had largely recovered from the effects of anesthesia at this time. They were still in a fasting state insofar as ingested food was concerned and by this time the feeding fluids had been discontinued. Fluids to maintain body hydration may have been in use at this time, however, depending upon the patient.

Fifth sample: The fifth sample of blood was collected before breakfast the morning after the operation. No ingested food had been taken before this time so the patients were still in a fasting state. Some patients were given intravenous feedings before this time if their condition indicated it.

This was the last sample taken as this was felt to represent the full recuperation from the acute aspect of physical stress. The

bleeding should have been mostly stopped by this time and the processes of healing in progress.

Ionic Fatty Acid Determination (23, 30, 31, 32) has been found to give reproducible results and is practicable. Place 2 ml. of serum in 25 ml. test tube; use Teflon stopper. Add with shaking 10 ml. of extraction mixture,[4] then add 6 ml. of heptane[4] and 4 ml. of distilled water. Shake at least two minutes. Let it stand to separate phases. Pipet 5 ml. upper heptane phase into a 15 ml. centrifuge tube. Add 5 ml. of dilute sulfuric acid;[4] shake for five minutes; centrifuge for five minutes. Pipet 3 ml. of supernatant washed heptane phase into a suitable test tube. Add two drops of 0.1 percent thymolphthalein in isopropanol.[4] Stir by washed nitrogen (alkali-ethanol). Titrate to faint blue with 0.02 normal potassium hydroxide in isopropanol.[4] End point persists five seconds. Calculate ionic fatty acid values from net alkali used on final 3 ml. of extract. Net alkali is determined by that amount used to titrate serum minus that amount needed to titrate the saline blank run with each test.

The theoretical amount of recovery of ionic fatty acids from the plasma is 3/8 of the actual amount in the plasma. This is true because after the first extraction the total amount of ionic fatty acid should be in the 8 ml. of heptane present. Five ml. of this heptane phase is drawn off, thus 5/8 of the ionic fatty acid is recovered. This 5/8 is then washed with sulfuric

acid and then 3 ml. of the 5 ml. washed are recovered, thus 3/5 of the 5/8 are recovered: 3/5 x 5/8 = 3/8 or .375 theoretical recovery value.

The actual recovery rate found by extracting and titrating known solutions of ionic fatty acids is .37, thus being only .005 short of recovering the theoretical .375.

The calculation of ionic fatty acid concentration in mEq/L is as follows using the standard concentration x volume = concentration x volume formula. Applying the figures available the calculation is as follows:

$$2 \text{ ml. serum} \times \text{normality} = \text{ml. of KOH} \times .02 \text{ Normal} \times \frac{1}{.37}$$

$$\text{Normality of serum} = \frac{\text{Ml. of KOH}}{.37} \times .01$$

$$\text{mEq/L of IFA} = \text{ml. } .02 \text{ NKOH} \times 27$$

Coagulation Time - Two small saline rinsed test tubes were used for the test. The timing was started at the beginning of withdrawal of non-citrated blood. When the blood was placed in the small tubes, the first of them was tilted every 30 sec. until a gel sufficiently firm not to flow with the tube in a horizontal position occurred. This time was noted and then the second tube was tilted each 30 sec. until it too formed such a gel. The time was again noted, this being the definitive coagulation time.

Recalcified Clot Times - The procedure requires two tubes per set, thus four tubes per plasma sample as follows:

Set A - 0.2 ml. of calcium chloride 5 plus 1.8 ml. of saline in one tube. A mixture of 0.1 ml. calcium chloride and 0.9 ml. of saline 5 may also be used. In the second tube is placed 1 ml. (or 0.5 ml.) of plasma.

Set B - 0.3 ml. (or 0.15 ml.) of calcium chloride plus 1.7 (or 0.85 ml.) of saline in one tube. Place in the second tube 1 (or .5) ml. of plasma.

Incubate the test tubes in a 37° C water bath for five minutes. Mix the two tubes of each set by pouring back and forth three times and record the time of mixing. Observe the mixture approximately every thirty seconds. When the mixture congeals to the degree that it won't flow upon holding the tube horizontally, it has reached the end point and this time is recorded.

As a routine, mix a set each thirty seconds and read them between mixing. Series up to ten or twelve can be run at one time.

The .2 ml. of calcium chloride gives a wide and seemingly quite sensitive range of clot times. The .3 ml. concentration yields much lower times with correspondingly small variation, thus being less sensitive. The values do tend to correlate with values from the lower concentration and therefore both concentrations are justified.

Fibrinogen Determination. - In the procedure (23,33, 34) two test tubes are used for each set, thus four for each plasma sample as follows:

Set A - 9 ml. saline [6] in one tube (or 4.5 ml.),

1 ml. of plasma in the other tube (or 5 ml.)

Set B - 9 ml. Fowell's solution [6] in one tube (or 4.5 ml.)

1 ml. of plasma in the other tube (or .5 ml.)

The spectrometer is set at 510 millimicrons.

Mix the contents of the two A tubes by pouring back and forth three times. Set the alarm for three minutes and mix the two B tubes in the same manner as the A tubes. Fill the cuvette with water. Set the instrument to optical density zero or at 100 percent transmission with the water filled cuvette in place. At one minute before the alarm is due to ring, pour the plasma-saline mixture into the cuvette and read and record the optical density. Then reset the instrument to zero optical density with this solution. This compensates for coloration, hemolysis, and turbidity in the plasma itself. Pour out the plasma-saline, rinse the cuvette with distilled water, and pour in the plasma-Fowell's solution mixture. Read and record the optical density at the time the alarm rings.

Fowell directly correlated the optical density readings to mgm. percent of fibrinogen by comparison with the Kjeldahl nitrogen assay of seprated fibrin. Thus an optical density of .200 is interpreted as 200 mgm. percent of fibrinogen in the plasma.

1. The carbohydrate solution used for this series was Baxter Laboratories Plasma-lyte with Travert 10 percent. The constituents of this are similar to Plasma-lyte except for the invert sugar and some preservative. The constituents are as follows:

NaCl	.496 gm/100 ml.	Na	145 mEq/L
Na Acetate	.64 gm/100 ml.	K	110 mEq/L
KCl	.03 gm/100 ml.	Ca	5 mEq/L
$CaCl_2$	.0368 gm/100 ml.	Mg	3 mEq/L
$MgCl \cdot 6H_2O$	.0305 gm/100 ml.	Cl	103 mEq/L
NaR-Lactate	.0896 gm/100 ml.	Acetate	47 mEq/L
Travert (invert sugar)	10 gm/100 ml.	Lactate	8 mEq/L
Na Bisulfite	.05% added	Bisulfite	5 mEq/L

2. As control solutions the following isotonic electrolyte solutions were used in weight-volume relationships identical to those of the carbohydrate solutions used. First used was Lactated Ringer's Solution (Cutter Laboratories) which contains the constituents shown below:

NaCl	.6 gm/100 ml.	Na	130 mEq/L
KCl	.03 gm/100 ml.	K	4 mEq/L
$CaCl_2$	.02 gm/100 ml.	Ca	3 mEq/L
Na lactate	.31 gm/100 ml.	Cl	109 mEq/L
		HCO_3	28 mEq/L

The other was Baxter Laboratories Plasma-lyte which contains the following:

NaCl	.496 gm/100 ml.	Na	140 mEq/L
Na Acetate	.64 gm/100 ml.	K	10 mEq/L
KCl	.0746 gm/100 ml.	Ca	5 mEq/L
$CaCl_2$	.368 gm/100 ml.	Mg	3 mEq/L
$MgCl \cdot 6H_2O$	.0305 gm/100 ml.	Cl	103 mEq/L
Na R-Lactate	.0895 gm/100 ml.	Acetate	47 mEq/L
		Lactate	8 mEq/L

3. Used as an anticoagulant for samples of blood to be used as plasma samples was 3.8 percent sodium citrate solution. To make this 19.00 grams of sodium citrate$\cdot 2H_2O$ (F.W. 94 was made to 500 ml. with distilled water). This is a non-sterile solution.
4. Ionic fatty acid determination reagents - The reagents used for this procedure were: the extraction mixture, consisting of 39 ml. isopropanol, 10 ml. heptane, and 1 ml. 1 N. sulfuric acid; heptane; diluted sulfuric acid (1 ml. sulfuric acid plus 2 L. distilled water), 0.02 N. potassium hydroxide or sodium hydroxide in isoproanol; thymolphthalein 0.1 percent in isopropanol. This is colorless at pH 9.3 and blue above pH 10.5.
5. Reagents for recalcified clot times: isotonic saline composed of 9 grams sodium chloride diluted to 1 L. with distilled water;

calcium chloride (0.1 N.) composed of calcium chloride 3.675 gm. plus water diluted to 500 ml. with a pH of 5.5 to 8.2

6. Fibrinogen determination - The reagents used for this procedure were: isotonic saline which was composed of 9 gms. sodium chloride diluted to one liter with distilled water and adjusted to a pH of 8 and Fowell solution composed of 133.3 gm. ammonium sulfate plus 9 gm. sodium chloride plus water, adjusted with sodium hydroxide to pH 7, dilute to 1 L. The 13.3 percent ammonium sulfate solution is diluted to 12 percent when 1 ml. of plasma is added to 9 ml. of Fowell's solution.

RESULTS

In this series, 21 randomly selected patients were used. Nine patients were in the control group. This group received electrolyte solution (either Hartman's solution or Plasma-lyte) at a rate of 5 ml./kg./hour.

Eleven patients were used in the experimental group given invert sugar at a rate of 0.5 gm./kg./hour or 5 ml./kg./hour.

The types of operations done varied in severity from a breast biopsy done with local anesthetic to an abdominoperineal resection of the colon and rectum and a thoracotomy.

The anesthetics used consisted almost uniformly of thiopental sodium which was variously supplemented by nitrous oxide, cyclopropane, and Fluothane. In two cases spinal and epidural anesthesia were used.

The series went quite smoothly although a few patients were dropped due to my inability to obtain blood samples by venapuncture. Some sources of error were detected during the course of the series. The main finding was that of clots in the citrated blood sample to be used for fibrinogen and recalcified clot time determinations. These findings were recorded and used to explain the low fibrinogen values and high recalcification times obtained on these samples.

Although the series was small, the results were uniform enough to show very strong tendencies. The ionic fatty acids,

shown in Tables I and II showed a definite decrease in sample 3 of the group given carbohydrate as opposed to the control group. Even in this small sample the probability of this occurrence by chance was 1.04 percent. The trends are shown in Graph A.

The Lee-White clot times shown in Tables III and IV showed a tendency for the control groups clot times to decrease in samples 2 and 3 while the clot times of the carbohydrate group fell in sample 4 and rose again in sample 5. These trends are shown in Graph B.

The recalcification times shown in Tables V and VI gave very divergent results. The values of the standard errors of the mean were quite large. Therefore, the significance of the trends indicated is small. Graph C shows erratic results. Except for samples 1 and 2, however, the recalcification times show the same general, although exaggerated trend as did the Lee-White clot times.

The fibrinogen values shown in Table VII and VIII while showing no significant differences between the two groups did show a rising trend in samples four and five. The standard errors of the mean on these results was small showing quite good consistency of results. Graph D illustrates this trend.

Total Lipids, shown in tables IX and X, showed a distinct decrease in the value for sample 3 of the group given carbohydrate

whereas such a decrease in the control group values did not occur until sample 5. This is illustrated in Graph E. This trend is not as precise in temporal relations as it might be since only samples 1, 3, and 5 were tested.

DISCUSSION OF RESULTS

The results of this study substantiate the direct relation between stress and ionic fatty acid levels. The correlation between ionic fatty acid levels and reduced clot time, while not perfect, was present.

The mean ionic fatty acid levels in the control group showed a substantial rise between the first sample, which served as baseline, and the second sample, which represented probable preoperative psychologic stress. An even further rise occurred in the third or immediately postoperative sample which would reflect the physical stress of operation. This would include blood loss and tissue damage. This was the peak value. The level began to descend by four hours postoperatively and was back to (actually below) base line value by 24 hours postoperatively. This would suggest that the patients had recovered from the acute stress phase by 24 hours postoperatively.

This rise was quite significant as the values went from .648 mEq/L at the first sample to a peak of 1.066 mEq/L at the immediately postoperative sample, and returned to .613 mEq/L at the 24 hour postoperative sample.

The mean Lee-White clot times in the control group showed an initial decrease from 8.06 minutes to 6.72 minutes by the second (immediately preoperative) sample. This corresponds with the rise in ionic fatty acids. Whereas the ionic fatty acids continuedto rise, the clot time increased between the second and third samples. This portion of the results does not substantiate the inverse relation between ionic fatty acid levels and clot time. As the ionic fatty acid levels begin to decrease, the clot time continued to rise, as might be expected, but it peaked at the fourth sample with a volume of 9.21 minutes and then fell again to 8.63 minutes by the fifth sample even though ionic fatty acids were also falling.

The clot time values are subject to error. One source of error was the amount of tissue thromboplastins which contaminated the sample. Others were the amount of disturbance of the blood in transfer and observation, and the determination of a constant end point. This can be somewhat difficult since not all end points appear the same.

Error in the results shown is suggested since even the base-line values between control and experimental groups were disparent. This will be discussed later.

Two patients of the control group who were rather debilitated, were given five percent dextrose solution over the 48 hours prior to their operations. This was discontinued just prior to

the operation. The thought regarding this was that the carbohydrate might spare the ionic fatty acid effect. The clot time shown would indicate that such was the case. The clot times in one case decreased from 7 minutes to 6 minutes but in the other they actually increased from 8 minutes to 12 minutes. The ionic fatty acids, however, showed a substantial increase in both cases. One went from .35 mEq/L to .59 mEq/L while the other went from .51 mEq/L to 1.11 mEq/L. Each had been given approximately 200 grams of glucose over 48 hours prior to operation. This amounted to approximately 4 gm./hour or divided by 50 kg. body weight, approximately .08 gm./kg./hour which is far from the optimum dose of .5 gm/kg./hour.

The recalcified clot times, which in theory are a more sensitive gauge of clot time due to their expanded time scale, did not clearly reflect the trend shown by the clot times or ionic fatty acids. There were numerous excessively high and low values. Several sources of error may effect this test and the errors are magnified by the increased sensitivity of the test. First, the amount of tissue thromboplastins liberated by venapuncture will lower the times. In several samples a partial clot occurred in the plasma sample despite the sodium citrate. The recalcified clot times were made a good deal higher by this since a fibrinogen deficiency was present (reflected by small fibrinogen values). The recalcified clot times are extremely sensitive to

the amount of calcium chloride used in re-calcifying the plasma sample. This could be affected in two ways. The amount of citrate in the plasma sample, not being precise due to measurement by a syringe, may be more or less in excess. Therefore, variable amounts of calcium chloride are required to overcome the excess. Small and difficult to measure amounts of calcium chloride are used. Even excess hanging drops or other minute flaws in technic could cause considerable error. This test has yet to be perfected, especially, I feel, in the area of eliminating excess sodium citrate.

If one were to omit the second sample, however, the mean re-calcification times would inversely reflect the ionic fatty acid values in the control group. The baseline value for the control group was 14 minutes. This dropped to 10.08 minutes by the third sample in which the ionic fatty acids had reached a peak. The recalcification times rose to 10.8 minutes in the fourth sample and to 14.3 minutes in the fifth sample as the ionic fatty acid levels descended back to baseline.

The total lipids, which were done just as an exercise due to their previous seeming lack of value, did show changes occurring between the two groups.

The mean baseline values of the control group was .685 percent while that of the carbohydrate group was .722 percent. The measurement made of the third sample showed the control group to have maintained its values at the baseline level. The ionic fatty

acid had risen quite significantly during this same period, however. The total lipids, by the fifth sample, were considerably lower and during this same period the ionic fatty acids also fell considerably.

The mean ionic fatty acid values shown by the experimental group show less rise between samples one (.533mEq/L) and two (.718 mEq/L) than does the control group. Analysis of the groups showed that the experimental group had a lower mean age (56 opposed to 65) and less obese subjects. Both groups did show a rise, however, as would be expected since both groups were treated the same. After carbohydrate administration the value shown by sample three dropped to .48 mEq/L. The values rose again after discontinuation of carbohydrate administration to .561 mEq/L in sample four and .698 mEq/L in sample five. This rise takes the level in sample five to above baseline whereas the control group showed values descending back to baseline. This, on the surface, might suggest a delayed or prolonged but less severe stress response. These last values are not significant statistically, however, as the probability of occurrence by chance is over 68 percent.

The mean Lee-White clot time of the experimental group decreased from 9.25 minutes to 8.292 minutes. This was as would be expected since the ionic fatty acids increase. The clot time rose to 9 minutes between samples two and three which again would be expected on the basis of ionic fatty acid levels since they

fell after administration of carbohydrate. The clot time fell to 6.33 minutes at sample four. This corresponded to a rise in the ionic fatty acids after discontinuation of carbohydrate administration. The clot time rose to 9.583 minutes again even though ionic fatty acids were still rising. This again goes counter to expectation just as did the last sample in the control group. This may suggest some other factor involved in the later phase of acute distress.

The mean recalcification time again made little sense unless the second sample is disregarded. Even with this the values do not reflect the Lee-White clot times or the ionic fatty acid values.

The group given carbohydrate showed a significant decrease in total lipids in the third sample and a slightly further decrease to the fifth sample. This initial fall was correlated with a fall of ionic fatty acids, but when the ionic fatty acids rose between the third and fifth samples, this was in no way reflected by the total lipids.

The only correlation shown by either group was a fall in ionic fatty acids accompanied by a fall in total lipids. The ionic fatty acid rise in the initial two samples showed no significant rise in total lipids and the rise in the ionic fatty acids between the third and fifth samples in the group given carbohydrates showed

no rise although the rate of decrease fell markedly. Therefore, the total lipids seemed to be somewhat directly variable with the ionic fatty acids. This may have, in part, been a reflection of the ionic fatty acid variation although the ionic fatty acid fraction of the total lipids is very small and the half life of these acids is only three minutes, (35) so the likelihood of their being responsible for this change is slight. Serum lipase activity may have a part in causing this phenomenon also by degrading lipoproteins and triglycerides to ionic fatty acids which are rapidly removed from the circulation.

The fibrinogen values suggest some response to the stress situation by showing a definite rise in both groups in samples four and five. This may well be in response to the bleeding which occurred as a result of the operation.

No correlation with clot times, ionic fatty acids, or carbohydrate administration was notable. The baseline values between the control and carbohydrate group were so divergent as to make interpretation of any changes rather difficult.

One uncontrollable variable introduced into the study was that of anesthetic agents. Certain of these are thought to be procoagulants. (22) Fluothane, cyclopropane, and sodium pentothal were used with some frequency in this study and thereby may have in some measure tended to lower the coagulation time in sample three. This, however, would have no effect upon ionic fatty acid levels.

A study was done using 15 surgical patients from whom blood was obtained preoperatively and ten days postoperatively. (36) The total lipids and the fatty acids were measured. At ten days the total lipids showed no change. The ionic fatty acids varied widely, half being above, half being below the original value with the mean change being only 0.5 mEq/L. The author concluded that although the changes are not consistent in direction, they are consistently present and must therefore be due to the operative stress. According to the findings of the project I have just completed, the ionic fatty acid effects are nearly exhausted within 24 hours postoperatively. Any changes thereafter could be due to numerous things varying from the patient's diet to his habitus.

In regard to the mechanism of ionic fatty acid elaboration upon stress, it has long been known that epinephrine causes the elaboration of ionic fatty acids. (1, 24) More recently a lipid mobilizer hormone has been found which is supposed to originate in the posterior lobe of the pituitary gland. A study done on hypophysectomized rats into which posterior lobe extract was injected showed a lipemia. No other hormone would cause this. Total plasma cholesterol, total fatty acids, and phospholipids were increased two fold.

The clinical implications of this hypercoagulability are quite important insofar as they relate to not only the common

thrombotic disease but also have been implicated in the pathogenesis of irreversible shock and renal failure.

One statistical study (37) showed that one in 100 major operations had associated postoperative pulmonary thrombosis. In this study, the blood changes found postoperatively were increased fibrinogen by the third or fourth day (found 24 hours postoperatively in our results), increased prothrombin activity with increased fibrinogen B (thrombin+fibrinogen=fibrinogen B), increased platelets and increased circulation time in limbs of bed patients.

In regard to venous thrombosis of the lower extremities a study was done on various positions and their effects upon this. Semi-Fowlers position was found to be quite detrimental insofar as it caused a "crimp" in the femoral vein where it passes beneath Poupart's ligament. This, combined with a hypercoagulable state, was important in causing thrombophlebitis.

Work has been done showing that disseminated intravascular coagulation is responsible for irreversible shock, renal failure, and many other conditions.

In one such study, (38) the femoral arteries of dogs were catheterized and india ink injected. Group A had nothing done to it, Group B had the left kidney freed of peritoneal attachments before injection. The trauma to the tissue caused vasospasm and

resulted in capillary thrombosis which inhibited cortical function. This is analogous to the vasospasm of secondary shock. Capillary thrombi appear to release a vasospasm producing peptide in the thrombin fibrinogen reaction. The vasospasm and thrombus formation becomes a vicious cycle.

Another series (39) in which dogs were bled for a two hour period to experimentally reproduce hemorrhagic shock revealed several possible causes of hypercoagulability. The blood was anti-coagulated and returned to the dog after two hours. First noted was an increased norepinephrine level followed by hypercoagulability, stasis, and intravascular coagulation thus giving decreased fibrinogen values. This coagulation resulted in the infarction of tissue in liver, kidney, and gastrointestinal mucosa. As both a test of what functions are involved and as a possible therapeutic agent, heparin was used. It drastically reduced the fall in fibrinogen and the fall in prothrombin, both of which are consumed in the process of coagulation. The survival rate in heparinized dogs was 63 percent whereas the unheparinized dogs had a 4 percent survival rate.

To break up the mechanisms indicated in these last two studies, the treatment would include heparin to prevent coagulation, ganglionic blocking agents to prevent vasospasm and possibility fibrinolysis to reverse minor areas of thrombi already formed. (38)

Glucose administration would help in the same manner as would heparin by preventing the destabilization of fibrinogen by ionic fatty acid excesses.

Dicumarol administration would help also by decreasing the amount of prothrombin available for the production of thrombi but its action is relatively slow in onset and its effects are slowly reversible in the event of hemorrhagic episodes secondary to therapy.

The evidence presented here would indicate to me the advisability of using maximal intravenous feeding with glucose or invert sugar starting possibly prior to operation and continuing such feeding until 24 hours after the operation to prevent the rebound seen in the group given carbohydrates for only four hours. If the person is diabetic, the control of this with insulin if possible would seem desirable as they are especially susceptible to thrombotic episodes.

Exercise (37) as soon postoperatively as possible is always indicated to prevent stasis or pooling of blood.

SUMMARY

The body response to stress includes a hypercoagulability of blood. This gives rise to thromboembolic diseases and plays a part in irreversibility of shock and renal failure.

The study done dealt with the body response to operative stress, both psychologic and physical. The correlation of ionic fatty acids, fibrinogen, total lipid, and coagulation time was studied. The ionic fatty acids were found to rise during stress and were correlated to a decrease in clot time.

Two parallel groups were studied, the experimental receiving a 10 percent solution of invert sugar in electrolytes, the control receiving only the electrolyte. The carbohydrate was found to reduce ionic fatty acid levels significantly and in turn reduce the hypercoagulable state.

No definite relationship was established with fibrinogen or total lipids.

The clinical significance of the study lies in the finding that intravenous feeding of glucose or invert sugar appears to be an effective way to reduce the danger of postoperative complications of the thrombotic variety such as phlebothrombosis and pulmonary embolism.

TABLE I

IONIC FATTY ACIDS

CONTROL GROUP

mEq./L.

Patient #	Sample # 1	2	3	4	5
I	.351	.729	.378	1.593	.756
II	.350	.590	.780	1.200	.650
III	.510	1.110	1.690	.890	.760
IV	1.400	1.130	1.940	----	----
XII	1.000	1.000	1.920	1.540	1.020
XIII	.410	.840	.570	.380	.490
XV	.430	.680	.940	.650	.490
XVII	.840	1.490	.320	.620	.730
XXI	.540	.590	1.060	1.020	.620
Total	5.831	8.159	9.598	7.893	5.516
Mean	.648	.907	1.066	.877	.613
Standard Error of the Mean	.1200	.1005	.2126	.1603	.0671

TABLE II

IONIC FATTY ACIDS

EXPERIMENTAL GROUP

mEq./L.

Patient #	Sample # 1	2	3	4	5
V	.410	.540	.460	.880	.510
VI	.380	.620	.320	.270	.590
VII	.320	.650	.270	.730	.570
VIII	.430	.950	.270	.350	.140
X	.810	.760	.270	----	1.030
XI	.590	.620	.540	----	1.080
XVI	.890	.700	.220	1.190	1.000
XVIII	.300	.840	1.110	.410	.780
XIX	.620	.970	.650	.389	.650
XX	.920	.540	.430	.540	.860
XXIII	.540	.430	.920	.540	.380
XXIV	.430	1.010	.320	.320	.780
Total	6.640	8.630	5.780	5.610	8.370
Mean	.553	.718	.481	.561	.698
Standard Error of the Mean	.0624	.0500	.0812	.0933	.0812
Standard Error of the Difference	.1353	.1122	.2276	.1855	.1054
Relative Deviate	.7025	1.81	2.535	1.705	.805
Probability	> 68%	6.86%	1.04%	9.18%	> 68%

TABLE III

LEE-WHITE CLOT TIMES

CONTROL GROUP

Minutes

Patient #	Sample # 1	2	3	4	5
I	7	4	4	6	6
II	7	6	8	6½	6
III	8	12	11	9	9
IV	7½	5½	-	-	-
XII	10	9	6	10	11
XIII	7	8	8	8	8
XV	8	6	12	10	10
XVII	9	6	8	15	12
XXI	9	4	5	-	7
Total	72½	60½	62	64½	69
Mean	8.06	6.72	7.75	9.21	8.63
Standard Error of the Mean	.3549	.8544	.9777	1.1358	.8012

TABLE IV

LEE-WHITE CLOT TIMES

EXPERIMENTAL GROUP

Minutes

Patient #	Sample # 1	2	3	4	5
V	5	8½	9	8	9
VI	9	9	8	-	10
VII	10	8	10	7	10
VIII	11	10	11	10	11
X	10	12	11	-	10
XI	15	12	8	-	11
XVI	8	7	10	9	9
XVIII	10	7	11	12	11
XIX	9	7	10	9	10
XX	10	5	7	9	9
XXIII	7	7	5	5	10
XXIV	7	7	8	9	5
Total	111	99½	108	78	115
Mean	9.25	8.292	9.0	6.33	9.58
Standard Error of the Mean	.7190	.6172	.4604	1.049	.4680
Standard Error of the Difference	.8019	1.054	1.082	1.546	.8724
Relative Deviate	1.48	1.49	1.69	1.86	1.10
Probability	13.78%	13.78%	9.18%	5.89%	28.05%

TABLE V

RECALCIFICATION TIMES

CONTROL GROUP

Minutes

Patient #	Sample # 1	2	3	4	5
I	7½	3½	3½	5	4
II	6	3½	4½	5	17½
III	4½	58	-	13½	47
IV	26	16	10	-	-
XII	6	4	12½	12	8
XIII	8	8	11½	9	10½
XV	29	12	18½	15	11
XVII	8½	18	-	8	8½
XXI	30½	32	-	19	9½
Total	126	155	60½	86½	116
Mean	14	17.2	10.08	10.8	14.3
Standard Error of the Mean	3.6606	5.9498	2.2605	2.0224	4.8270

TABLE VI

RECALCIFICATION TIMES

EXPERIMENTAL GROUP

Minutes

Patient #	Sample # 1	2	3	4	5
V	16	28½	10	15	8
VI	16	7½	26	8½	12
VII	30½	5½	12	-	8
VIII	7	11	10½	8	7
X	30	11	6	-	8
XI	7	4½	17½	-	20
XVI	19	5	11½	13	7
XVIII	9	7	9	8	34
XIX	5	5½	-	5	4½
XX	-	-	21	12	15
XXIII	16	-	7	9	15
XXIV	24	11	29	12	16
Total	179½	96½	159½	90½	154½
Mean	16.32	9.65	14.5	10.06	12.54
Standard Error of the Mean	2.7129	2.2539	2.3431	1.0440	2.3516
Standard Error of the Difference	4.5607	6.3640	3.2558	2.2760	5.3759
Relative Deviate	.5152	1.189	1.36	.3642	.3278
Probability	> 50.00%	22.47%	17.73%	> 50.00%	> 50.00%

TABLE VII

PLASMA FIBRINOGEN

CONTROL GROUP

mgm.%

Patient #	Sample # 1	2	3	4	5
I	.030	.080	----	.080	.088
II	.325	.325	.320	.280	.330
III	.220	.230	----	.212	.220
IV	.220	.115	.155	----	----
XII	.285	----	.290	.282	.400
XIII	.105	.120	.110	.130	.155
XV	.282	.330	.305	.292	.340
XVII	.170	.125	.115	.128	.250
XXI	.125	.110	----	----	.200
Total	1.762	1.435	1.295	1.404	1.983
Mean	.196	.179	.216	.202	.248
Standard Error of the Mean	.0316	.0361	.0412	.0346	.0361

TABLE VIII

PLASMA FIBRINOGEN

EXPERIMENTAL GROUP

mgm.%

Patient #	Sample # 1	2	3	4	5
V	.160	.132	.132	.135	.190
VI	.300	---	.270	.305	.320
VII	.240	.320	.280	----	.315
VIII	.130	.120	.092	.100	.182
X	.150	.175	.120	----	.200
XI	.188	.205	.200	----	.240
XVI	.108	----	.170	.100	.150
XVIII	.370	.370	.310	.305	.370
XIX	.320	.320	----	.310	.320
XX	.175	----	.130	.170	.300
XXIII	.330	----	.270	.295	.360
XXIV	.215	.190	.135	.210	.225
Total	2.686	1.832	2.109	1.930	3.172
Mean	.224	.229	.192	.213	.267
Standard Error of the Mean	.0245	.0332	.0224	.0300	.0224
Standard Error of the Difference	.0400	.0490	.0469	.0458	.0424
Relative Deviate	.700	1.022	.5125	.240	.449
Probability	>68%	31.17%	>68%	>68%	>68%

TABLE IX

TOTAL LIPIDS

CONTROL GROUP

Percent

Patient #	Sample # 1	3	5
I	.70	.63	.50
II	.47	.49	.45
III	.50	.63	.44
IV	---	---	---
XII	.85	.82	.61
XIII	.72	.58	.57
XV	.81	.77	.60
XVII	.62	.77	.54
XXI	.81	.81	.56
Total	5.48	5.50	4.27
Mean	.685	.688	.534
Standard Error of the Mean	.0510	.0424	.0245

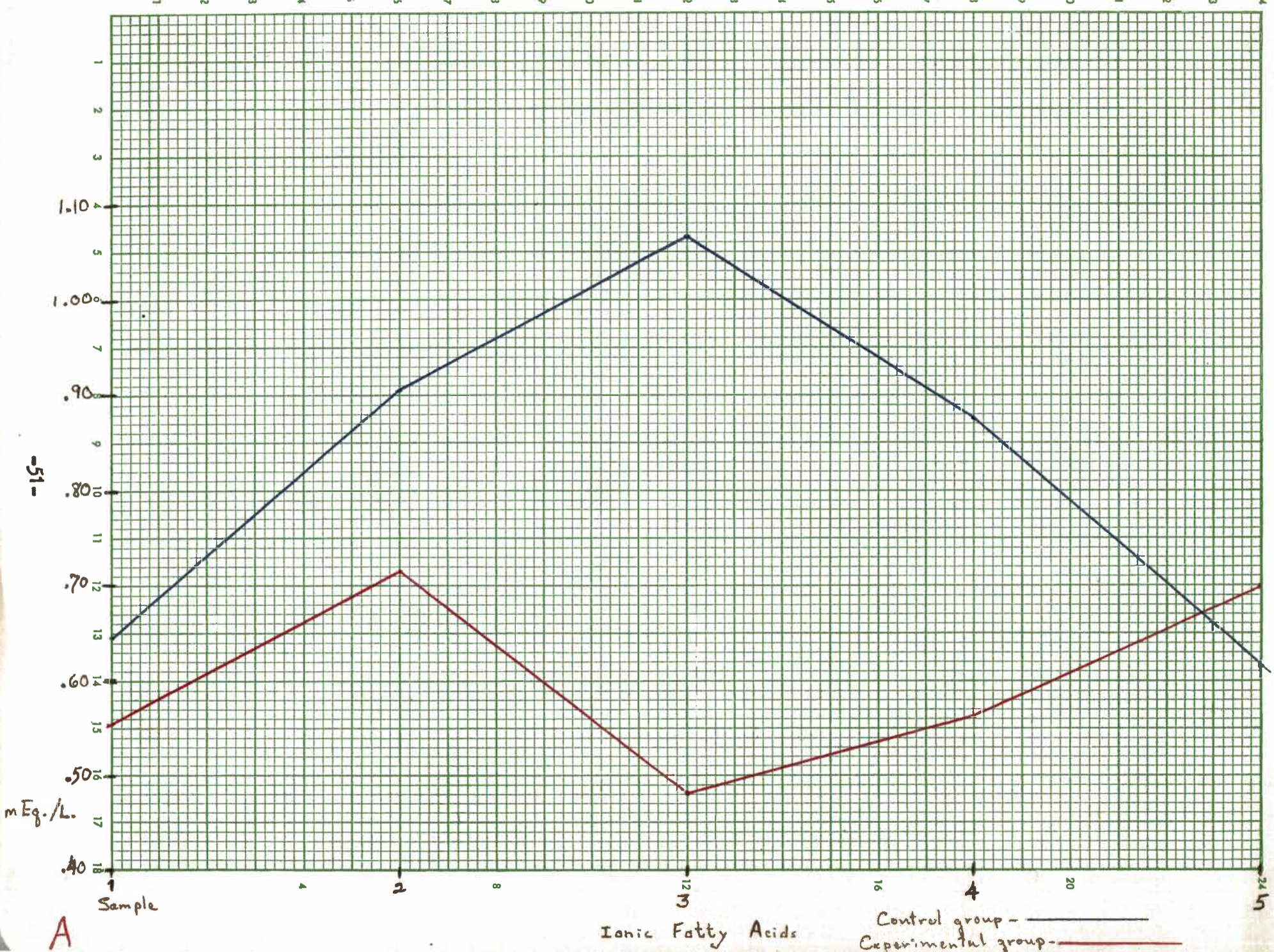
TABLE X

TOTAL LIPIDS

EXPERIMENTAL GROUP

Percent

Patient #	Sample # 1	3	5
V	.82	---	.80
VI	.82	.56	.58
VII	.55	.53	.76
VIII	---	.45	.30
X	.52	.41	.35
XI	.83	.83	.76
XVI	.85	.40	.635
XVIII	.65	.60	.40
XIX	.58	.52	.45
XX	.73	---	.50
XXIII	.65	.62	.48
XXIV	1.04	.92	.88
Total	7.94	5.84	6.895
Mean	.722	.584	.575
Standard Error of the Mean	.0480	.0539	.0520
Standard Error of the Difference	.0700	.0686	.0574
Relative Deviate	.52	1.518	.715
Probability	> 68.00%	13.78%	> 68.00%


1.10
1.00
.90
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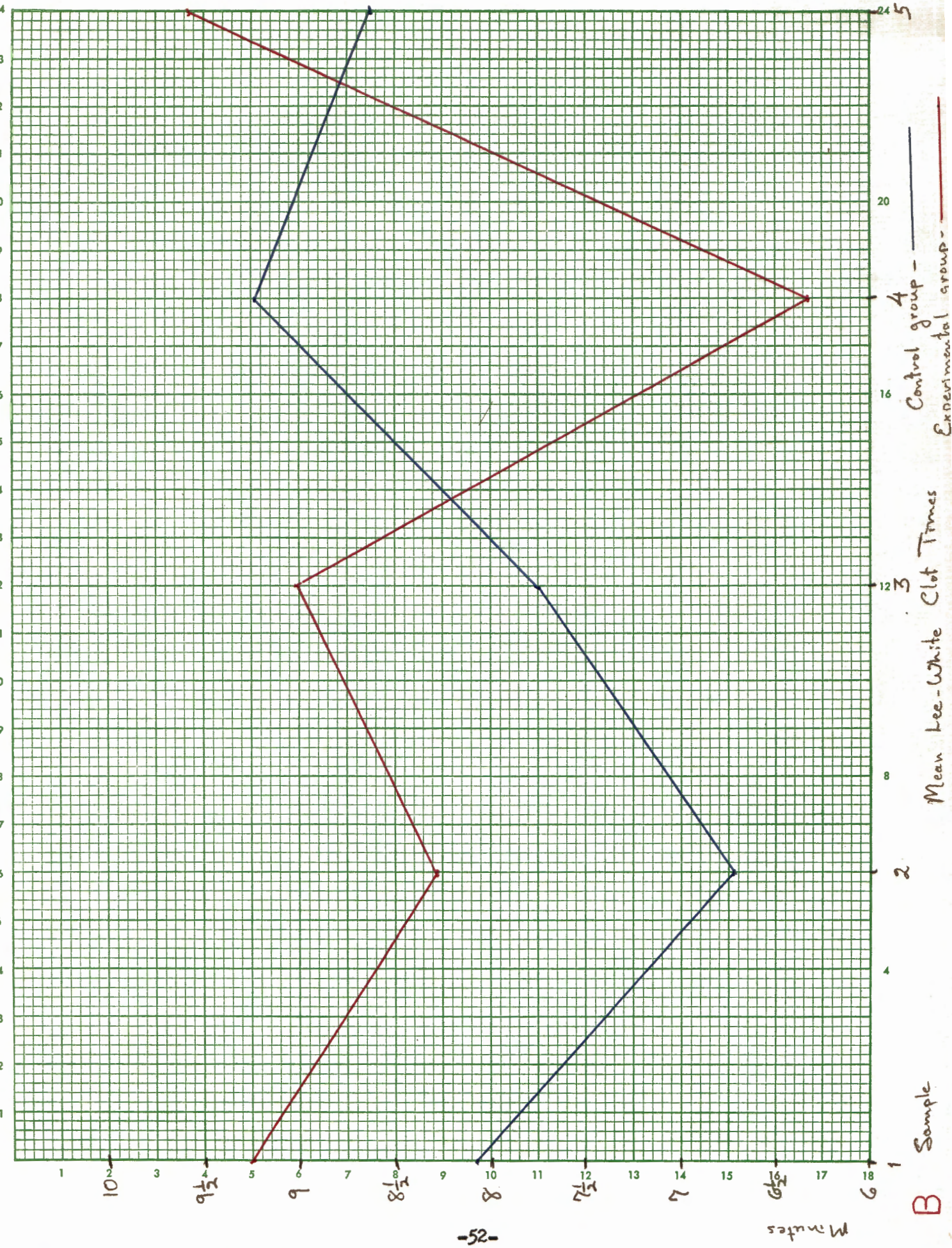
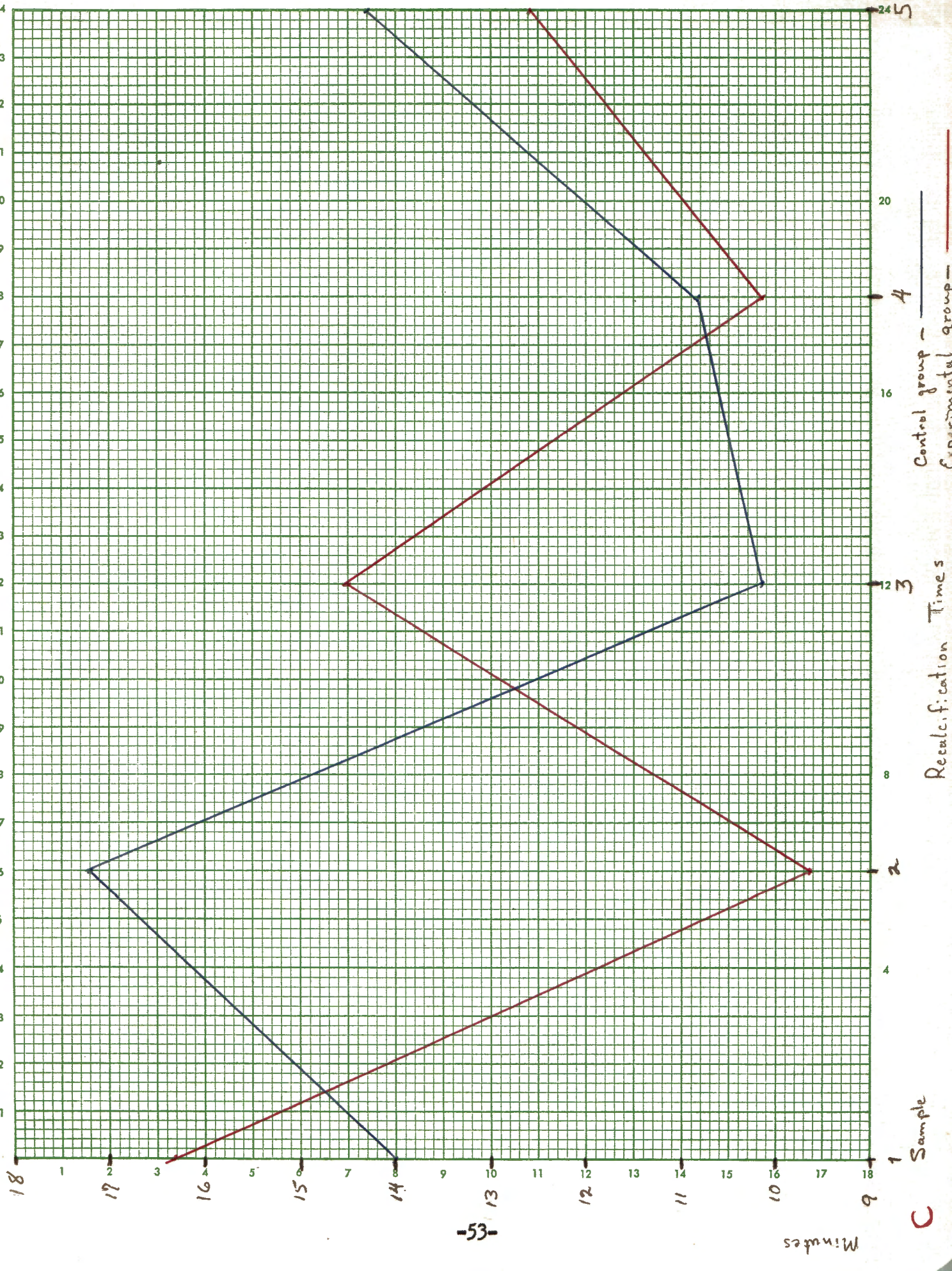
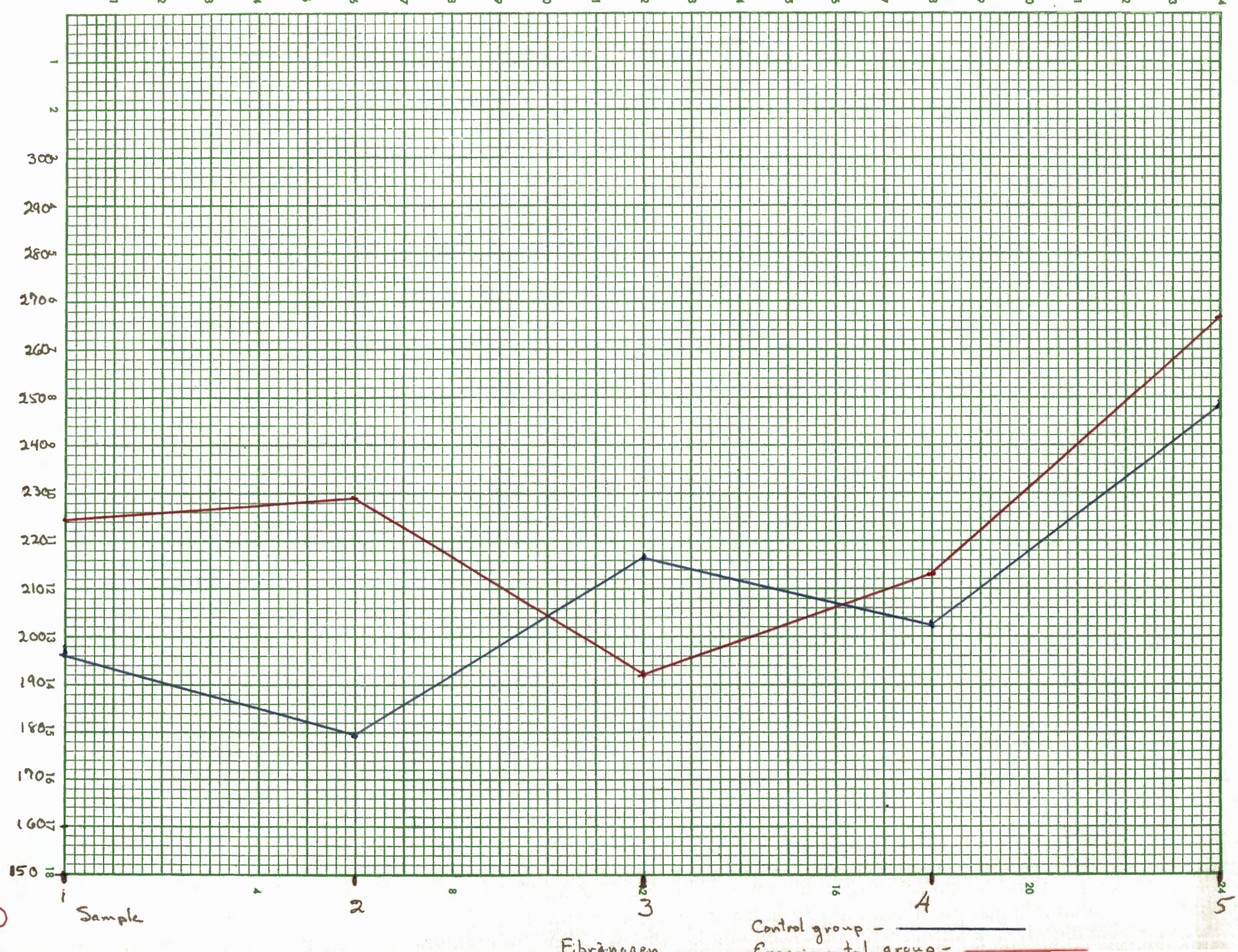
.50
.40
mEq./L.
1
2
3
4
5
Sample
Ionic Fatty Acids
Control group -
Experimental group -

A


Mean Lee-White Clot Times
Control group
Experimental group
Minutes
Sample
B
-52-


Control group
Experimental group
Recalcification Times
Sample
Minutes
1
2
3
4
5
C


% · mgm
300
290
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270
260
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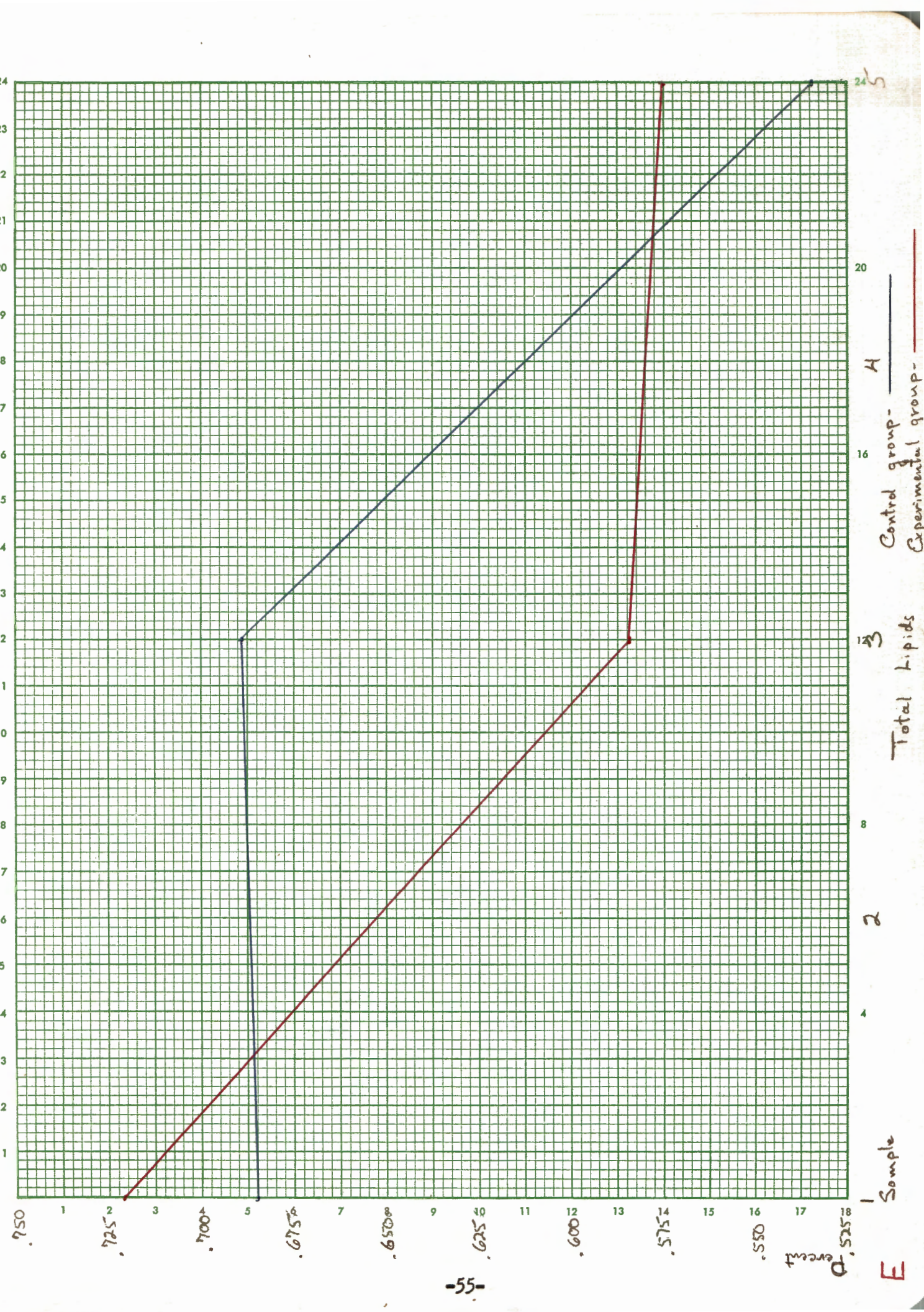
230
220
210
200
190
180
170
160
150
1
2
3
4
5
Sample
Control group –
Experimental group –
Fibrinogen

D


Control group-
Experimental group-
Total Lipids
Sample
Percent
.750
.725
.700
.675
.650
.625
.600
.575
.550
.525

ACKNOWLEDGMENTS

Prepared in the Department of Surgery under the direction of Merle M. Musselman, M.D. and Herbert L. Davis, Ph.D.

I am also indebted to Mrs. Nora L. Davis, Peter Quintero, and Harold Kaiman for valuable assistance in the analysis of blood samples.

I deeply appreciate the help and cooperation received from the staff and resident physicians of the surgery and anesthesiology departments.

I owe deepest gratitude to my wife, Lynne, who devoted much time and effort to the statistical evaluation of the results of the project.

I thank the secretarial staff of the Department of Surgery for preparation of the text of this thesis.

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