ALTERATIONS IN THE MECHANISM OF HAEMOSTASIS IN THE POST-SURGICAL PATIENT ASSOCIATED WITH THE DEVELOPMENT OF A HYPERCOAGULABLE STATE

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by

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ABSTRACT

Sequential testing, using a wide spectrum of clotting tests, most of which were designed for this study, was performed on dilute whole blood samples obtained from a series of 25 patients who underwent a variety of major elective surgical procedures. Testing commenced prior to surgery and thereafter at intervals during the first 7 to 10 days postoperatively. Clotting test results were compared to the pre-operative mean values so that each patient acted as his own control.

Very shortly after surgery, increased in vitro coagulability was demonstrated in almost all patients by the use of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests. A peak increase in coagulability was found between days 3 to 5, with a smaller peak occurring on or around the eight day postoperative. The results of the phase 3 test showed marked dispersion in some patients, while others gave results consistent with increased coagulability.

Plasma fibrinogen concentration and the platelet count both increased early in the postoperative period and thereafter increased progressively. The results of the two universally used tests of haemostasis, namely the prothrombin time and the kaolin-cephalin clotting time

were comparatively poor indicators of the postoperative increase in coagulability demonstrated by the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests, although the results of the phase 3 test were somewhat similar to those found using the prothrombin time.

This study has demonstrated that in the majority of patients a postoperative increase in the concentration of serum fibrin(ogen) degradation products is found at some time during the first 7 days following surgery.

Clot retraction, studied with an original technique, was found to increase in approximately 50% of patients during the first 3 postoperative days.

During the first 7 days following major elective surgery, the rise in the fibrinogen concentration and in the platelet count, and the increase in the concentration of serum fibrin(ogen) degradation products indicates that, in attempting to detect haemostatic failure in the postoperative patient, evaluation of haemostatic tests should not be made using the so-called "normal values". The author suggests that postoperative evaluation of haemostasis be compared to values obtained prior to surgery.

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INTRODUCTION

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The Issue of Hypercoagulability.

Haemorrhagic disorders are often associated with a deficiency of one or more of the blood clotting factors which have been officially recognized by the International Committie on Nomenclature, and dignified by the assignment of a roman numeral. Usually such disorders are demonstrated by the finding of an increase in the time required for whole blood or plasma to clot in tests which are sensitive to the factor or factors which are reduced in concentration. Such disorders are examples of the hypocoagulable state.

In contrast, the blood of certain individuals is characterized by an increase in either, or both of the activity or concentration of one or more of the blood clotting factors. This increase may sometimes be demonstrated by noting a shortening of the time required for whole blood or plasma to clot in tests which are sensitive to the factor or factors which are increased in concentration or activity. Such a state has been referred to as a hypercoagulable state. Although the demonstration of *in vitro* hypercoagulability may be acheived, hypercoagulability in the patient is still

hypothetical.

To define hypercoagulability in this way however, may represent an oversimplification. The term has acquired a diversity of connotations and has become popular during a period of active experimental investigations of *in vivo* coagulation, as well as during widespread studies of clinical conditions associated with thrombosis or disseminated intravascular coagulation. Specifically, the term has been applied to; 1) changes in the concentration of clotting and/or fibrinolytic factors such that *in vitro* clotting tests are accelerated (Spittell *et al* 1961, Hume & Chan 1967, Skjodt 1967), 2) a stage of intravascular coagulation during which clotting factors are activated (Botti & Ratnoff 1964, Niemetz & Nossel 1967), and 3) the experimental initiation of intravascular clotting by the injection of *tissue thromboplastin*, (Penick *et al* 1958, Rapaport *et al* 1963, Izak & Falewsky 1966, Nossel 1967), *thrombin*, (Warner *et al* 1939, Rapaport *et al* 1963, Kowalski *et al* 1965, Margaretten *et al* 1967), and *activated clotting factors* (Arakawa & Spaet 1964, Deykin 1966).

On the other hand, many clinicians would prefer to define hypercoagulability as a clinical state in which we know intravascular thrombi are prone to develop and would welcome clues to its presence.

The term hypercoagulability is highly subjective, having a somewhat nebulous character and has remained

difficult to define by laboratory investigations for the following reasons. Hypercoagulability does not mean the same state when used by different people. There is a wide variation in the normal levels of blood clotting factors in persons of the same age and sex (though one person's level may remain constant). So that a patient with a usual level at the upper end of the normal range of any clotting test might be said to be in a hypercoagulable state if his test result changed to the mid normal range. Whereas a patient with a usual level in the mid normal range would have to change to the low normal range before one could define the state of his blood as hypercoagulable. Thus unless one knows a patient's usual level, it is difficult to comment on the result of a test in the postoperative period or during a serious illness.

In spite of the somewhat nebulous nature of the hypercoagulable state, it is generally held by clinicians that such a state does exist in certain types of patients. The lineage of the hypercoagulable state does appear to have some measure of respectability. Wiseman (1676), described the properties of a thrombus and stated that intravascular occlusion was related, in part, to systemic alterations in the circulating blood. Virchow (1856), some 200 years later, made almost passing reference to hypercoagulability and ennunciated his famous triad of factors influencing the pathogenesis of venous occlusion. In the same paper he introduced the term "thrombosis",

probably in an attempt to emphasize his opposition to the concepts prevailing in his time, regarding the mechanisms of spontaneous intravascular occlusion. He refuted Hunter's theory of "inflammation of the inner layer of the vein" as being the fundamental pathogenetic process in thrombosis. He denied Bichat's concept of "excessive tendency of the vein for inflammation. Virchow was the first to realize that an alteration in the balance between procoagulant and anticoagulant forces within the circulating bloodstream could play a role in the establishment of intravascular thrombi. Virchow's original hypothesis, that it was mainly contact with air or oxygen that induced blood to clot was modified since he was unable to explain all cases of thrombosis with this blood-gas contact hypothesis. His modification, the oft cited "Triad of Virchow", remains as valid today as at the time of its ennunciation, and states that the formation of intravascular fibrinous occlusions results from the interplay of alterations in a) blood flow, b) the vessel and c) the biochemical factors in the blood.

The Hypercoagulable State in Medical Patients

Since the end of World War II, blood clotting factors have been discovered in rapid successsion, largely by the recognition of congenital deficiency states inindividual patients with haemorrhagic diatheses.

The identification and partial characterization of each new clotting factor has often raised hopes that the factor may play a key role in the pathogenesis of thromboembolic diseases, most of which appear to be characterized by some laboratory manifestation of hypercoagulability. It has been assumed that the hypercoagulable state predisposes to thromboembolism, although direct evidence for a cause and effect relationship is still lacking. Nevertheless, certain clinical conditions are known to be associated with an increased incidence of thromboembolism including; malignancy (Sproul 1938, Byrd et al 1947, Amundsen et al 1963, Levin & Conley 1964, Thornes et al 1967 and Brugarolas et al 1973), arteriosclerosis obliterans (Pascuzzi et al 1961), coronary artery disease (Wright & Marple 1954, McDonald & Edgill 1957 a, b, Mersky et al 1960, Cooperberg & Teitelbaum 1961, Chakrabati et al 1968), thrombophlebitis (Durham 1955, Egeberg 1965, Hume 1966), pregnancy and the peurperium (Dieckman & Wagner 1934, Pechet & Alexander 1961, Strauss & Diamond 1963, Todd et al 1965, Nossel et al 1966, Poller & Thomson 1966, Bonnar et al 1970), ulcerative colitis (Lee et al 1968), homocystinuria (McDonald & Bray 1968), polycythaemia (Dawson & Ogston 1970), diabetes mellitus (Moolten et al 1963, Egeberg 1963, Fearnley et al 1963, Hellem et al 1964, Bridges et al 1965, Shaw et al 1967, Chmielewski & Farbiszewski 1970), trauma (Innes & Sevitt

1964, Sevitt 1969), and in women who use oral contraceptives (Rutherford et al 1964, Houghie et al 1965, Kaulla & Kaulla 1970, Fagerhof & Abilgaard 1970, and Howie et al 1970).

The Hypercoagulable State in Postoperative Patients

Following surgical operations, there are changes in many of the properties of the blood which are important in haemostasis. Many studies have been performed to determine the physiological response to surgical trauma, and a wide variety of haemostatic parameters have been studied. Perhaps the most consistent change found in the postoperative period is the rapid rise in the level of circulating fibrinogen (Warren et al 1950, Atkins & Hawkins 1969, Ygge 1970 a, b, Hammer et al 1973). Another well documented change involves the circulating numbers of platelets. Dawbarn et al (1928), Potts & Pearl (1941), Ham & Slack (1967) and Fogliano et al (1973) all reported increases in the numbers of circulating platelets in the postoperative period. The increase in the platelet count is commonly subsequent to an early postoperative decrease. Increases in the adhesiveness of platelets in the postoperative patient have been documented by Wright (1942), Emmons & Mitchell (1963), Hirsh et al (1966), Bygdeman et al (1968), Negus et al (1969),

and by O'Brien et al (1974). The property of platelet aggregation in response to the addition of certain stimuli, such as collagen, adrenalin and adenosine diphosphate, the subject of much intensive study during the past decade, has also been shown to alter following surgical trauma. Enticknap et al (1970), demonstrated an increase, in the early postoperative period, in the numbers of platelets that aggregate in response to the addition of adenosine diphosphate. to O'Brien et al (1971), demonstrated a transient period occurring in the late operative and early postoperative period, in which platelets became refractory to the addition of such stimuli as collagen and adenosine diphosphate. Additional changes which have been documented as part of the haemostatic alteration in response to surgical trauma include; increased concentrations of factors VIII, IX and X (Egeberg 1962, Davidson & Tomlin 1963, Nilchen & Nilsson 1967, and Ygge 1970 a,b), increased consumption of prothrombin (Williams & Warren 1957), and a decrease in plasminogen and spontaneous fibrinolytic activity (Ygge 1970 a, b).

Another approach to the study of in vitro hypercoagulability during the postoperative period has been to examine changes in the activity of clotting factors in whole blood or plasma samples. Shortening of the clotting times of whole blood and plasma has been reported by Poller (1956), Gardikas et al (1959), Feruglio et al (1960),

Eastham & Johnson (1963) and by Eastham & Morgan (1964). Shortening of the clotting times of whole blood and plasma in the presence of very small amounts of heparin (heparin resistance test) have also been documented by DeTakats (1943), Waugh & Ruddick (1944), DeTakats & Marshall (1952), Gardikas et al (1959), Holger-Madsen & Schiolar (1959), Gormsen & Haxholdt (1960), Feruglio et al (1960), Godal & Fichera (1961), Gormsen & Haxholdt (1961), Eastham & Johnson (1963). Postoperative shortening of the clotting times of whole blood held in siliconized test tubes has been documented by McClerry et al (1952). The prothrombin time of whole blood or dilute plasma may also shorten during the postoperative period as was demonstrated by Brambell & Loker (1943), Sandrock & Maloney (1948) and by Warren et al (1950).

Aims of the Study.

The greater majority of studies directed towards determining the haemostatic changes resulting from surgical operations have been characterized by one or more of the following; a) study of the concentration of specific clotting factors in plasma samples using specific factor assays, b) study of the activity of groups of clotting factors by the use of non-specific tests performed on plasma samples. Some of the latter tests, e.g. the prothrombin time and partial thromboplastin time, are universally used and have proven sensitivity to clotting factor deficiencies, but not to increases in clotting factors and/or activity, and c) comparison of results of clotting tests performed on patient samples with normal values obtained from the testing of samples drawn from "healthy" persons.

Studies having these characteristics may be open to criticism on the following grounds. Cell poor plasma is an artificial sample and is not representative of the circulating blood. The use of well established tests, such as those cited above, without modification, to follow the development of a hypercoagulable state is questionable since it is likely that the kinetics of clotting in factor deficient plasma samples is quite different from those in plasma samples containing an excess of one or more factors.

Since the normal range of clotting factors is so wide, very significant alterations in the level of one or more clotting factors, following some appropriate stimulus, might be described as being within "normal limits" in spite of changes which may readily exceed 100% of the pre-stimulus value.

With these thoughts in mind, this study was performed in order to follow the haemostatic changes following surgery using; a) whole blood samples, b) test systems utilizing dilute whole blood, in an effort to increase the sensitivity of the tests to increases in clotting factors or activity, and c) the patient's pre-operative haemostatic test results as the baseline to which all postoperative results would be compared, that it, the patient acts as his own control. Finally, the entire spectrum of clotting tests would be used with each patient in a sequential manner.

MATERIALS AND METHODS

A) MATERIALS

De-ionized water.

Water used for the preparation of all solutions and reagents was freshly de-ionized by passage through a Barnstead de-mineralizer charged with a mixed bed resin. The resistance of such water was always in excess of 1 megohm.

Tris (Tris Hydroxymethyl Aminomethane) Buffer

(Fisher Scientific Co., Toronto, Ontario).

Tris buffer was made up according to Gomori (1946). A stock solution of 0.1M tris was prepared. To 500ml. of this stock solution, 414ml. of 0.1M HCl and 6.58 grams of dried sodium chloride was added. Following complete dissolution of the added chemicals, the volume was made up to 1000ml. with de-ionized water. The pH of the buffer solution was checked with a pH meter and adjusted to pH 7.4 if necessary.

Heparin Solution

Heparin, 1,000 international units per ml. (M.T.C.Pharmaceuticals, Hamilton, Ontario) was diluted with tris buffer to give a solution of 1,000 international units per 100ml.

Calcium Chloride Solution

A 0.025M solution was prepared by adding 2.75 grams of anhydrous calcium chloride to de-ionized water. Following complete dissolution of the chloride, the volume was made up to 1,000ml. with de-ionized water.

Thrombo-Wellcotest Kit

Used in the determination of serum fibrin(ogen) degradation products. (Warner Chilcott Laboratories).

Castor Oil (U.S.P.).

1% w/v Celite in Tris Buffer.

For the preparation of this reagent, Celite 512 A.W. (John's Manville Product, Hartman Leddon Co., Philadelphia), was used.

Tissue Thromboplastin (Simplastin).

(Warner Chilcott Laboratories, Toronto, Ontario)

This preparation of rabbit brain thromboplastin contains an added optimum concentration of calcium chloride and was used only for the phase 3 test.

Brain Thromboplastin

(Ortho Pharmaceuticals, Don Mills, Ontario).

Used in the determination of the one stage prothrombin time.

Hypertonic Sodium Chloride Solution.

A 1.8% w/v solution was prepared using anhydrous sodium chloride.

Sodium Citrate Anticoagulant

A 3.8% w/v solution was prepared and sterilized in small volumes.

Prothrombin Test Unit Reaction Chambers

(Fisher Scientific Co., Toronto, Ontario).

Micro-projector, Tri-Simplex, Bausch & Lomb

(Canadian Laboratory Supplies, Toronto, Ontario)

The micro-projector was modified in the following manner. A clear plastic box 18 inches long, by 9 inches wide and 2 inches high was fixed to the stage of the micro-projector. By means of a pump, water maintained at 37°C was delivered to the box and returned to the pump. The arrangement of the delivery and return water lines, permitted a constant depth of water of 1½ inches.

Automatic Pipettes

For dispensing 0.1ml. and 0.2ml. volumes, a Fibrometer automatic pipette (Canadian Laboratory Supplies, Toronto, Ontario) with disposable tips was used. For the dispensing of 0.5ml. and 1.0ml. volumes, Oxford automatic pipettes with disposable tips were used. (Canadian Laboratory Supplies, Toronto, Ontario). For the dispensing of the heparin solution, a 100 micro-litre, Hamilton gas-tight syringe, fitted with a Chaney adaptor and delivery stops was used (Canadian Laboratory Supplies, Toronto, Ontario).

Polystyrene Tubes with Polyethylene Caps.

(Luckham Ltd., Burgess Hill, Sussex, England).

B) METHODS

Collection of Venous Blood Samples.

Venous blood samples were obtained from a prominent ante-cubital vein using a two-plastic syringe technique, with minimal tourniquet induced stasis. Following the light application of a tourniquet and cleansing of the puncture site, the first plastic syringe, bearing a 19 gauge, 1 inch needle, was used to collect 5ml. of venous blood, 2ml. of which were immediately added to a vial containing thrombin/trayslol (supplied with the Thrombo-Wellcotest Kit), the vial contents were then thoroughly mixed. The remaining 3mls. were rapidly discharged into vacutainer tubes containing 1% ethylene-diamine tetra-acetic acid and thorough mixing accomplished. The second syringe, containing 1ml. of 3.8% sodium citrate was then attached to the needle, left *in situ*, and 9mls. of venous blood were slowly collected. Following sample collection, the needle was removed and the syringe piston was withdrawn slightly to permit mixing of the syringe contents, which was accomplished by slowly inverting the syringe 10 times. The anticoagulated blood was then slowly dispensed into a polystyrene tube and closed with a polyethylene cap.

Blood samples were then taken to the laboratory

for immediate processing and testing. The testing of all blood samples during the study commenced within 30 minutes of phlebotomy.

Preparation of Blood for Testing

a) Preparation of non-contact activated whole blood.

Into a polystyrene tube containing 2.5ml. of tris buffer, 2.5ml. of venous whole blood was added. The tube was closed with a polyethylene cap and the contents mixed by inverting the tube 10 times. The tube was then placed in the 37°C water bath.

b) Preparation of contact activated whole blood.

Into a polystyrene tube containing 2.5ml. of 1% celite in tris buffer prewarmed to 37°C was added 2.5ml. of venous whole blood. Following capping, the tube was placed on a rotator operating at 15 revolutions per minute. During each cycle of the rotator, the tube was immersed in the water bath, maintained at 37°C, for 180 degrees of each cycle. Rotation (contact activation) was allowed to proceed for exactly 5 minutes. Following the termination of contact activation, the blood was

tested immediately.

c) Preparation of platelet poor citrated plasma.

2.5mls of citrated venous whole blood was transferred to another polystyrene tube and centrifuged at 2,000g for 15 minutes. The supernatant platelet poor plasma was removed with a plastic pipette and stored in a polystyrene tube in ice water until testing commenced.

Test Methods

Phase 1 Test.

0.2ml of the non-contact activated dilute whole blood was added to a polystyrene tube containing 0.6ml. of tris buffer and 0.2ml. of 0.025M calcium chloride solution, all prewarmed to 37°C. A stopwatch was started simultaneously and the tube was gently tilted at one second intervals by means of a special holding plate. During tilting, the tube was examined by means of a x5 large hand lens mounted on a laboratory stand. The stopwatch was stopped when a firm fibrin clot formed. The time taken for the formation of the fibrin clot was recorded.

Phase 2 Test.

0.2ml. of contact activated dilute whole blood was added to a polystyrene tube containing 0.6ml. tris buffer and 0.2ml. 0f 0.025M calcium chloride solution prewarmed to 37°C. A stopwatch was started simultaenously and the tube was treated as already described for the phase 1 test.

Phase 3 Test

0.2ml. of contact activated dilute whole blood was added to a polystyrene tube containing 0.4ml. of tris buffer and 0.4ml. of tissue thromboplastin (Simplastin), containing calcium chloride. Reagents in the tube were prewarmed to 37°C. A stopwatch was started simultaneously and the tube was then treated as already described for the phase 1 test.

Heparin Resistance Test

(Modified from Marbet & Winterstein 1950).

1.0ml. of contact activated dilute whole blood was added to a polystyrene tube containing 1.0ml. of 0.025 M calcium chloride solution and 0.01ml. of heparin solution, both prewarmed to 37°C. A stopwatch was started simulataneously and the tube was treated in the same manner as already described for the phase 1 test.

Autocoagulography Test

0.1ml. of contact activated dilute whole blood was added to a polystyrene tube containing 0.2ml. of deionized water prewarmed to 37°C. The tube contents were quickly mixed and allowed to incubate at 37°C

for 60 seconds. 0.2ml. of hypertonic (1.8%) sodium chloride solution was then added to restore isotonicity to the haemolysate, followed immediately by 0.5ml. of contact activated dilute whole blood. The tube contents were then mixed by tilting the tube twice and 0.5ml. of 0.025M calcium chloride solution was added. A stop-watch was started simultaneously and the tube was treated as already described for the phase 1 test.

Autocoagulography Control Test.

0.1ml. of contact activated dilute whole blood was added to a polystyrene tube containing 0.4ml. of isotonic saline solution prewarmed to 37°C. Following a 60 second period of incubation, 0.5ml. of contact activated dilute whole blood was added followed immediately by 0.5ml. of 0.025M calcium chloride solution. A stop-watch was started simultaneously and the tube was treated as already described for the phase 1 test.

Figures 1, 2, 3, 4, 5, and 6 found on pages 19 to 24 respectively, illustrate the principles, reagents used and the clotting activity thought to be measured by each of the phase 1, phase 2, phase 3, heparin resistance, autocoagulography and autocoagulography control tests.

Gravimetric Estimation of Fibrinogen as Fibrin.

Method based on that of Ingram (1952).

2.0ml. of citrated platelet poor plasma was recalcified by the addition of 1.0ml. of 0.025M calcium

PHASE I

Principle. Dilute non-contact activated whole blood is added to a relatively large volume of tris buffer containing calcium ions. The time required for the formation of a fibrin clot is recorded.

Clotting activity thought to be measured by the Test.

Clotting activity resulting from the activation of all factors that participate in the intrinsic mechanism of blood coagulation, i.e. Factors XII, XI, IX, VIII, X, V, II, I, and platelets.

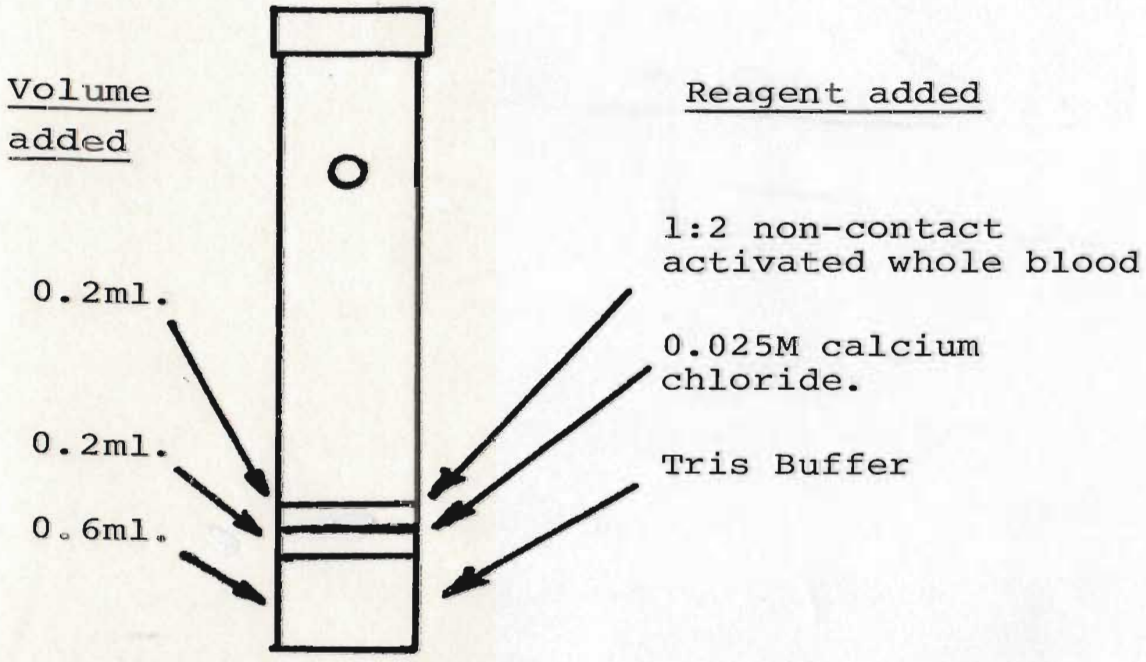

Fig.1. Illustrating the principle, reagents and the clotting activity thought to be measured by the phase 1 test.

PHASE 2.

Principle. Dilute contact activated whole blood is added to a relatively large volume of tris buffer containing calcium ions. The time required for the formation of a fibrin clot is recorded.

Clotting Activity thought to be measured by the Test

Clotting activity resulting from the activation of Factors IX, VIII, X, V, II, I and platelets in the presence of pre-activated Factors XII and XI.

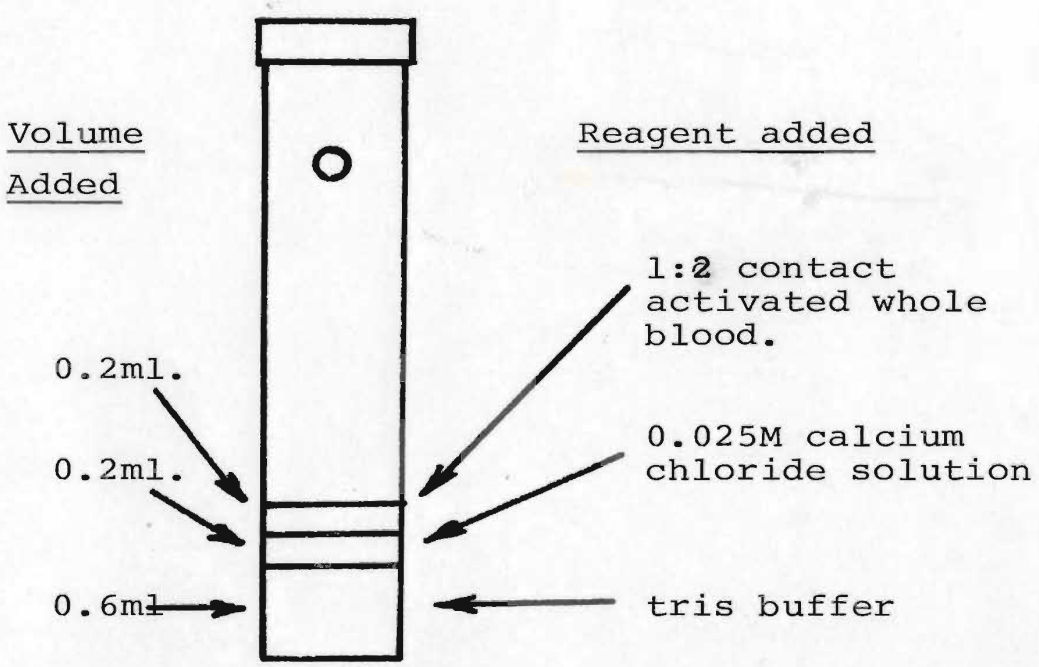

Fig.2. Illustrating the principle, reagents and the clotting activity thought to be measured by the phase 2 test.

Phase 3.

Principle. Dilute contact activated whole blood is added to tris buffer containing tissue thromboplastin and calcium ions. The time required for the formation of a fibrin clot is recorded.

Clotting activity thought to be measured by the Test

Clotting activity resulting from the activation of all factors that participate in the extrinsic mechanism of blood coagulation, i.e. Factors X, VII, V, II and I.

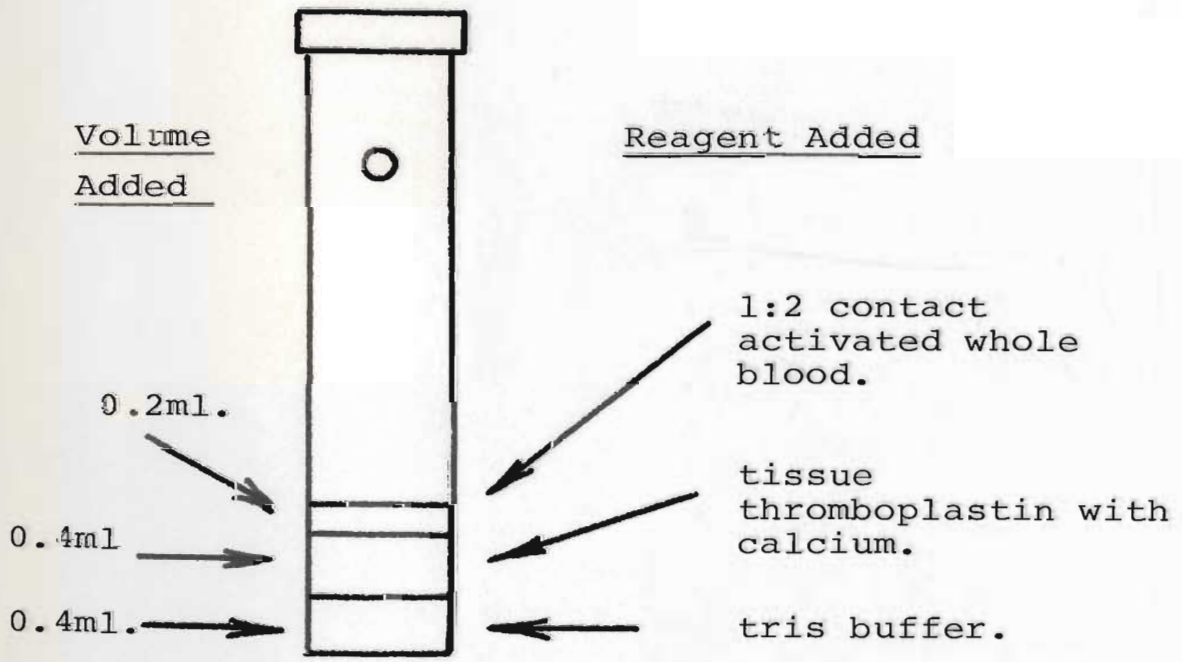

Fig.3. Illustrating the principle, reagents and the clotting activity thought to be measured by the phase 3 test.

Heparin Resistance Test.

Principle Dilute contact activated whole blood is added to calcium chloride containing a small concentration of heparin. The time required for the formation of a fibrin clot is recorded.

Clotting activity thought to be measured by the test

Clotting activity resulting from the activation of Factors IX, VIII, X, V, II, I and platelets in the presence of pre-activated Factors XII and XI and in the presence of a small amount of heparin.

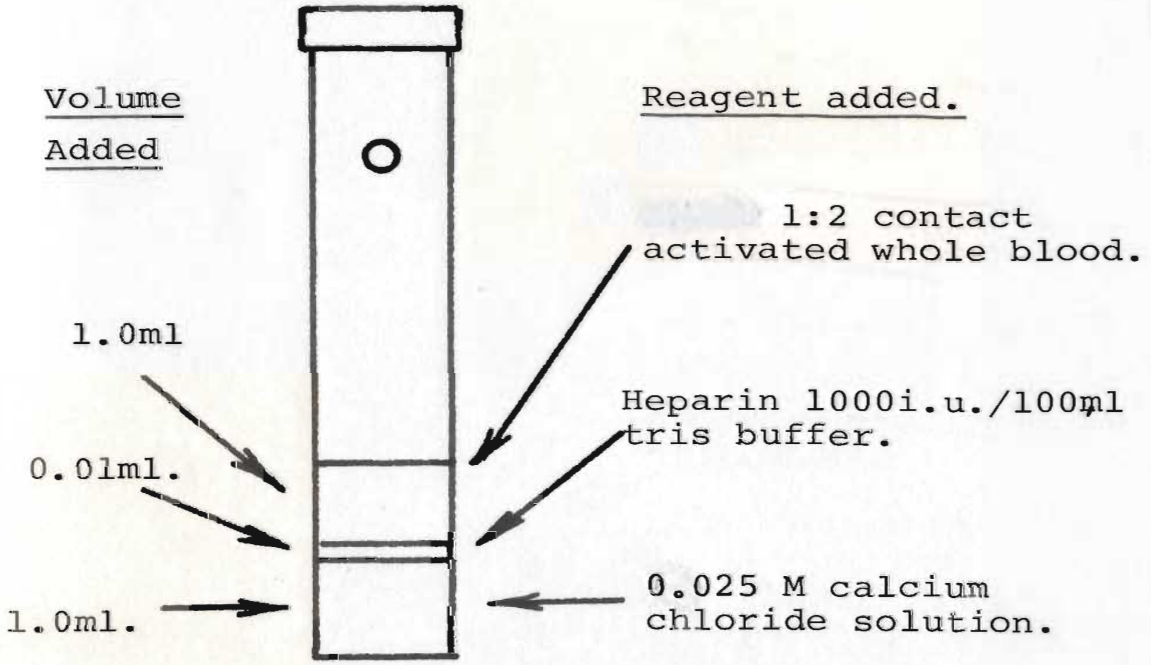

Fig.4. Illustrating the principle, reagents and the clotting activity thought to be measured by the heparin resistance test.

Autocoagulography Test.

Principle. dilute contact activated whole blood is added to a small volume of autologous haemolysate. The mixture is recalcified and the time required for the formation of a fibrin clot is recorded.

Clotting activity thought to be measured by the test.

Clotting activity resulting from the activation of Factors IX, VIII, X, V, II, I and platelets in the presence of autologous haemolysate and pre-activated factors XII and XI.

The difference between the autocoagulography control test and the autocoagulography test will reflect the coagulant activity of the red cells.

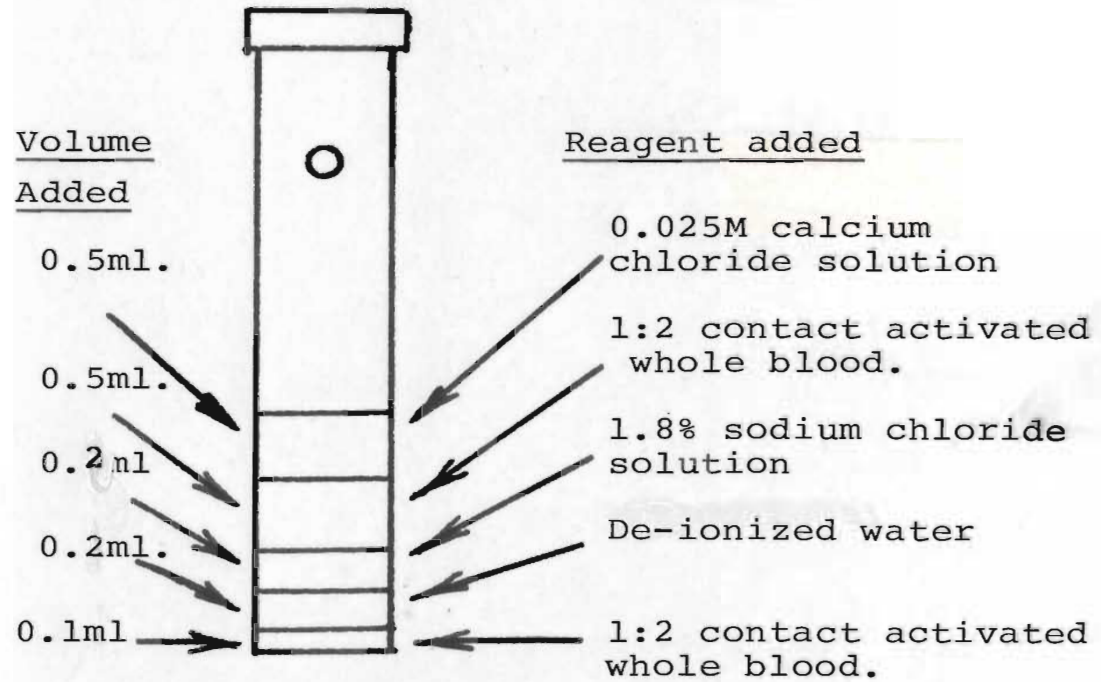

Fig.5. Illustrating the principle, reagents and the clotting activity thought to be measured by the autocoagulography test.

Autocoagulography Control Test.

Principle. Dilute contact activated whole blood is added to an equal volume of the same blood diluted with isotonic saline solution. The mixture is recalcified and the time required for the formation of a fibrin clot is recorded.

Clotting activity thought to be measured by the test.

Clotting activity resulting from the activation of Factors IX, VIII, X, V, II, I and platelets in the presence of pre-activated Factors XII and XI.

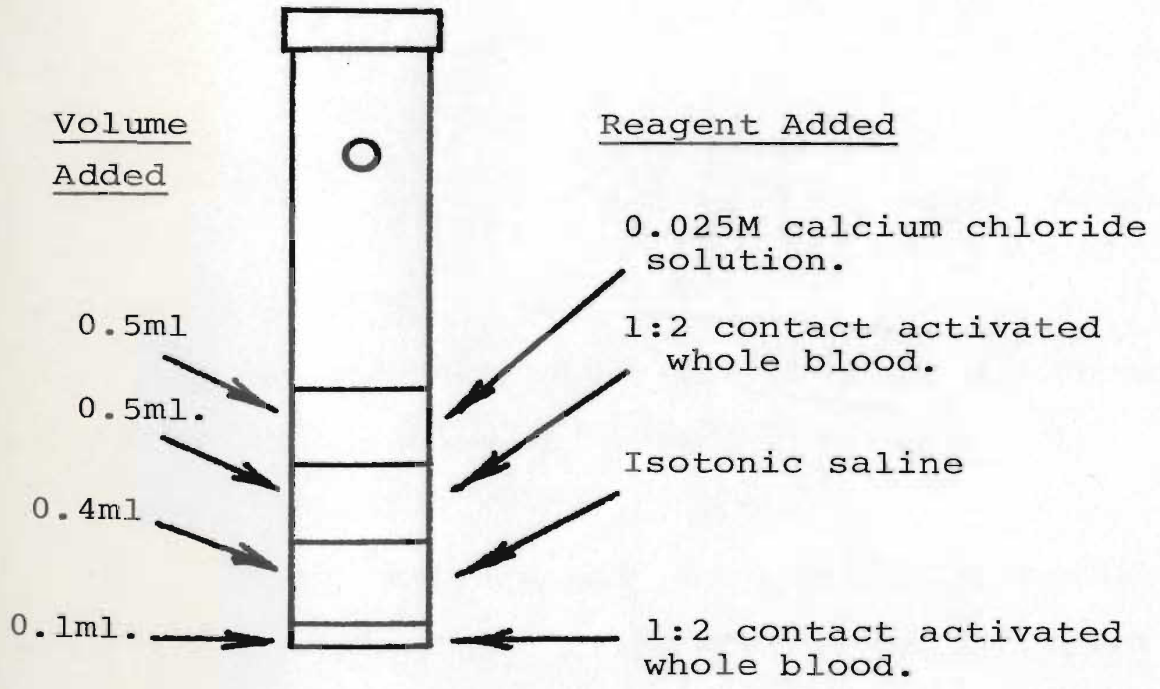

Fig.6. Illustrating the principle, reagents and the clotting activity thought to be measured by the autocoagulography control test.

chloride solution. The tube was corked and inverted a number of times to effect thorough mixing. The tube was then placed in a water bath maintained at 37°C and left to incubate for 30 minutes. Following incubation, the clot was carefully removed and the serum expressed from the clot by repeatedly pressing the clot between circles of filter paper. The fibrin clot was then washed in large volumes of de-ionized water for 5 minutes, followed by rinsing in acetone for the same time period. The fibrin clot was then allowed to dry in a 37°C incubator for 24 hours, following which the clot was weighed on an analytical balance. The concentration of fibrinogen (as fibrin) was calculated by the use of the following formula:

$$\frac{\text{Weight of fibrin(mg)}}{2} \times 100 = \text{Fibrinogen mg/100ml. plasma.}$$

Platelet Counts.

Platelet counts were performed using the Thrombo-counter. (Coulter Electronics, Miami, Florida).

Kaolin-cephalin Clotting Time.

The method was performed in accordance with the manufacturer's instructions. The kaolin-cephalin reagent used was obtained from Diagnostic Reagents, Thame, Oxon, England. Each vial of lyophilized kaolin-cephalin reagent was reconstituted with 10ml. of freshly de-ionized

water and was thoroughly mixed just prior to use. 0.2ml. of the kaolin-cephalin reagent was pipetted into a 10 x 75mm. glass test tube and was incubated in a water bath maintained at 37°C. Following incubation for 3 minutes, 0.1ml. of test plasma was added and the tube was tilted 5 times and returned to the water bath: 60 seconds after the addition of the plasma, the tube was re-tilted 5 times and again returned to the water bath. At exactly 2 minutes after the addition of the plasma, 0.1ml. of 0.025M calcium chloride solution was added and a stop-watch started simultaneously. The tube was tilted once a second and the time required for the formation of the fibrin endpoint was recorded.

One Stage Prothrombin Time

The method was performed in accordance with the instructions issued by the manufacturer of the brain thromboplastin (Ortho Pharmaceuticals, Don Mills, Ontario). The lyophilized brain extract/calcium chloride reagent was reconstituted with 12ml. of freshly de-ionised water. 0.2ml. of the reconstituted reagent was pipetted into a 10 x 75mm. glass test tube and incubated for 3 minutes at 37°C. 0.1ml. of the test plasma was then pipetted into the tube and a stopwatch started simultaenously. The time required for the formation of a fibrin clot was recorded.

Clot Retraction.

1.0ml. of non-contact activated dilute whole blood was dispensed into a polystyrene tube containing 0.5ml. of 0.025M calcium chloride solution pre-warmed to 37°C. By means of a fibrometer pipette, fitted with a disposable plastic tip bearing a 21 gauge, 1 inch needle, 0.1ml. of the well mixed blood-calcium mixture was carefully dispensed into the bottom of a prothrombin test unit reaction chamber filled with castor oil warmed to 37°C. (See Figure 7, page 28). The reaction chamber was then placed into the plastic box containing water circulating constantly and being maintained at 37°C which was mounted on the stage of the micro-projector. The remaining blood in the polystyrene test tube, still held in the water bath, was examined until clotting resulted. At this time the micro-projector was switched on, focussed, and the projected image of the clot in the reaction chamber was carefully drawn on paper. This outline represents the size of the clot at time zero (the time of clotting). The image of the clot, maintained at 37°C throughout the period of observation, was redrawn at 10, 20, 30 and 60 minutes. (See Figure 8, page 29). Only the image of the clot was drawn, no attempt being made to outline the image of extruding serum. After the period of observation, the clot outline were carefully cut out and weighed on an analytical balance. The percent

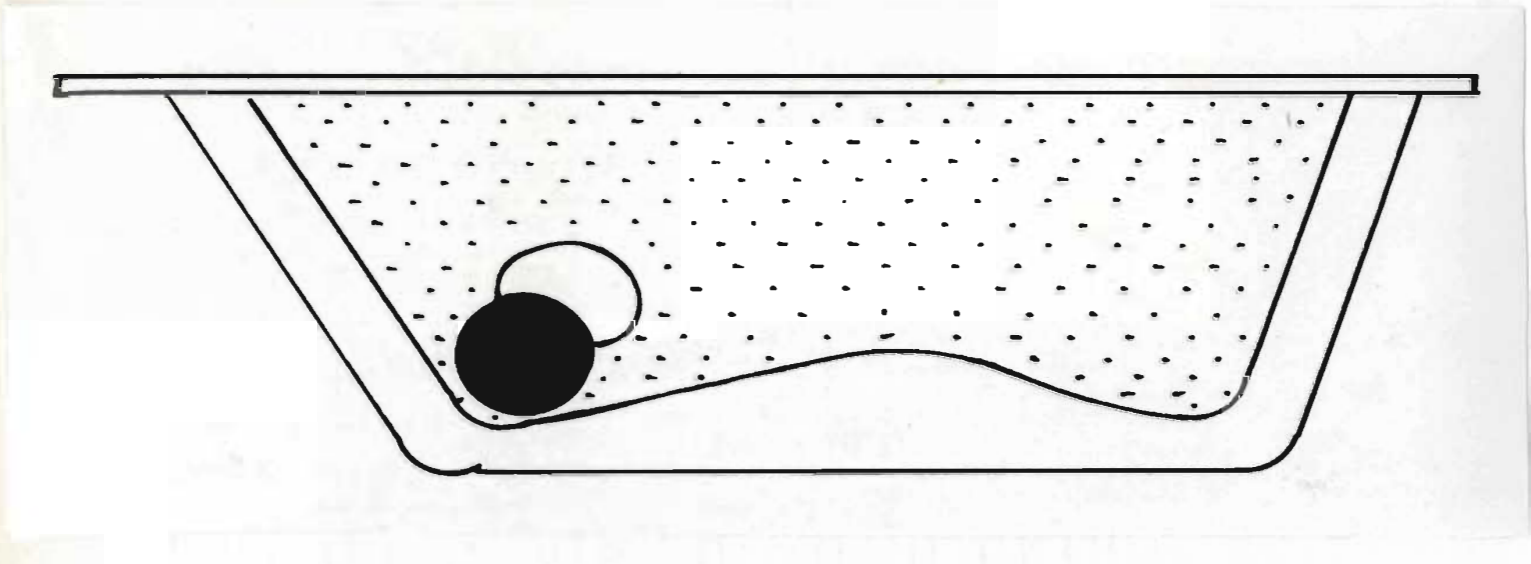

Fig 7. Showing the clear plastic reaction chamber, filled with castor oil and containing a clot which is actively retracting as evidenced by the extrusion of serum. The width of the chamber as seen from the top is 2 mm.

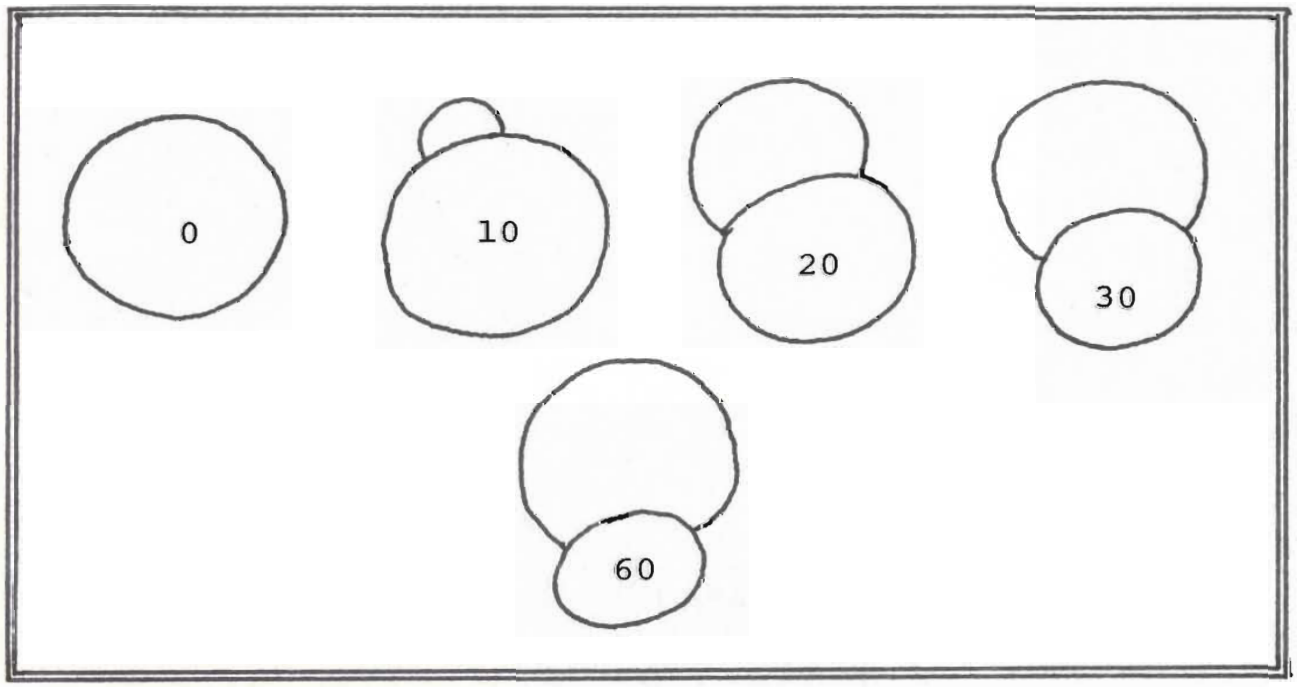

Fig. 8. Showing the drawn outlines of the projected image of a retracting clot at time zero and at 10 minute intervals following clotting for the first 10 minutes and again at 60. The times in the figure are superimposed on the clot itself.

retraction occurring at any time interval was calculated by the use of the following formula:

$$\frac{Wt.C.O.T._{o} - Wt.C.O.T._{in}}{Wt.C.O.T._{o}} \times 100 = \%\ \text{Retraction at } T_{in}$$

Where $C.O.T._{o}$ = weight of clot outline at time zero and $C.O.T._{in}$ = weight of clot outline at any particular time interval.

The percent retraction values were then plotted against time (See Figure 9, page 30), and the median retractive index found. The median retractive index (M.R.I.) represents the time at which 50% retraction occurs. A second indice, the maximum retractive activity time (M.R.A.T.) was also recorded. This indice was found by noting the time at which peak retractive activity resulted based on the % retraction values obtained for each 10 minute interval. Thus, if 20% retraction occurred at 10 minutes and 35% retraction was found at 20 minutes, 50% at 30 minutes and 56% at 60 minutes, then 20% retraction occurred during the first 10 minutes, 15% during the second 10 minutes, 15% during the third 10 minutes and 6% during the last 30 minutes. These values were also graphically displayed (See Graph line 2, Figure 9, page 30). In Figure 9, the median retractive index

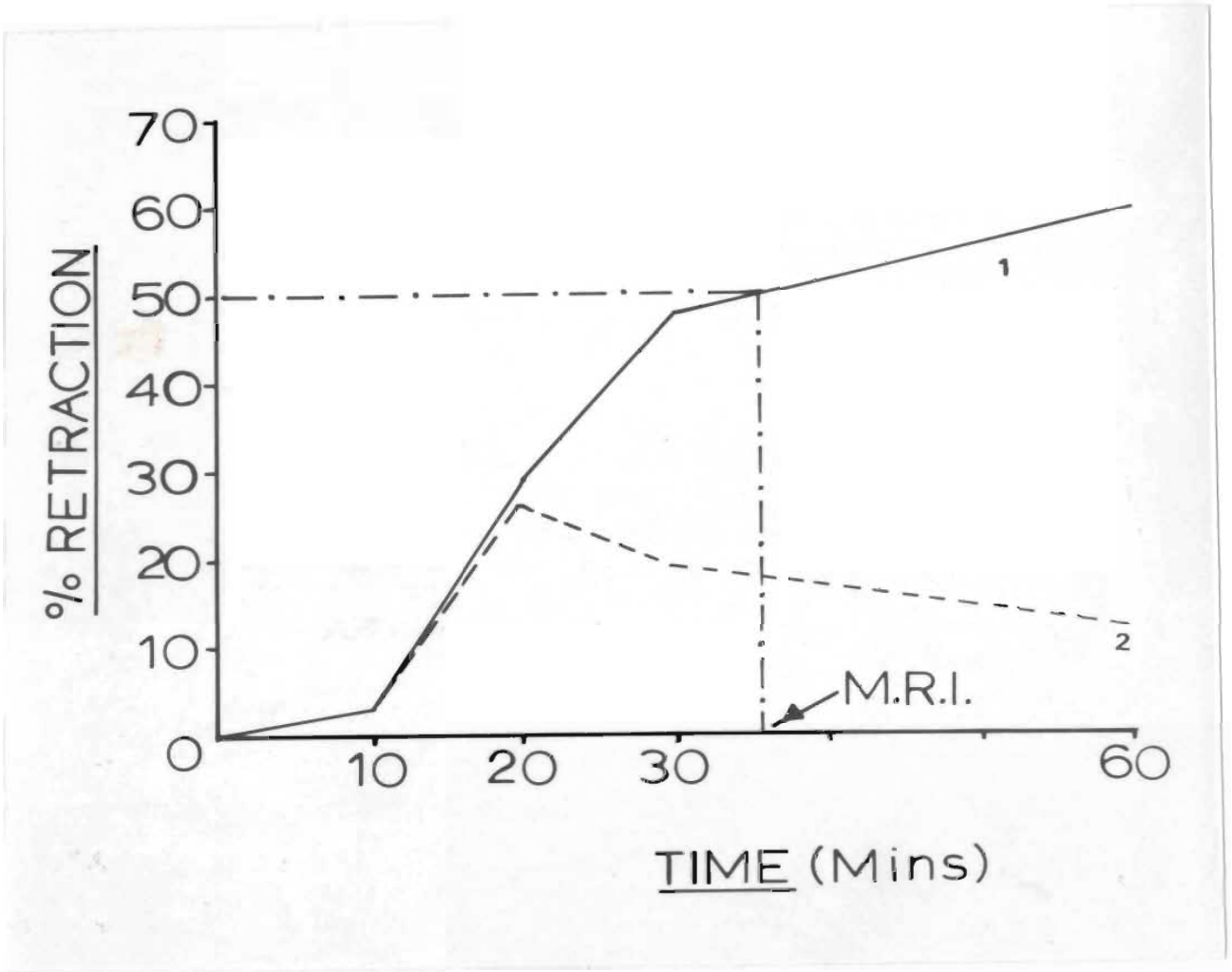

Fig.9. Showing the manner of plotting progressive clot retraction over the 60 minutes period of observation (Graph line 1). Graph line 2 shows a plot of the % retraction that occurs in each 10 minute period up to 30 minutes. The last point on this graph line shows the % retraction that occurred during the last 30 minutes of the period of observation.

is 35 minutes and the maximum retractive activity time is 20 minutes.

Assay of Serum Fibrin(ogen) Degradation Products

For this assay the Thrombo-Wellcotest kit supplied by Warner Chilcott Laboratories, Toronto, Ontario was used.

Following incubation for 2 hours at 37^{o}C, the blood collected into the vial containing thrombin/trayslol was centrifuged at 2,000g for 10 minutes to obtain cell free serum. Using the glycine buffer as a diluent, serum dilutions of 1:5, 1:10, 1:20, and 1:40 were routinely prepared. Occasionally additional dilutions were prepared. The serum dilutions were tested in the following way. Using droppers supplied with the kit, one drop of each serum dilution was delivered into separate wells on a glass slide. To each drop of serum dilution, 1 drop of sensitized latex particles was added. Using wooden applicator sticks, the contents of each well were well mixed. At exactly 2 minutes after mixing, the reaction patterns were evaluated against a dark background. If an unagglutinated latex pattern was observed the test was recorded as being negative. If, however, the latex particles agglutinated, the reaction was recorded as positive. A positive and negative control serum was always included with each test. The method of recording the results can be seen in Table 2, (page 34).

Table 1. Summary of blood collection, processing and tests performed on the various subsamples of blood.

Syringe	One		Two		
Volume blood drawn	5ml.		9ml.		
Subsample	2ml.	3ml.			
Added to	Thrombin/ Trayslol	EDTA	1ml. 3.8% sodium citrate solution		
Subsample			2.5ml.	2.5ml.	2.5ml
Diluent			2.5ml Tris Buffer	2.5ml 1% Celite in Tris buffer	
Subsequent Treatment				Contact Activation	
Tests Performed.	F.D.P.	Platelet Count	Phase 1 Clot Retraction	Phase 2 Phase 3 Autocoagulography Autocoagulography Control Heparin Resistance	Prothrombin Time Kaolin-cephalin clotting time. Fibrinogen Determination.

Table 2. Showing the method of recording the levels of serum fibrinogen degrada-products.

Serum Dilution	Reaction	Serum FDP Level
1:5	Negative	Less than 10micrograms/ml.
1:5 1:10	Positive Negative	10 to 20 micrograms/ml.
1:10 1:20	Positive Negative	20 to 40 micrograms/ml.
1:20 1:40	Positive Negative	40 to 80 micrograms/ml
1:40 1:80	Positive Negative	80 to 160 micrograms/ml.
1:80 1:160	Positive Negative	160 to 320 micrograms/ml

Table 1, (page 33), summarizes the methods of blood collection, processing of the various sub-samples and the tests performed on each of the sub-samples.

Methods employed for the Addition of Blood and Other Reagents to the Polystyrene Tubes and for the Visualization of Fibrin Clot Endpoints.

For the performance of the phase 1, phase 2, phase 3, heparin resistance, autocoagulography and autocoagulography control tests, 10ml. polystyrene tubes, closed with polyethylene caps, were used. A ¼ inch hole was made in each tube about one third of the way down from the stopper, prior to its use. The purpose of the hole was to facilitate the addition of blood (and other reagents) directly into the reaction mixtures. (See Figure 10, page 36). When performing the clotting tests referred to above, the 6 appropriate tubes were held onto a glass plate by means of a stout rubber band. The plate, in turn, was fixed to a bakelite rod by means of epoxy cement. The ends of the rod rested on the edge of the water bath during a series of tests. This arrangement allowed easy and rapid access to all tubes and permitted simultaneous tilting and observation of all the tubes. (See Figure 11, page 37).

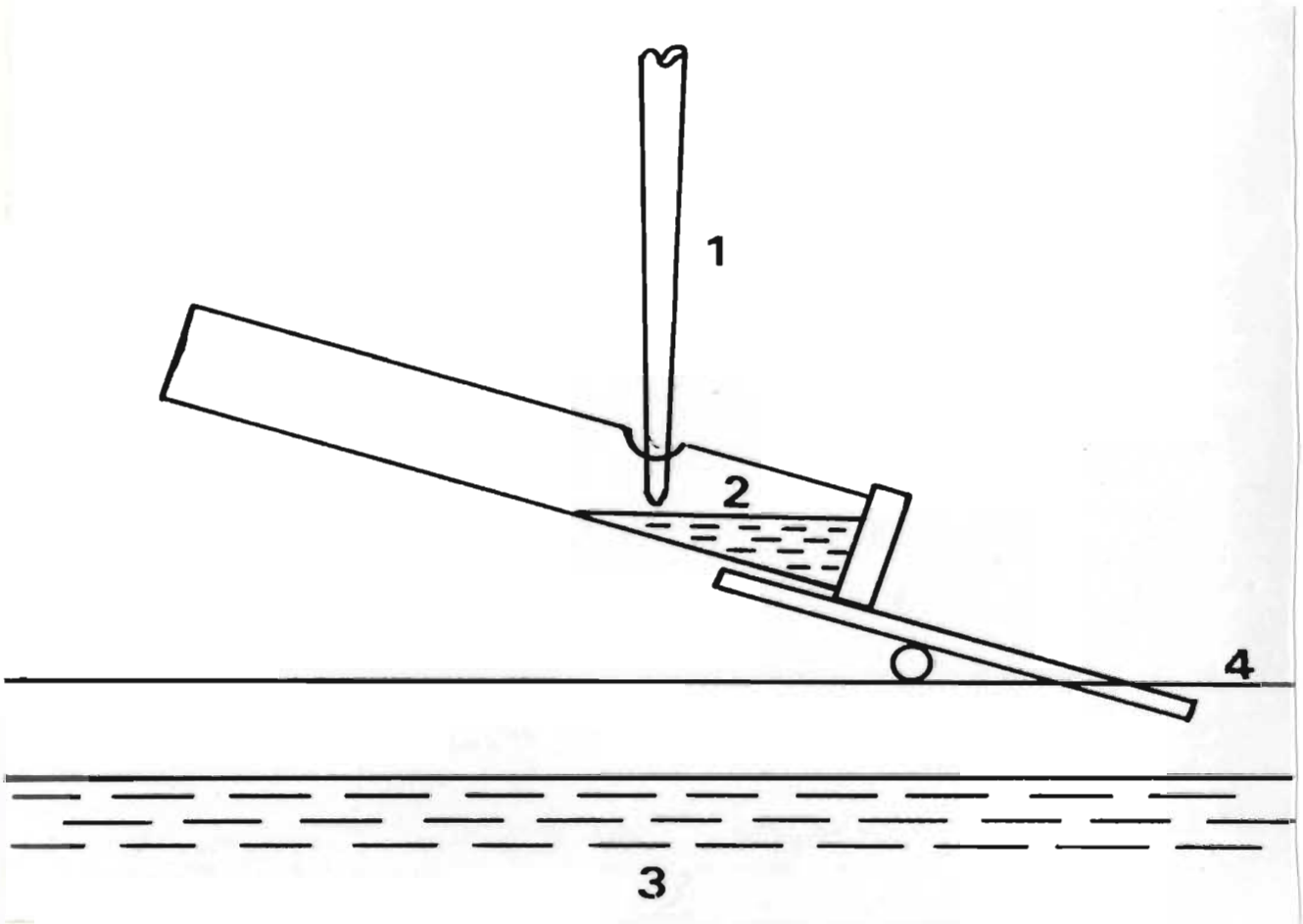

Fig. 10. Illustrating the manner in which blood and other reagents were added to the plastic test tubes.

1) The dispensing pipette tip
2) Plastic test tube containing reagents.
3) Water bath maintained at 37°C.
4) Top edge of water bath.

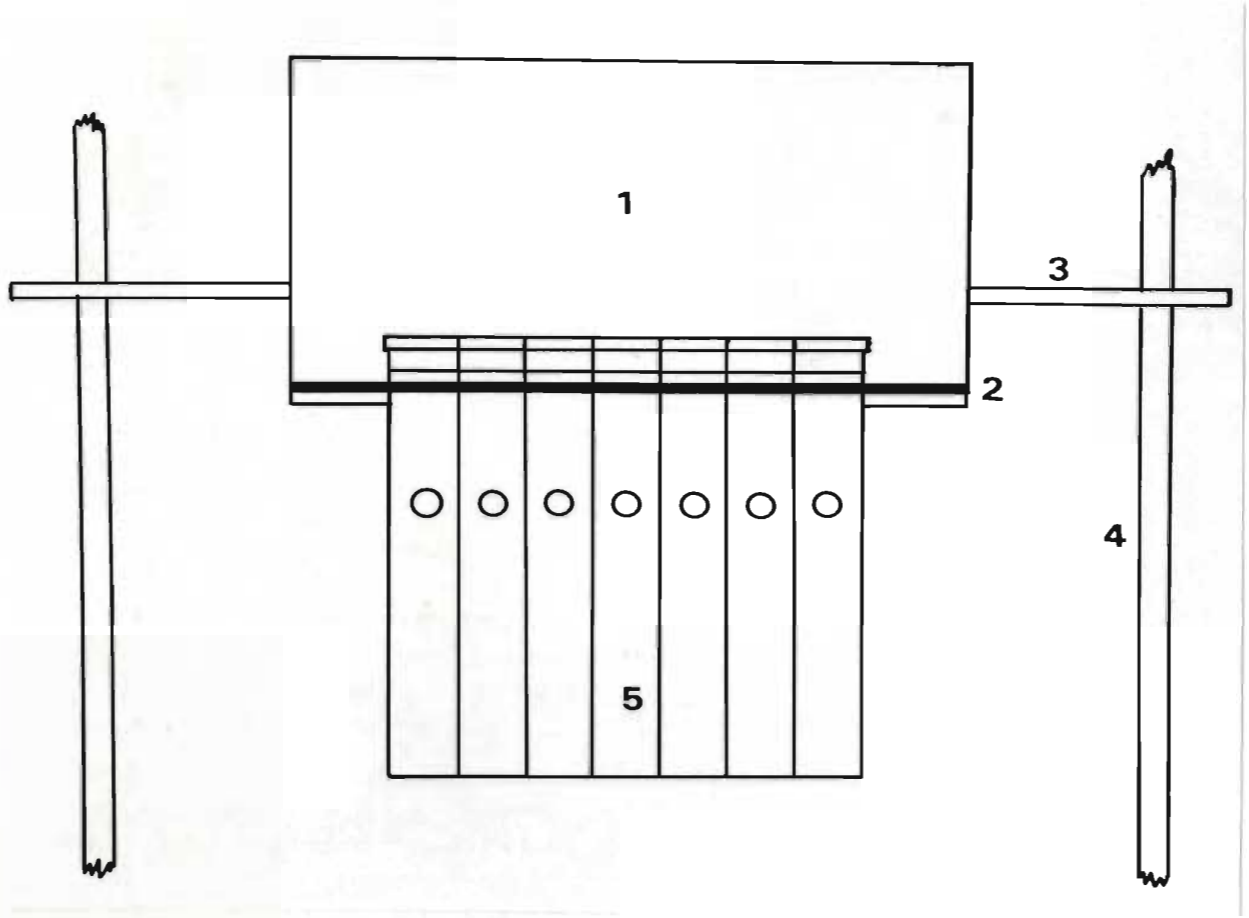

Fig. 11. Plan view, showing the method of holding a number of polystyrene tubes over the water bath for the simultaneous performance of a number of tests.

1) Glass plate
2) Rubber band
3) Bakelite rod fixed to plate
4) Top edges of water bath
5) Polystyrene tubes

By means of a mirror placed at an angle in the water bath, light from an external light source was directed upwards and passed through the tubes under observation. This arrangement removed the difficulty, often experienced, in observing the formation of fibrin clot endpoints in tests utilizing whole blood.

Throughout the study all tests were performed in a definite sequence. Phase 1, phase 2, heparin resistance test and phase 3 were performed simultaneously, using separate stopwatches for each test. As soon as the endpoint was obtained for phase 3, the autocoagulography and autocoagulography control tests were commenced using separate stopwatches. After the completion of these tests, the kaolin-cephalin clotting time and prothrombin time were performed, followed closely by the clot retraction study. The estimation of fibrinogen, the level of serum fibrino(gen) degradation products and the platelet count were carried out last. No difficulties were experienced in the simultaneous performance of certain tests referred to above because of the wide differences in the clotting times.

Patients Selected for the Study.

Patients used in the study were between the ages of 35 to 60 years, and were admitted for elective surgery, with the exception of patient # 24 who had

surgery for a ruptured appendix. The types of surgical procedures together with the numbers of patients having a particular surgical operation are shown in Table 3,(page 40).

The entire spectrum of clotting tests were performed on each patient at least once prior to surgery, and when possible, twice before surgery. With the exception of the kaolin-cephalin clotting time, prothrombin time, fibrinogen degradation products, clot retraction, fibrinogen and platelet count, all post-operative results were expressed as a percentage change from the pre-operative values, or, when two pre-operative tests were performed, the percentage change from the mean of the pre-operative values. Whenever possible, the entire spectrum of clotting tests were repeated at two to three day intervals following the day of surgery.

Table 3. Showing the numbers of patients undergoing various surgical procedures.

Number of Patients.	Surgical Procedure or Event
2	Scheduled, but did not go to surgery
2	Minor surgery with general anaesthesia
5	Gastrectomy
4	Cholecystectomy
4	Splenectomy
2	Vagotomy and pyloroplasty
2	Laparotomy
1	Laparotomy and partial gastrectomy
1	Bowel ressection
1	Removal of large pelvic tumour
1	Removal of abdominal tumour
1	Right nephrectomy
1	Removal of intestinal polyps and adhesions
1	Thoracotomy
1	Appendectomy

RESULTS

REPRODUCIBILITY STUDIES

In order to determine the precision of the tests employed in the study, many of which are original or are modifications of existing techniques, the following experiments were performed and the standard deviation calculated using the following formulae.

For the calculation of the standard deviation using split samples:

$$\text{Standard deviation} = \sqrt{\frac{\sum d^2}{2N}}$$

For the calculation of the standard deviation using values obtained from consecutive analyses of a single sample:

$$\text{Standard deviation} = \sqrt{\frac{\sum d^2}{N-1}}$$

1) Reproducibility of the Phase 1 Test.

Blood samples, collected from 18 healthy persons, were each divided into two equal parts. The phase 1 test was performed on each of the subsamples. The mean value was 510 seconds with a standard deviation of $\pm$ 31 seconds.

2) Reproducibility of the Phase 2 Test

Blood samples, collected from 21 healthy individuals were each divided into two equal parts. The phase 2 test was performed on each subsample. The mean value was 210 seconds with a standard deviation of ± 10.5 seconds.

3) Reproducibility of the Phase 3 Test.

Blood samples, collected from 19 healthy persons, were each divided into two equal parts. The phase 3 test was performed on each subsample. The mean value was 33.6 seconds with a standard deviation of ± 3.1 seconds.

4) Reproducibility of the Heparin Resistance Test.

Blood samples collected from 21 healthy persons, were each divided into two equal parts. The heparin resistance test was performed on each subsample. The mean value was 135 seconds with a standard deviation of ± 7 seconds.

5) Reproducibility of the Autocoagulography Test.

Blood samples collected from 16 healthy persons were each divided into two equal parts. The autocoagulography test was performed on each subsample. The mean value was 16.3 seconds with a standard deviation of ± 4 seconds.

6) Reproducibility of the Autocoagulography Control Test.

Blood samples, collected from 16 healthy persons, were each divided into two equal parts. The autocoagulography control test was performed on each subsample. The mean value was 88.4 seconds with a standard deviation of ± 7.8 seconds.

7) Gravimetric Determination of Fibrinogen as Fibrin.

Plasma, obtained from a single unit of blood from the blood abnk, was centrifuged at 2,000 g for 15 minutes to obtain cell poor plasma. Twenty three consecutive determinations were performed on 2ml. aliquots of the plasma. The mean value was 235 mg./100ml. plasma with a standard deviation of ± 15.5 mg.

8) Clot Retraction.

Blood samples, collected from 10 healthy persons, were each divided into two equal parts. The clot retraction test was performed on each subsample. The mean value was 59% with a standard deviation of ± 4.5%. These values are based on clot retraction figures obtained by calculating the % retraction occurring at 60 minutes after clotting.

Variability of Test Results in Single Healthy Persons Studied Sequentially.

Since the study is concerned with the changes in clotting activity in individuals during the postoperative period, and since the changes in activity are cal-

culated using the pre-operative results as a baseline for comparison, it is useful to determine the pattern of variability occurring in the spectrum of tests under normal physiological conditions. Six healthy persons, two males and four females, two of whom used oral contraceptive pills, were studied sequentially. The results are shown in Tables 4 to 9 on pages 45 to 50.

Patient Results.

Patients 1 and 2 were scheduled for surgery and were informed of this. Subsequently the surgery was cancelled. The results of limited sequential testing of these patients are shown in Tables 10 and 11 on pages 51 and 52. They were included in the study because of the possible influence of psychic factors (related to pre-surgical apprehension) on the haemostatic mechanism.

Patients 3 and 4 both underwent minor surgery under general anaesthesia. Patient 3 had a small tumour on the upper lip removed and Patient 4 went to surgery for the correction of a right hydrocele. Results of limited sequential testing are shown in Tables 12 and 13. (See pages 53 and 54). Data from both patients were included to determine if any pronounced changes in the spectrum of clotting tests resulted from the combination of minor surgery and general anaesthesia.

The results of the spectrum of clotting tests performed sequentially on the main group of surgical

Table 4. Showing the results of sequential testing on Normal Male # 1.

TEST	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	MEAN	RANGE
Phase 1	543	497	565	460	509	514.8	460-565
Phase 2	223	219	235	190	202	213.8	190-235
Phase 3	38	36	39	35	37	37	35-39
Autocoagulography	75	74	78	71	73	74.2	71-78
Autocoagulography Control	98	88	108	87	83	91.2	83-108
Heparin Resistance.	128	110	148	113	125	124.8	110-148

Table 5. Showing the results of sequential testing on Normal Male # 2.

TEST	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	MEAN	RANGE
Phase 1	501	473	547	519	464	581	514.1	464-581
Phase 2	209	200	224	197	185	239	209	185-239
Phase 3	35	32	37	36	31	38	34.8	31-38
Autocoagulography	80	78	83	77	76	85	79.8	76-85
Autocoagulography Control	100	98	109	110	99	117	105.5	98-117
Heparin Resistance	139	114	143	140	117	151	133.5	111-151

Table 6. Showing the results of sequential testing on Normal Female # 1. (Not using oral contraceptives).

TEST	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	MEAN	RANGE
Phase 1	494	570	495	540	495	518.8	494-570
Phase 2	233	227	216	240	220	227.2	216-240
Phase 3	36	38	37	38	39	37.6	36-39
Autocoagulography	67	59	62	64	63	63	59-67
Autocoagulography Control	100	99	93	107	96	99	93-107
Heparin Resistance	125	151	139	149	150	142.8	125-151

Table 7. Showing the results of sequential testing on Normal Female # 2. (Using oral contraceptives. Last pill taken on Day 4).

TEST	DAY 0	DAY 2	DAY 4	DAY 8	MEAN	RANGE
Phasel	527	381	454	690	563	381-690
Phase 2	258	156	219	257	235	156-307
Phase 3	29	27	30	42	32	27-42
Autocoagulography	66	56	63	69	63.5	56-69
Autocoagulography Control	103	70	90	112	93.7	70-112
Heparin Resistance	114	125	119	190	142	119-210

Table 8. Showing the results of sequential testing on Normal Female # 3. (Using oral contraceptives).

TEST	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	MEAN	RANGE
Phase 1	430	442	520	490	355	447.4	355-520
Phase 2	302	289	263	262	236	270.4	236-302
Phase 3	30	31	37	39	38	35	30-39
Autocoagulography	61	54	53	57	59	56.8	53-61
Autocoagulography Control	97	90	91	82	86	89.2	82-97
Heparin Resistance	211	175	191	153	200	186	153-211

Table 9. Showing the results of sequential testing on Normal Female # 4. (Not using oral contraceptives).

TEST	DAY 0	DAY 2	DAY 4	DAY 6	MEAN	RANGE
Phasel	532	527	567	490	529	490-567
Phase 2	199	240	230	185	213.5	199-240
Phase 3	34	32	34	34	33.5	32-34
Autocoagulography	57	62	65	57	60.2	57-65
Autocoagulography Control	89	103	98	85	93.7	85-103
Heparin Resistance	165	150	120	119	138.5	119-165

Table 10. Showing results of sequential testing in Patient # 1, who was scheduled but did not go to surgery.

TEST	Day 0	Day 2	Day 3	Day 5	Mean
Phase 1	475	464	493	483	478.8
Phase 2	181	173	194	189	184.3
Phase 3	36	35	39	38	37.3
Autocoagulography	51	50	52	49	50.5
Autocoagulography Control	66	64	67	63	65
Heparin Resistance Test	120	117	119	122	119.5
Kaolin-cephalin clotting time	45	43	45	46	44.7
Prothrombin Time	12	13	13	12	12.5
Fibrinogen Conc.	309	325	330	317	320
Fibrin. Degradation products	N	N	N	N	N
Platelet Count	321	339	317	299	319
Clot Retraction at 10 minutes	7	5	6	4	
Clot Retraction at 20 minutes	11	14	12	10	
Clot Retraction at 30 minutes	41	39	43	38	
Clot Retraction at 60 minutes	67	65	66	66	
Median Retractive Index.					
Maximum Retractive Activity Time	30	60	30	30	

Table 11. Showing the results of sequential testing in Patient # 2, who was scheduled but did not go to surgery.

TEST	Day O	Day 2	Day 4	Mean.
Phase 1	375	361	387	384.3
Phase 2	130	133	125	129.3
Phase 3	38	40	37	38.3
Autocoagulography	51	53	49	51
Autocoagulography Control	59	60	57	58.6
Heparin Resistance Test	105	99	103	102.3
Kaolin-cephalin clotting time	41	43	45	43
Prothrombin Time	12	13	13	12.6
Fibrinogen Conc.	362	384	351	365
Fibrin. Degradation Products	N	N	N	
Platelet Count	291	330	319	313
Clot Retraction at 10 minutes	3	5	2	
Clot Retraction at 20 minutes	29	31	30	
Clot Retraction at 30 minutes	47	44	46	
Clot Retraction at 60 minutes	61	59	58	

Table 12. Showing the results of limited sequential testing in Patient # 3 who had a small tumour on the lip removed under general anaesthesia.

TEST	Pre-operative Result	Day 1 Post-operative
Phase 1	605	645
Phase 2	185	180
Phase 3	41	39
Autocoagulography	52	53
Autocoagulography Control	58	59
Heparin Resistance Test	112	115
Kaolin-cephalin clotting time	43	44
Prothrombin Time	13	13
Fibrinogen Conc.	207	223
Fibrin. Degradation Products	N	N
Platelet Count	311	328
Clot Retraction at 10 minutes	5	7
Clot Retraction at 20 minutes	16	19
Clot Retraction at 30 minutes	29	33
Clot Retraction at 60 minutes	46	48
Median Retractive Index	-	-
Maximum Retractive Activity Time	60	60

Table 13. Showing the results of limited sequential testing in Patient # 4 who underwent surgical correction of a right hydrocele.

TEST	Pre-operative	Postoperative	
		Day 0	Day 3
Phase 1	464	431	420
Phase 2	110	115	109
Phase 3	41	37	39
Autocoagulography	39	41	39
Autocoagulography	43	44	42
Heparin Resistance Test	159	161	165
Kaolin-cephalin clotting time	41	43	41
Prothrombin Time	13	13	13
Fibrinogen Conc.	319	330	334
Fibrin. Degradation Products.	N	N	N
Platelet Count	289	301	288
Clot Retraction at 10 minutes	2	3	10
Clot Retraction at 20 minutes	16	18	29
Clot Retraction at 30 minutes	36	39	48
Clot Retraction at 60 minutes	55	53	60
Median Retractive Index.	-	-	-
Maximum Retractive Activity Time	30	30	30

patients (#5 to #29), are shown individually in Tables 14 to 38 (pages 56 to 80), and as a group for each test, in Figures 12 to 21 (pages 81 to 90).

TABLE 14. Showing the results of sequential testing for Patient # 5. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following cholecystectomy. Columns headed '%' show the percentage change from the pre-operative mean.

TEST	PRE	PRE	PRE X	1	%	3	%	5	%	7	%
Phase 1	-	586	586	406	31	416	29	564	4	532	9
Phase 2	-	220	220	165	25	132	40	116	47	158	28
Phase 3	-	40	40	38	5	35	13	36	10	35	13
H.R.T.	-	128	128	124	3	85	17	114	11	90	30
A.C.G.	-	45	45	45	0	43	4	47	4	45	0
A.C.G.C.	-	-	-	-	-	-	-	-	-	-	-
K.C.C.T.	-	44	44	41		40		39		41	
P.T.	-	13	13	12		12		13		14	
F.D.P.	-	Neg	Neg	10-20		Neg		Neg		Neg	
G.E.F.	-	281	281	275		290		354		403	
Plats.	-	197	197	200		189		253		301	
C.R. 10	-	0	0	1		5		4		2	
C.R. 20	-	3	3	12		17		22		12	
C.R. 30	-	18	18	48		40		44		20	
C.R. 60	-	51	51	69		59		58		55	
M.R.I.	-	58	58	33		45		43		55	
M.R.A.T.	-	60	60	30		30		30		60	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 15. Showing the results of sequential testing for Patient # 6. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following bowel resection. Columns headed '%' show the percentage change from the preoperative mean.

TEST	PRE	PRE	PRE X	1	%	3	%	5	%	7	%
Phase 1	478	466	472	380	19	378	20	298	37	309	35
Phase 2	190	200	195	141	28	121	38	112	43	119	39
Phase 3	36	34	35	30	14	29	20	30	14	33	6
H.R.T.	165	155	160	150	6	106	34	65	59	75	53
A.C.G.	50	46	48	46	4	42	12	45	6	47	2
A.C.G.C.	68	58	63	61	3	52	17	50	21	50	21
K.C.C.T.	42	42	42	40		40		38		40	
P.T.	12	-	12	14		15		13		14	
F.D.P.	Neg	Neg	Neg	10-20		Neg		Neg		Neg	
G.E.F.	330	-	330	359		434		559		601	
Plats.	215	-	215	183		197		241		361	
C.R. 10	2	-	2	6		4		6		8	
C.R. 20	33	-	33	25		17		42		40	
C.R. 30	48	-	48	42		37		50		51	
C.R. 60	64	-	64	53		53		64		60	
M.R.I.	34	-	34	52		54		30		26	
M.R.A.T.	20	-	20	20		30		20		20	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 16. Showing the results of sequential testing for Patient # 7. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following removal of a large pelvic tumour. Columns headed '%' show the percentage change from the preoperative mean.

TEST	PRE	PRE	PRE X	2	%	4	%	7	%	9	%
Phase 1	301	370	335	250	25	229	32	263	21	260	22
Phase 2	125	146	136	98	28	78	43	115	15	116	15
Phase 3	24	26	25	23	8	20	20	23	8	26	4
H.R.T.	112	108	110	84	24	70	36	85	23	82	25
A.C.G.	50	50	50	46	8	42	16	44	12	43	14
A.C.G.C.	59	69	64	53	17	51	20	53	17	54	16
K.C.C.T.	38	40	39	37		38		41		40	
P.T.	13	15	14	14		13		14		14	
F.D.P.	Neg	Neg		10-20		10-20		Neg		Neg	
G.E.F.	541	-	541	579		636		784		839	
Plats.	401	-	401	417		509		616		670	
C.R. 10	4	-	4	5		7		6		3	
C.R. 20	33	-	33	35		42		39		29	
C.R. 30	49	-	49	52		58		53		48	
C.R. 60	62	-	62	64		66		64		60	
M.R.I.	32	-	32	24		25		27		36	
M.R.A.T.	20	-	20	20		20		20		20	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 17. Showing the results of sequential testing for Patient # 8. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following gastrectomy. Columns headed '%' show the percentage change from the pre-operative mean.

TEST	PRE	PRE	PRE X	1	%	3	%	4	%	6	%	8	%	10	%
Phase 1	-	487	487	380	22	360	26	410	16	389	20	374	23	393	19
Phase 2	-	230	230	214	7	160	30	169	27	175	24	169	27	172	25
Phase 3	-	25	25	26	4	25	0	29	16	27	8	26	4	24	4
H.R.T.	-	95	95	92	3	80	16	76	20	75	21	79	17	84	12
A.C.G.	-	72	72	80	11	61	15	68	6	66	8	64	11	70	3
A.C.G.C.	-	107	107	104	3	75	30	82	23	84	21	82	23	109	2
K.C.C.T.	-	43	43	42		39		35		36		40		41	
P.T.	-	12	12	14		15		13		15		13		13	
F.D.P.	-	N	N	10-20		20-40		10-20		N		N		N	
G.E.F.	-	489	489	522		601		716		773		685		676	
Plats.	-	260	260	195		371		346		593		599		579	
C.R. 10	-	5	5	7		3		1		4		6		3	
C.R. 20	-	25	25	28		24		20		24		25		26	
C.R. 30	-	41	41	43		39		36		41		40		39	
C.R. 60	-	51	51	52		50		47		53		52		49	
M.R.I.	-	58	58	55		60				52		56			
M.R.A.T.	-	20	20	20		20		20		20		20		20	

(See Appendix of meanings of abbreviations and units of measurement)

TABLE 18. Showing the results of sequential testing for Patient # 9. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following vagotomy and pyloroplasty. Columns headed '%' show the percentage change from the preoperative mean.

TEST	PRE	PRE	PRE X	1	%	3	%	5	%	7	%	9	%	10	%	11	%
Phase 1	458	424	441	396	10	385	13	398	10	430	2	375	15	368	17	330	25
Phase 2	158	130	144	175	22	115	20	113	22	131	9	127	12	106	26	113	22
Phase 3	23	25	24	24	0	40	67	38	58	23	4	25	4	23	4	24	0
H.R.T.	116	100	108	129	19	100	8	89	18	90	17	116	7	83	23	82	24
A.C.G.	53	51	52	52	0	45	13	48	8	47	10	41	21	45	13	44	15
A.C.G.C.	59	55	57	51	10	52	9	52	9	51	10	46	19	45	21	43	25
K.C.C.T.	51	49	50	41		42		47		40		40		41		42	
P.T.	14	12	13	13		15		13		13		13		13		12	
F.D.P.	N	-	N	N		10-20		N		N		N		N		N	
G.E.F.	363	367	365	391		481		509		700		815		801		765	
Plats.	301	325	313	298		300		359		523		448		420		500	
C.R. 10	0	-	0	0		0		9		-		3		1		2	
C.R. 20	1	-	1	2		3		28		-		25		21		18	
C.R. 30	9	-	9	13		34		40		-		42		40		29	
C.R. 60	46	-	46	48		54		52		-		56		58		56	
M.R.I.	-	-	-	-		55		55		-		47		46		51	
M.R.A.T.	60	-	60	60		30		20		-		20		20		30	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 19. Showing the results of sequential testing for Patient # 10. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following thoracotomy. Columns headed '%' show the percentage change from the pre-operative mean.

TEST	PRE	PRE	PRE X	1	%	4	%	6	%	8	%	10	%
Phase 1	519	475	497	376	24	370	25	390	21	420	15	347	30
Phase 2	240	228	234	212	9	151	35	153	35	157	33	175	25
Phase 3	25	23	24	26	8	25	4	26	8	29	21	25	4
H.R.T.	97	97	97	92	5	80	18	77	21	75	23	78	20
A.C.G.	75	69	72	81	12	61	15	65	10	68	6	64	11
A.C.G.C.	109	107	108	105	3	75	31	79	27	82	24	82	24
K.C.C.T.	36	40	38	38		34		35		34		42	
P.T.	11	11	11	11		12		13		13		14	
F.D.P.	N	-	N	N		10-20		N		N		N	
G.E.F.	620	-	620	654		850		925		1168		1041	
Plats.	250	190	220	136		325		369		410		440	
C.R. 10	1	-	1	7		12		11		13		12	
C.R. 20	4	-	4	25		31		32		33		35	
C.R. 30	11	-	11	38		46		48		45		47	
C.R. 60	32	-	32	50		60		61		57		60	
M.R.I.	-	-	-	60		40		35		43		37	
M.R.A.T.	60	-	60	20		20		20		20		20	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 20. Showing the results of sequential testing for Patient # 11. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following gastrectomy. Columns headed '%' show the percentage change from the pre-operative mean.

TEST	PRE	PRE	PRE X	1	%	4	%	6	%	8	%
Phase 1	490	440	465	401	14	466	0	435	6	345	26
Phase 2	195	179	187	175	6	230	23	200	7	173	7
Phase 3	29	33	31	41	32	36	16	35	13	34	10
H.R.T.	90	84	87	62	29	90	3	70	20	75	14
A.C.G.	55	51	53	54	2	56	6	50	6	59	11
A.C.G.C.	78	74	76	78	3	88	16	77	1	79	4
K.C.C.T.	35	37	36	53		41		33		35	
P.T.	11	11	11	16		14		12		12	
F.D.P.	N	N	N	N		40-80		20-40		10-20	
G.E.F.	543	483	513	362		340		330		478	
Plats.	174	-	174	134		165		294		330	
C.R. 10	4	-	4	6		20		25		21	
C.R. 20	26	-	26	27		35		41		36	
C.R. 30	36	-	36	43		50		53		52	
C.R. 60	45	-	45	58		65		64		62	
M.R.I.	-	-	-	44		30		27		29	
M.R.A.T.	20	-	20	20		10		10		10	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 21. Showing the results of sequential testing for Patient # 12. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following right nephrectomy. Columns headed '%' show the percentage change from the preoperative mean.

TEST	PRE	PRE	PRE X	2	%	4	%	6	%	8	%	12	%	14	%
Phase 1	503	543	523	410	22	480	8	464	11	365	30	379	27	408	22
Phase 2	239	249	244	205	16	255	5	253	4	162	34	179	27	211	14
Phase 3	33	-	33	34	3	32	3	37	12	43	30	33	0	32	3
H.R.T.	98	-	98	77	21	82	16	85	13	76	22	77	21	78	2
A.C.G.	61	-	61	61	0	56	8	75	30	63	3	67	10	72	18
A.C.G.C.	109	-	109	92	16	110	1	113	4	79	28	86	21	108	1
K.C.C.T.	36	-	36	34		36		34		34		34		34	
P.T.	13	-	13	14		14		13		14		14		13	
F.D.P.	N	-	N	10-20		10-20		N		N		N		N	
G.E.F.	681	-	681	769		963		1131		888		813		708	
Plats.	138	-	138	101		124		266		377		400		391	
C.R. 10	0	-	0	1		3		8		8		12		14	
C.R. 20	5	-	5	2		18		25		26		24		28	
C.R. 30	23	-	23	27		37		39		42		44		45	
C.R. 60	46	-	46	52		54		50		56		58		61	
M.R.I.	-	-	-	48		47		60		47		43		39	
M.R.A.T.	60	0	60	60		30		30		30		30		30	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 22. Showing the results of sequential testing for Patient # 13. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following cholecystectomy. Columns headed '%' show the percentage change from the preoperative mean.

TEST	PRE	PRE	PRE X	2	%	4	%	6	%	8	%
Phase 1	-	430	430	405	6	328	24	447	4	401	7
Phase 2	-	197	197	202	3	182	8	205	4	199	1
Phase 3	-	35	35	41	17	33	6	35	0	34	3
H.R.T.	-	129	129	132	2	113	12	131	2	122	5
A.C.G.	-	72	72	52	28	60	17	62	14	59	18
A.C.G.C.	-	106	106	80	25	75	29	93	12	83	22
K.C.C.T.	-	37	37	34		38		39		36	
P.T.	-	13	13	14		13		12		12	
F.D.P.	-	N	N	10-20		N		N		N	
G.E.F.	-	352	352	451		570		610		653	
Plats.	-	230	230	171		220		319		361	
C.R. 10	-	12	12	6		11		13		11	
C.R. 20	-	36	36	32		29		32		32	
C.R. 30	-	48	48	50		44		46		48	
C.R. 60	-	58	58	59		56		59		60	
M.R.I.	-	36	36	30		46		40		35	
M.R.A.T.	-	20	20	20		20		20		20	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 23. Showing the results of sequential testing for Patient # 14. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following laparotomy and removal of intestinal polyps and adhesions. Columns headed '%' show the percentage change from the preoperative mean.

TEST	PRE	PRE	PRE X	1	%	3	%	4	%	5	%	6	%	8	%
Phase 1	460	560	510	383	25	415	19	386	24	370	27	393	23	437	14
Phase 2	199	233	216	182	16	170	21	173	20	185	14	142	34	170	21
Phase 3	35	35	35	38	9	37	6	38	9	35	0	35	0	33	6
H.R.T.	138	148	143	151	6	97	32	126	12	110	23	94	34	124	13
A.C.G.	55	53	54	58	7	53	2	54	0	40	26	52	4	56	4
A.C.G.C.	79	83	81	82	1	81	0	75	7	63	22	66	19	79	2
K.C.C.T.	47	45	46	42		41		42		41		39		46	
P.T.	12	12	12	13		11		12		13		11		12	
F.D.P.	N	-	N	N		10-20		N		N		N		N	
G.E.F.	366	-	366	360		549		507		502		438		306	
Plats.	308	364	336	278		231		310		413		473		515	
C.R. 10	16	-	16	10		6		4		12		17		14	
C.R. 20	33	-	33	30		24		22		32		36		32	
C.R. 30	59	-	59	55		50		47		52		54		58	
C.R. 60	70	-	70	63		58		54		60		59		66	
M.R.I.	26	-	26	27		30		24		29		27		27	
M.R.A.T.	30	-	30	30		30		30		20-30		20		30	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 24. Showing the results of sequential testing for Patient # 15. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following splenectomy. Columns headed '%' show the percentage change from the pre-operative mean.

TEST	PRE	PRE	PRE X	1	%	3	%	5	%	7	%
Phase 1	380	401	390	358	8	360	7	371	5	360	7
Phase 2	166	168	167	135	19	117	30	120	28	116	30
Phase 3	50	48	49	40	22	50	2	51	4	49	0
H.R.T.	139	-	139	90	32	77	42	94	29	92	31
A.C.G.	53	57	55	51	8	56	1	54	3	55	1
A.C.G.C.	57	-	57	57	0	52	9	55	4	55	4
K.C.C.T.	46	-	46	53	15	49	6	45	2	44	4
P.T.	13	13	13	15		19		18		16	
F.D.P.	N			N		N		10-20		N	
G.E.F.	301		301	290		339		401		519	
Plats.	277		277	270		339		414		503	
C.R. 10	3		3	8		16		16		18	
C.R. 20	12		12	20		30		36		38	
C.R. 30	33		33	40		49		48		53	
C.R. 60	59		59	60		63		60		66	
M.R.I.	49		49	45		32		35		28	
M.R.A.T.	60		60	30		30		20		20	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 25. Showing the results of sequential testing for Patient # 16. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following cholecystectomy. Columns headed '%' show the percentage change from the preoperative mean.

TEST	PRE	PRE	PRE X	1	%	3	%	5	%	7	%
Phase 1	561	599	580	457	21	471	19	493	15	469	19
Phase 2	202	238	220	171	22	129	41	119	46	143	35
Phase 3	41	39	40	37	7.5	34	15	37	7.5	35	12.5
H.R.T.	-	-	125	121	2	88	30	109	13	92	26
A.C.G.	47	47	47	44	6	48	2	46	2	45	4
A.C.G.C.	69	71	70	65	7	62	11	68	3	67	4
K.C.C.T.	43	45	44	42	4.5	40	9	39	11	38	14
P.T.	13.5	12.5	13	12		12		13		14	
F.D.P.			N	10-20		10-20		N		N	
G.E.F.	221	239	230	217	6	264	15	331	44	409	78
Plats.	201		201	223	11	269	34	331	65	427	112
C.R. 10	0		0	2		5		5		4	
C.R. 20	5		5	14		19		22		19	
C.R. 30	21		21	39		40		44		39	
C.R. 60	54		54	56		58		57		54	
M.R.I.	56		56	49		46		44		52	
M.R.A.T.	60		60	30		30		30		30	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 26. Showing the results of sequential testing for Patient # 17. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following sub-total gastrectomy. Columns headed '%' show the percentage change from the preoperative mean.

TEST	PRE	PRE	PRE X	1	%	3	%	4	%	6	%	8	%	10	%
Phase 1	457	517	487	361	26	352	28	400	18	373	23	359	26	401	18
Phase 2	236	206	221	209	5	152	31	159	28	161	27	158	28	174	21
Phase 3	27	29	28	30	7	28	0	30	7	26	7	25	11	23	18
H.R.T.	99	91	95	90	5	78	18	74	22	76	20	78	18	83	13
A.C.G.	74	76	75	80	7	63	16	67	11	65	13	63	16	68	9
A.C.G.C.	110	104	107	101	6	78	27	81	24	82	23	81	24	93	13
K.C.C.T.	41	43	42	41	2	38	9	36	14	36	14	39	7	42	0
P.T.	12	12	12	14		15		14		15		14		13	
F.D.P.			N	10-20		20-40				10-20	N			N	
G.E.F.	431	452	441	452	2	519	18	639	45	702	59	773	75	791	79
Plats.	231		231	219	5	235	2	343	15	378	64	424	83	561	143
C.R. 10	5		5	7		3				5		4		3	
C.R. 20	25		25	26		25				26		27		26	
C.R. 30	49		49	47		42				39		41		39	
C.R. 60	51		51	50		51				49		52		49	
M.R.I.	46		46	60		40						41			
M.R.A.T.	20		20	30		20				20		20		20	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 27. Showing the results of sequential testing for Patient # 18. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following vagotomy. Columns headed '%' show the percentage change from the pre-operative mean.

TEST	PRE	PRE	PRE X	1	%	3	%	5	%	7	%	9	%	10	%
Phase 1	431	473	452	371	18	369	18	383	15	402	11	350	22	331	27
Phase 2	142	164	153	159	4	109	29	108	29	115	24	114	29	103	33
Phase 3	26	26	27	27	4	38	46	36	38	25	4	24	8	22	15
H.R.T.	104	116	110	117	16	98	11	83	24	90	18	101	8	84	24
A.C.G.	55	61	58	57	2	43	26	45	22	46	21	43	26	46	21
A.C.G.C.	61	67	64	62	3	62	3	63	2	61	1	52	19	50	22
K.C.C.T.	50	-	50	46	8	47	6	46	8	45	10	43	14	43	14
P.T.	13	-	13	13		15		14		13		13		12	
F.D.P.	-	-	N	N		10-20		N		N		N		N	
G.E.F.	351	-	351	378	8	431	15	497	42	563	60	621	77	598	70
Plats.	291	-	291	298	2	313	7	371	27	470	61	469	61	474	63
C.R. 10	-	0	0	0		2		3		1		1		3	
C.R. 20	-	6	6	4		9		13		14		17		11	
C.R. 30	-	31	31	24		32		34		38		41		34	
C.R. 60	-	54	54	51		52		55		56		59		55	
M.R.I.	-	54	54	59		57		52		50		45		52	
M.R.A.T.	-	30	30	60		30		30		30		30		30	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 28. Showing the results of sequential testing for Patient # 19. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following cholecystectomy. Columns headed '%' show the percentage change from the preoperative mean.

TEST	PRE	PRE	PRE X	1	%	4	%	6	%	8	%
Phase 1	510	482	496	413	17	450	9	423	15	398	20
Phase 2	149	179	164	151	8	185	13	169	3	161	2
Phase 3	33	33	33	40	21	34	3	32	3	31	6
H.R.T.	89	101	95	69	27	94	1	72	24	73	23
A.C.G.	-	-	55	56	2	54	2	50	9	49	11
A.C.G.C.	-	-	78	77	1	81	4	76	3	77	1
K.C.C.T.	34	36	35	47	34	37	6	34	3	34	3
P.T.	11	11	11	15		13		13		12	
F.D.P.	-	-	N	N		20-40		40-80		10-20	
G.E.F.	535	-	535	417	22	434	19	490	8	549	3
Plats.	183	-	183	131	38	191	4	233	27	347	90
C.R. 10	6	-	6	8		17		21		20	
C.R. 20	24	-	24	26		34		40		37	
C.R. 30	38	-	38	42		50		55		49	
C.R. 60	49	-	49	53		59		59		60	
M.R.I.	-	-	-	52		30		26		33	
M.R.A.T.	20	-	20	60		10		10		10	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 29. Showing the results of sequential testing for Patient # 20. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following laparotomy. Columns headed '%' show the percentage change from the pre-operative mean.

TEST	PRE	PRE	PRE X	1	%	2	%	4	%	6	%	8	%
Phase 1	448	490	469	413	12	381	19	319	32	391	17	337	28
Phase 2	-	-	181	182	0	152	16	134	36	177	22	171	5
Phase 3	35	37	36	42	17	35	3	31	14	34	6	32	11
H.R.T.	139	121	130	430	0	122	6	115	11	126	3	117	10
A.C.G.	-	-	79	73	8	61	23	65	18	63	20	60	24
A.C.G.C.	119	103	111	87	22	74	33	72	35	75	32	73	34
K.C.C.T.	37	37	37	35	5	38	3	39	5	38	3	36	3
P.T.	13	13	13	13		14		14		13		12	
F.D.P.		N	N	10-20		N		N		N		N	
G.E.F.	-	360	360	411	14	471	31	539	50	624	73	679	89
Plats.	-	201	201	181	10	193	4	254	26	371	84	439	118
C.R. 10	-	10	10	7		-		12		9		8	
C.R. 20	-	37	37	33		-		35		40		37	
C.R. 30	-	49	49	45		-		49		52		29	
C.R. 60	-	61	61	57		-		62		63		57	
M.R.I.	-	32	32	36		-		32		28		43	
M.R.A.T.	-	20	20	20		-		30		20		20	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 30. Showing the results of sequential testing for Patient # 21. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following removal of an abdeominal tumour. Columns headed '%' show the percentage change from the preoperative mean.

TEST	PRE	PRE	PRE X	1	%	2	%	3	%	5	%	7	%
Phase 1	361	398	379	309	18	287	24	269	29	281	26	272	28
Phase 2	143	119	131	109	17	100	24	97	26	105	20	104	21
Phase 3	40	34	37	29	22	26	30	22	40	24	35	24	25
H.R.T.	98	104	101	85	16	71	30	62	39	65	36	66	35
A.C.G.	-	-	53	43	19	39	26	36	32	41	23	38	28
A.C.G.C.	-	-	70	58	17	52		50	14	54	23	53	24
K.C.C.T.	41	43	42	39	7	39	7	38	12	40	5	41	2
P.T.	14	14	14	15		14		15		13		14	
F.D.P.	-	N	N	N		N		10-20		N		N	
G.E.F.	-	489	489	501	2.4	507	4	569	16	636	30	709	45
Plats.	-	434	434	411	5	436	0	574	32	689	59	730	68
C.R. 10	-	4	4	4		-		10		8		11	
C.R. 20	-	12	12	12		-		20		21		29	
C.R. 30	-	39	39	33		-		42		44		48	
C.R. 60	-	63	63	59		-		65		62		59	
M.R.I.	-	43	43	50		-		40		40		35	
M.R.A.T.	-	30	30	60		-		60		30		30	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 31. Showing the results of sequential testing for Patient # 22. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following splenectomy. Columns headed '%' show the percentage change from the pre-operative mean.

TEST	PRE	PRE	PRE X	1	%	2	%	3	%	5	%	7	%	9	%
Phase 1	-	458	458	413	10	379	17	321	30	359	22	373	18	350	23
Phase 2	-	194	194	170	12	159	18	161	17	173	11	179	8	160	17
Phase 3	-	50	50	42	16	40	20	47	6	49	2	47	6	52	4
H.R.T.	-	133	133	90	32	81	39	77	42	94	29	90	32	87	34
A.C.G.	-	59	59	52	12	54	8	57	3	57	3	52	12	53	10
A.C.G.C.	-	61	61	60	2	55	8	56	16	57	6.5	55	10	56	16
K.C.C.T.	-	46	46	53		51		49		47		46		48	
P.T.	-	13	13	15		17		19		18		16		14	
F.D.P.	-	N	N	N		N		N		N		N		N	
G.E.F.	-	301	301	290		-		393		456		439		501	
Plats.	-	279	279	270		265		339		401		519			
C.R. 10	-	6	6	9		-		16		16		18			
C.R. 20	-	18	18	17		-		30		36		38			
C.R. 30	-	42	42	40		-		49		48		53			
C.R. 60	-	60	60	57		-		63		60		66			
M.R.I.	-	43	43	47		-		47		35		28			
M.R.A.T.	-	30	30	30		-		20		20		20			

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 32. Showing the results of sequential testing for Patient # 23. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following laparotomy. Columns headed '%' show the percentage change from the pre-operative mean.

TEST	PRE	PRE	PRE X	1	%	3	%	5	%	7	%
Phase 1	504	-	504	470	7	403	20	393	22	441	12
Phase 2	219	-	219	184	16	162	26	158	28	179	18
Phase 3	30	-	30	28	6	27	10	29	3	31	3
H.R.T.	120	-	120	109	9	89	26	71	41	88	27
A.C.G.	55	-	55	45	18	40	27	40	27	43	22
A.C.G.C.	73	-	73	67	8	62	15	60	18	68	7
K.C.C.T.	46	-	46	44		42		43		46	
P.T.	12	-	12	13		14		13		13	
F.D.P.	N	-	-	N		10-20		N		N	
G.E.F.	319	-	319	316		336		430		561	
Plats.	271	-	271	283		291		353		419	
C.R. 10	0	-	0	1		5		7		5	
C.R. 20	5	-	5	7		13		13		15	
C.R. 30	31	-	31	29		33		41		44	
C.R. 60	51	-	51	53		56		59		56	
M.R.I.	58	-	58	56		52		45		45	
M.R.A.T.	30	-	30	60		60		30		30	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 33. Showing the results of sequential testing for Patient # 24. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following surgery for a ruptured appendix. Columns headed '%' show the percentage change from the preoperative mean.

TEST	PRE	PRE	PRE X	1	%	3	%	5	%	7	%
Phase 1	398	-	398	370	7	309	22	354	11	383	4
Phase 2	194	-	194	162	16	141	27	161	16	181	7
Phase 3	31	-	31	29	6	28	10	28	10	32	3
H.R.T.	125	-	125	103	18	87	30	85	32	96	23
A.C.G.	61	-	61	57	6	49	20	53	13	58	5
A.C.G.C.	98	-	98	83	15	76	22	79	19	87	11
K.C.C.T.	47	-	47	45		43		41		44	
P.T.	13	-	13	15		15		14		14	
F.D.P.	-	-	N	N		N		N		N	
G.E.F.	360	-	360	369		397		451		483	
Plats.	217	-	217	201		231		269		288	
C.R. 10	7	-	7	8		-		11		9	
C.R. 20	19	-	19	17		-		24		28	
C.R. 30	42	-	42	40		-		45		43	
C.R. 60	54	-	54	53		-		56		51	
M.R.I.	50	-	50	53		-		43		55	
M.R.A.T.	30	-	30	30		-		30		20	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 34. Showing the results of sequential testing for Patient # 25. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following laparotomy and gastrectomy. Columns headed '%' show the percentage change from the preoperative mean.

TEST	PRE	PRE	PRE X	1	%	3	%	5	%	7	%
Phase 1	361	425	393	353	10	323	18	330	16	312	21
Phase 2	135	141	138	121	12	110	20	118	14	112	19
Phase 3	40	30	35	25	28	23	34	26	26	25	28
H.R.T.	99	115	107	85	21	62	42	71	34	69	36
A.C.G.	63	49	55	41	27	38	32	42	25	40	29
A.C.G.C.	78	62	70	52	26	49	30	55	21	52	26
K.C.C.T.	40	42	41	39		38		42		42	
P.T.	14	14	14	15		15		14		15	
F.D.P.	N	-	N	N		10-20		N		N	
G.E.F.	485	-	485	499		537		658		663	
Plats.	410	-	410	370		390		521		619	
C.R. 10	4	-	4	4		10		9		5	
C.R. 20	12	-	12	7		19		21		20	
C.R. 30	39	-	39	31		42		43		46	
C.R. 60	63	-	63	62		66		62		65	
M.R.I.	43	-	43	48		40		41		36	
M.R.A.T.	30	-	30	60		60		30		30	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 35. Showing the results of sequential testing for Patient # 26. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following splenectomy. Columns headed '%' show the percentage change from the pre-operative mean.

TEST	PRE	PRE	PRE X	1	%	3	%	5	%	7	%
Phase 1	380	401	390	358	8	360	8	371	5	360	8
Phase 2	166	168	167	135	19	117	30	120	28	117	30
Phase 3	50	48	49	40	18	50	2	51	4	49	0
H.R.T.	139	127	133	90	26	77	42	94	29	92	31
A.C.G.	55	57	56	51	9	56	0	54	4	55	2
A.C.G.C.	57	59	58	57	2	52	10	55	5	55	5
K.C.C.T.	46	46	46	53		49		45		44	
P.T.	13	13	13	15		19		18		16	
F.D.P.	N	-	N	N		N		N		N	
G.E.F.	301	-	301	290		393		456		439	
Plats.	277	-	277	270		339		401		519	
C.R. 10	3	-	3	8		16		16		18	
C.R. 20	12	-	12	20		30		36		38	
C.R. 30	33	-	33	40		49		48		53	
C.R. 60	59	-	59	60		63		60		66	
M.R.I.	50	-	50	45		32		35		28	
M.R.A.T.	60	-	60	30		30		20		20	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 36. Showing the results of sequential testing for Patient # 27. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following gastrectomy. Columns headed '%' show the percentage change from the pre-operative mean.

TEST	PRE	PRE	PRE X	1	%	3	%	5	%	7	%
Phase 1	-	480	480	387	19	357	26	428	11	443	8
Phase 2	-	150	150	147	2	125	17	125	17	133	11
Phase 3	-	36	36	35	3	33	8	32	11	32	11
H.R.T.	-	88	88	110	25	97	10	84	5	73	17
A.C.G.	-	58	58	58	0	57	2	60	3	65	12
A.C.G.C.	-	65	65	64	2	62	5	65	0	62	5
K.C.C.T.	-	50	50	68		44		38		41	
P.T.	-	14	14	14		13		13		13	
F.D.P.	-	N	N	10-20		10-20		N		N	
G.E.F.	-	600	600	590		719		835		792	
Plats.	-	600	600	609		1194		960		1020	
C.R. 10	-	31	31	49		27		20		21	
C.R. 20	-	67	67	74		52		42		44	
C.R. 30	-	77	77	80		64		57		58	
C.R. 60	-	82	82	85		72		66		70	
M.R.I.	-	15	15	10.5		19		25		24	
M.R.A.T.	-	20	20	10		10		20		20	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 37. Showing the results of sequential testing for Patient # 28. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following splenectomy. Columns headed '%' show the percentage change from the pre-operative mean.

TEST	PRE	PRE	PRE X	0	%	2	%	4	%	6	%	8	%
Phase 1	477	570	523	450	14	385	26	365	30	430	18	432	17
Phase 2	110	140	125	140	12	95	24	84	33	130	4	104	17
Phase 3	34	34	34	36	6	33	3	23	32	25	26	24	29
H.R.T.	102	102	102	94	8	50	51	63	38	76	25	74	27
A.C.G.	68	56	62	54	13	40	35	44	29	51	18	49	21
A.C.G.C.	-	-	-	-	-	-	-	-	-	-	-	-	-
K.C.C.T.	40	44	42	37		36		38		39		39	
P.T.	12	12	12	13		12		11		12		13	
F.D.P.	N	N	N	N		N		N		N		N	
G.E.F.	351		351	331		401		498		509		597	
Plats.	260		260	201		289		356		473		537	
C.R. 10	0		0	2		4		1		1		3	
C.R. 20	2		2	5		37		31		15		19	
C.R. 30	31		31	41		57		49		40		45	
C.R. 60	65		65	69		65		57		61		63	
M.R.I.	46		46	40		26		33		44		38	
M.R.A.T.	60		60	30		20		20		30		30	

(See Appendix for meanings of abbreviations and units of measurement)

TABLE 38. Showing the results of sequential testing for Patient # 29. 'Pre' refers to preoperative tests and the numerals following the preoperative mean column indicate days following gastrectomy. Columns headed '%' show the percentage change from the pre-operative mean.

TEST	PRE	PRE	PRE X	0	%	3	%	6	%	8	%
Phase 1	508	500	504	450	11	394	22	335	34	430	15
Phase 2	160	170	165	150	9	107	35	162	2	164	0
Phase 3	44	37	40	36	10	35	13	32	20	35	13
H.R.T.	108	100	104	89	14	74	29	125	20	105	1
A.C.G.	50	50	50	46	8	35	30	40	20	39	22
A.C.G.C.	75	75	75	60	20	35	53	34	55	53	29
K.C.C.T.	45	45	45	41		39		41		43	
P.T.	15	13	14	13		14		13		14	
F.D.P.	N	-	N	N		N		10-20		N	
G.E.F.	370	-	370	393		431		497		524	
Plats.	391	-	391	376		398		437		506	
C.R. 10	1	-	1	4		25		23		20	
C.R. 20	4	-	4	23		48		56		51	
C.R. 30	34	-	34	38		59		68		67	
C.R. 60	64	-	64	56		65		75		70	
M.R.I.	46	-	46	50		22		18		20	
M.R.A.T.	30	-	30	20		10		20		20	

(See Appendix for meanings of abbreviations and units of measurement.)

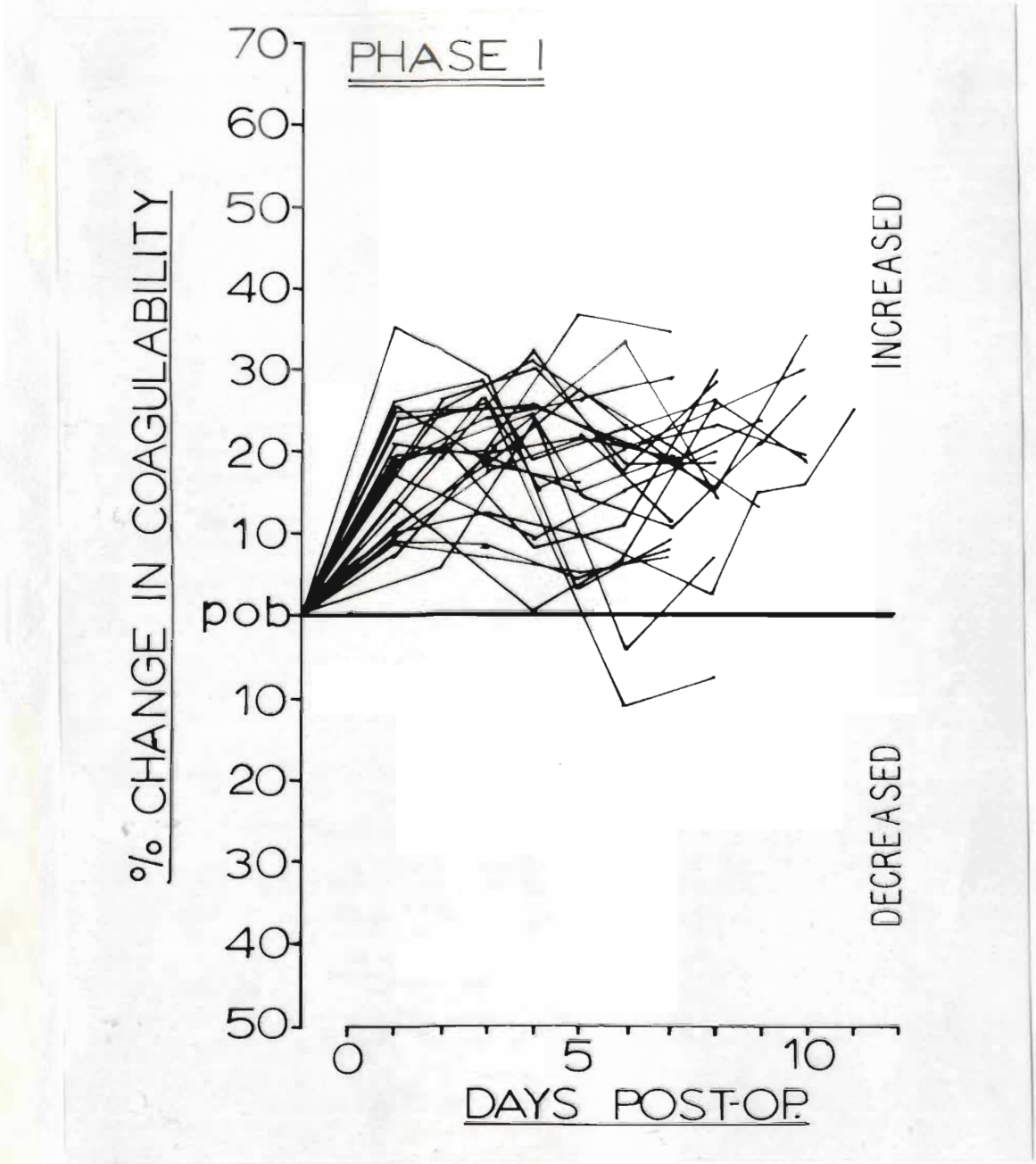

Fig. 12. Showing the general pattern of results of the phase 1 test in the group of 25 patients who had major elective surgery.

p.o.b. = pre-operative baseleine.

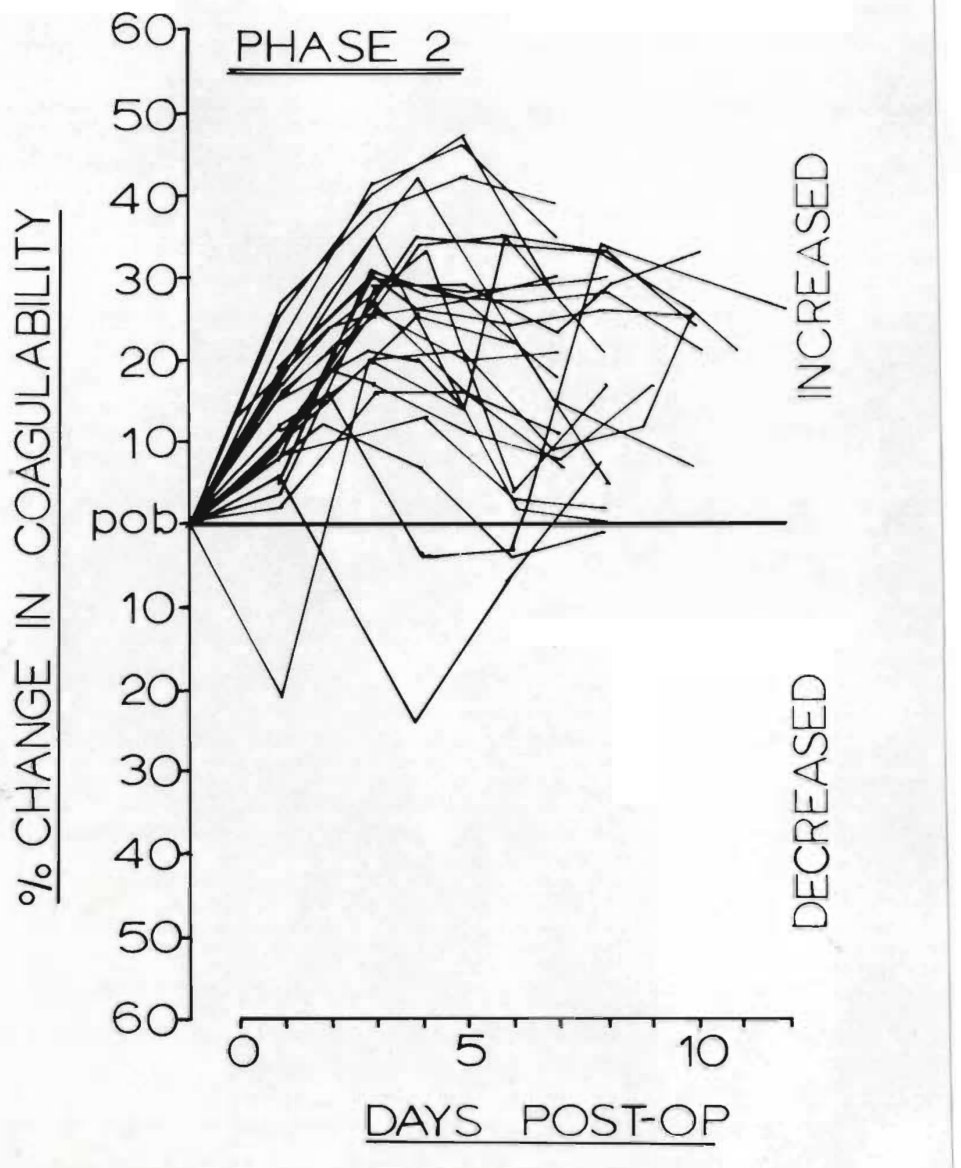

Fig. 13. Showing the general pattern of results of the phase 2 test in the group of 25 patients who had major elective surgery.

p.o.b. = pre-operative baseline.

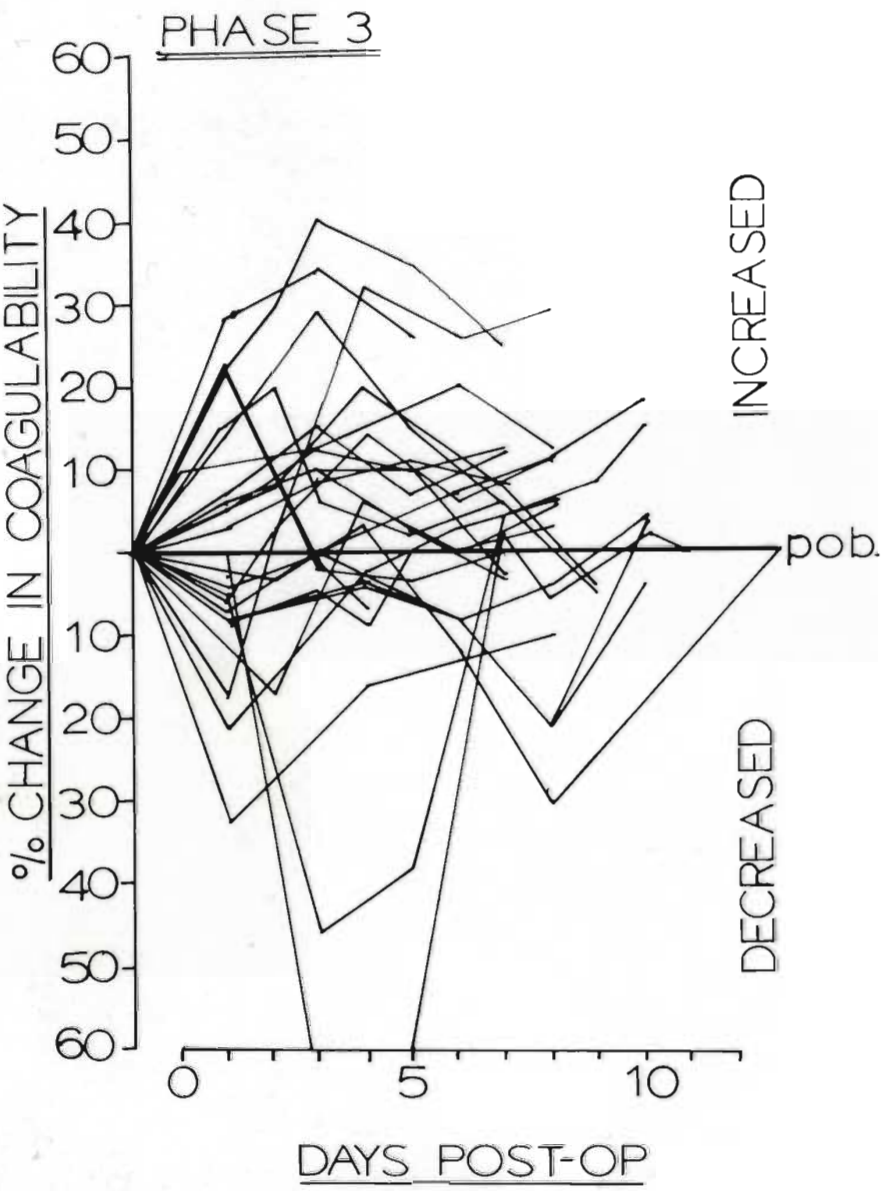

Fig. 14. Showing the general pattern of results of the phase 3 test in the group of 25 patients who had major elective surgery.

p.o.b.= pre-operative baseline.

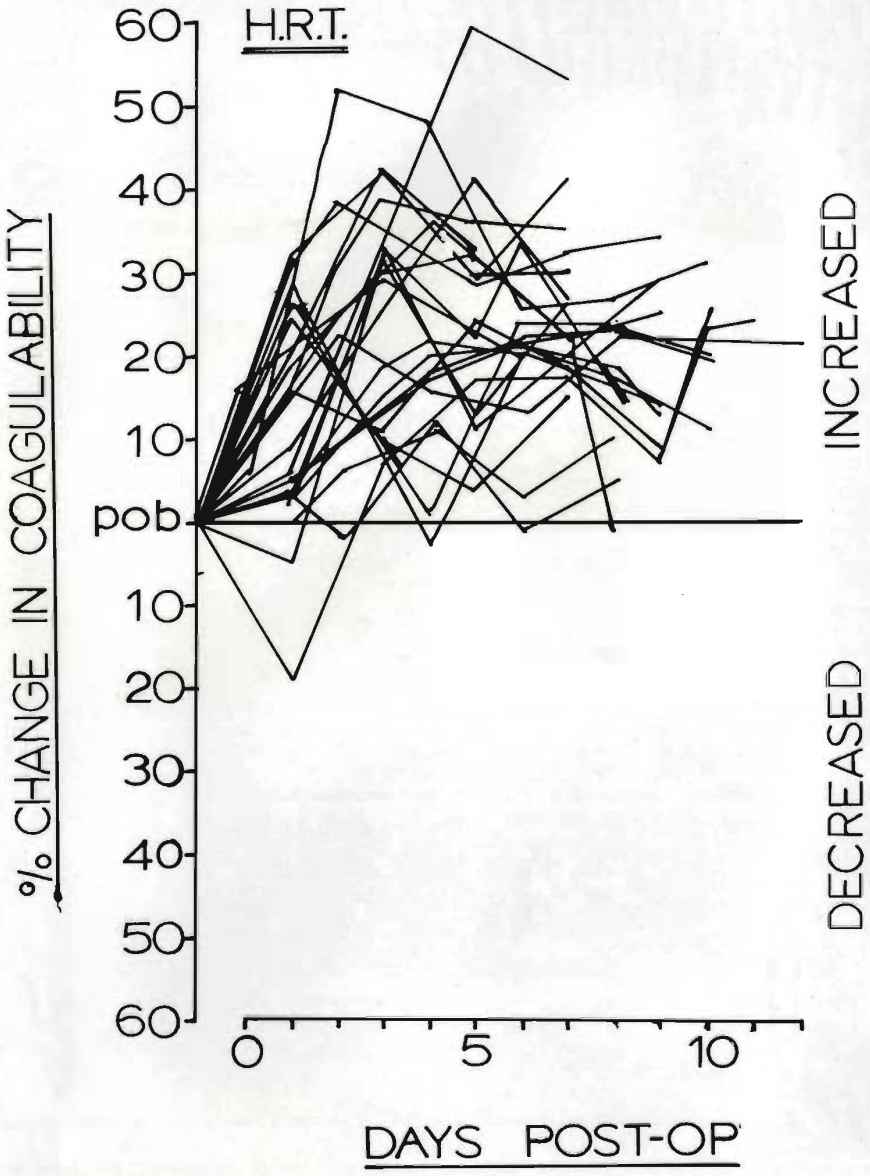

Fig. 15. Showing the general pattern of results of the heparin resistance test in the group of 25 patients who had major elective surgery.

p.o.b. = pre-operative baseline.

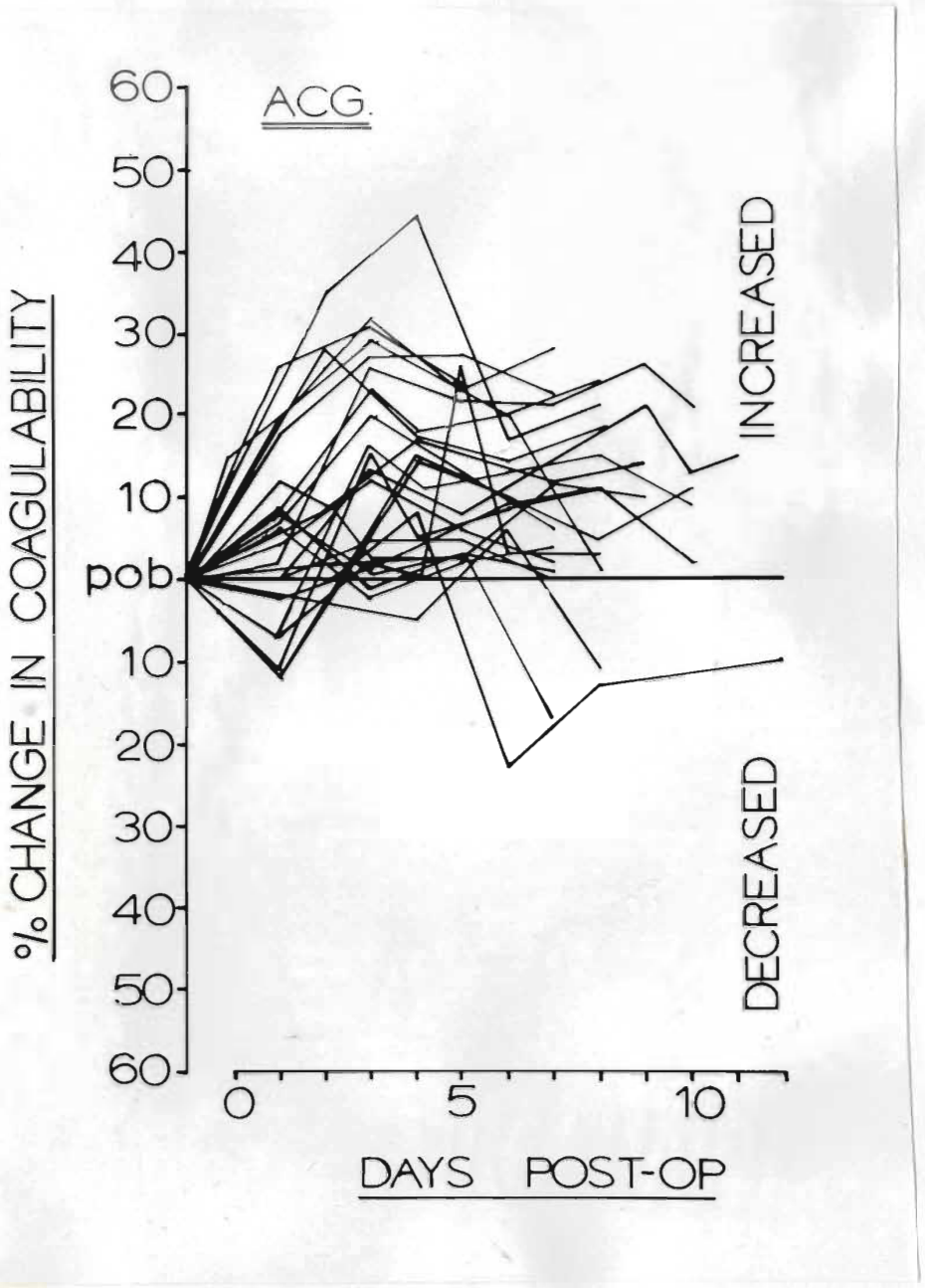

Fig. 16. Showing the general pattern of results of the autocoagulography test in the group of 25 patients who had major elective surgery.

p.o.b. = pre-operative baseline.

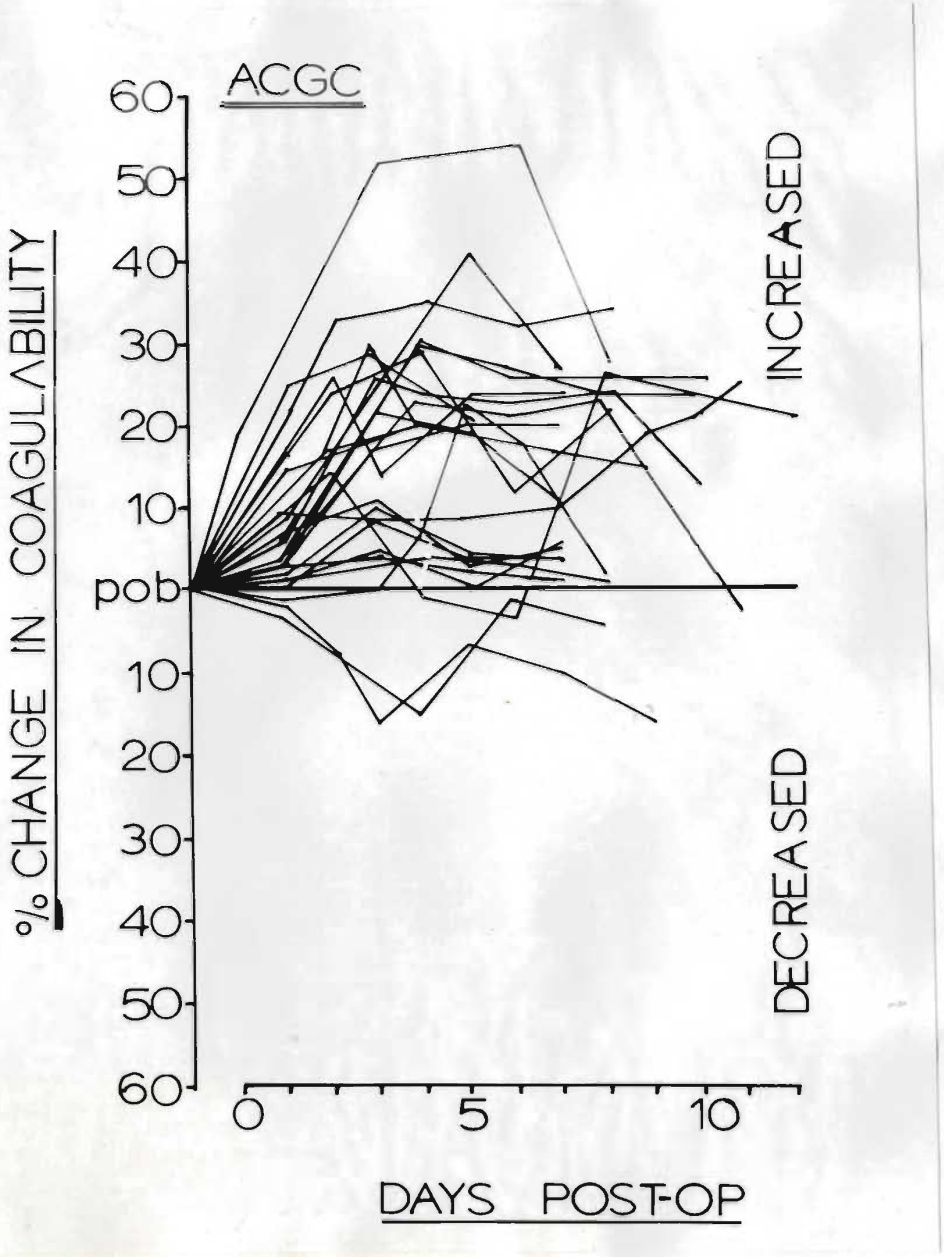

Fig 17. Showing the general pattern of results of the autocoagulography control test in the group of 25 patients who had major elective surgery.

p.o.b. = pre-operative baseline.

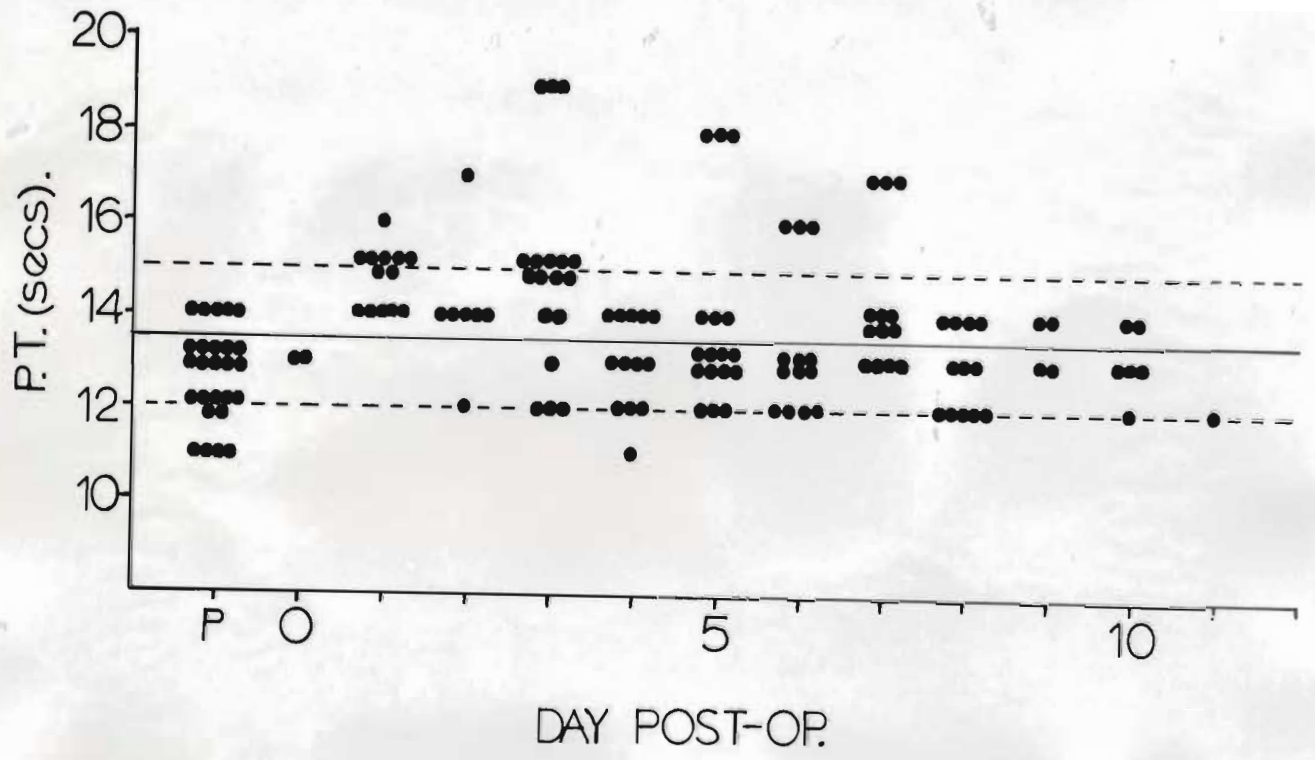

Fig. 18. Showing the results of the one stage prothrombin time in the group of 25 patients who had major elective surgery. The dashed lines represent the extent of the normal range (2 S.D.), and the full line, the mean of the normal range. P = pre-operative. O = day of surgery.

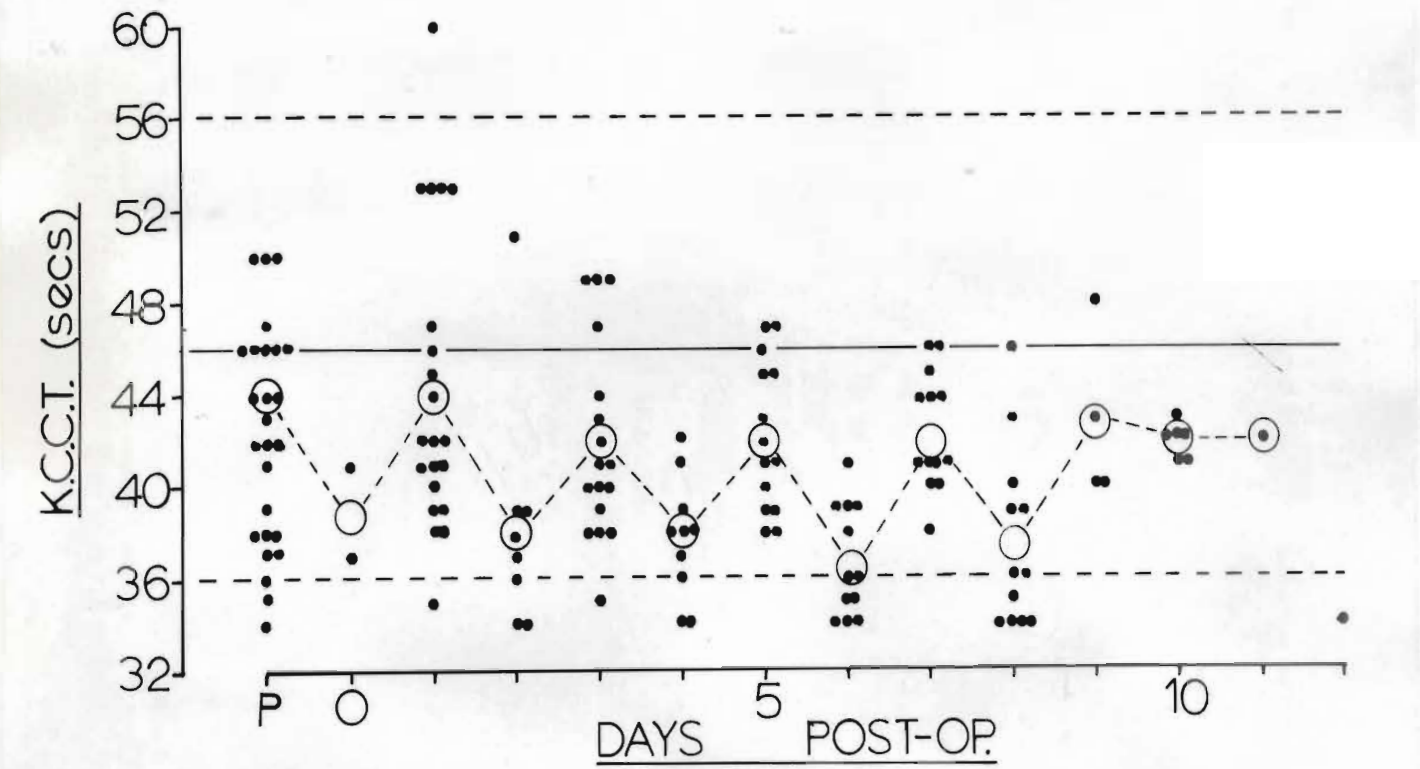

Fig. 19. Showing the results of the kaolin-cephalin clotting time in the group of 25 patients who had major elective surgery. The parallel dashed lines represent the limits of the normal range (2.S.D.), the full line, the mean of the normal range and the oscillating line, the daily mean of all patients for each day. P = pre-operative. O = day of surgery.

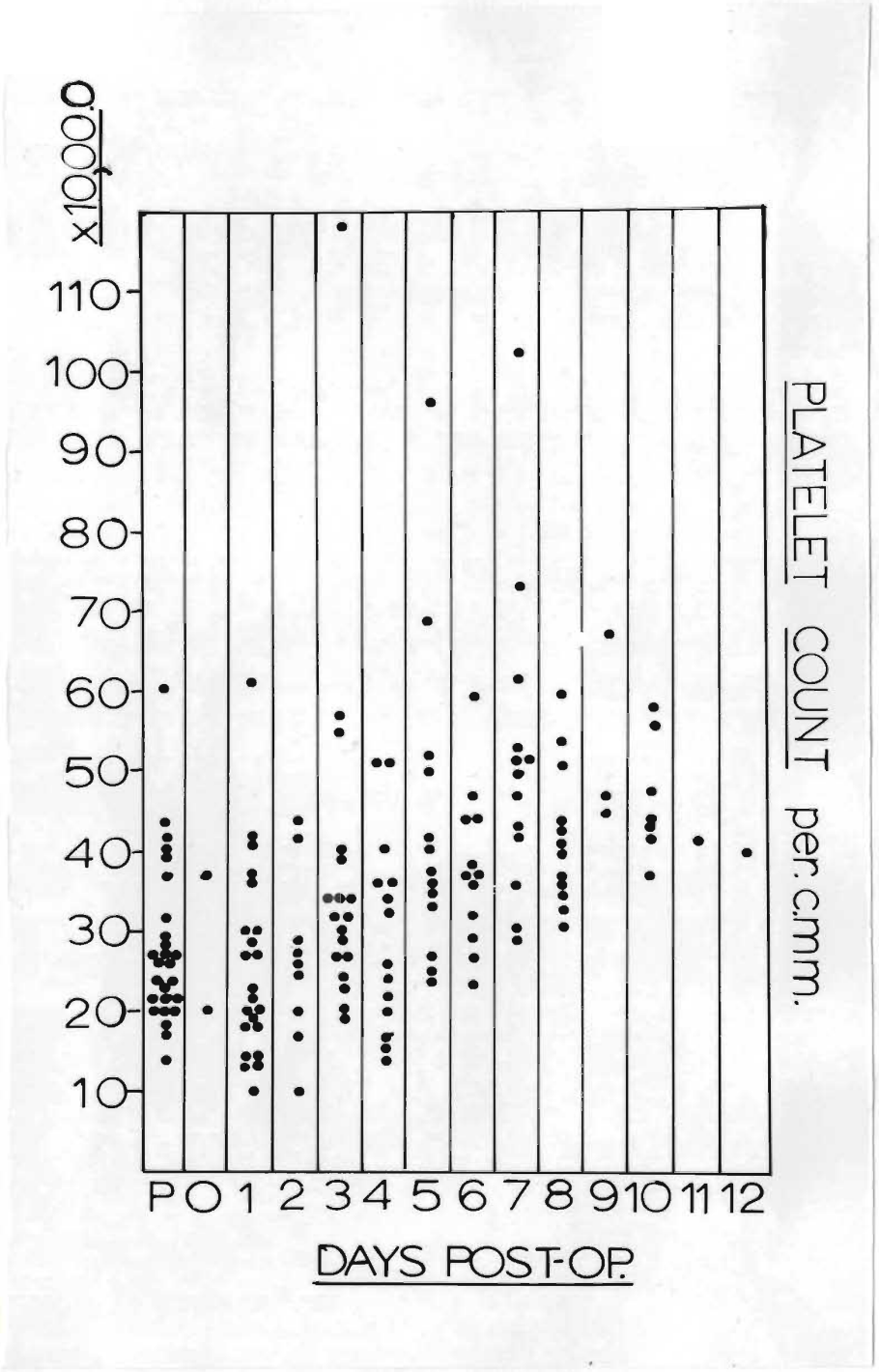

Fig. 20. Showing the results of the platelet count for the group of 25 patients who had major elective surgery.

P = pre-operative
O = day of surgery.

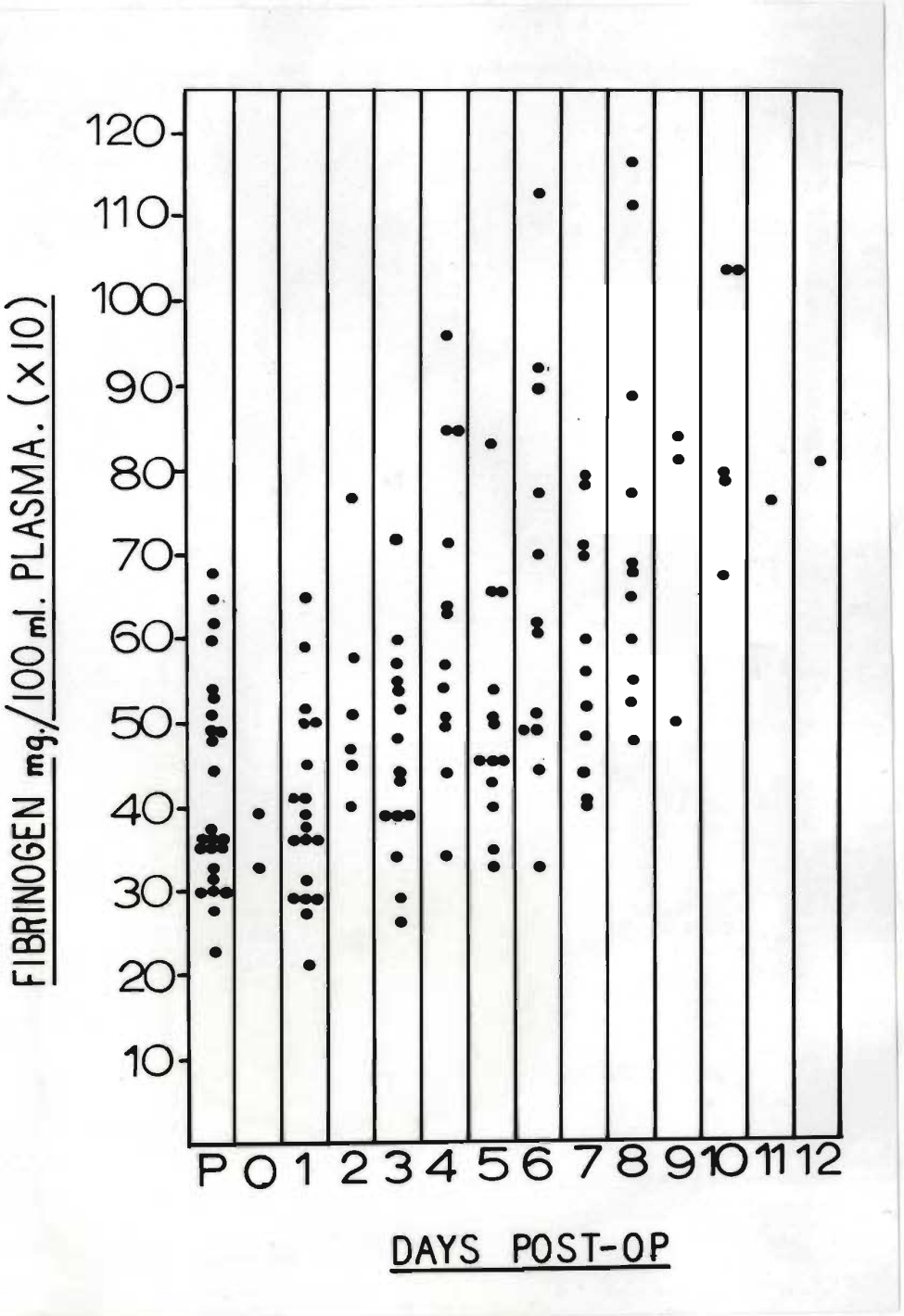

Fig. 21. Showing the results of the fibrinogen determinations in the group of 25 patients who had major elective surgery.

P = pre-operative
O = day of surgery

DISCUSSION

Many laboratory tests, the results of which have been claimed to indicate a hypercoagulable state, have been predicated on the assumption that clotting tests *in vitro* show acceleration.

If hypercoagulability in the postoperative patient is a manifestation of increases in either the concentration of circulating levels of certain procoagulants, or their activity, then it would be expected that, in the postoperative patient, the rise in the levels and/or activity of these procoagulants, would result in accelerated clotting tests, where such factors are measured, providing that the assumption referred to in the first paragraphy is correct.

It is widely accepted that the concentration of the known clotting factors in normal individuals is considerably in excess of that required for adequate haemostasis. The further increase in the concentration of one or more procoagulants, following surgical operations, may not accelerate clotting tests which utilize whole blood or plasma samples. Shapiro *et al* (1942), demonstrated a shortening of the prothrombin time, lasting for a few days to three weeks, in patients with mycardial infarction using blood samples diluted with saline. Overman and Wright (1951), using undiluted

blood samples, failed to show a shortening of the prothrombin time. One may speculate that the reason for this paradox of results between the two tests could be related to the non-specific increase in circulating fibrinogen which follows after tissue trauma. The prothrombin time using diluted blood samples is sensitive to further increases in fibrinogen concentration, whereas the test using undiluted blood is not, unless the fibrinogen concentration begins to fall. If adsorbed plasma, containing fibrinogen, is used as the diluent instead of saline, no shortening of the prothrombin time is observed (Tuft and Rosenfeld 1947).

This study was performed without the knowledge of the work just referred to. However, the phase 1, phase 2, phase 3, heparin resistance, autocoagulography and autocoagulography control tests were designed to provide clotting test that may have increased sensitivity to rises in the levels of clotting factors or their activity. In all of the tests just referred to, dilution of whole blood samples, processed in a standard way, forms the key element in each of the tests.

Although the variation in the concentration of clotting factors is very wide in groups of individuals of the same age and sex groups, there is evidence that the activity of the clotting factors in the same individual is similar when tested sequentially. Thus

Gormsen (1959), using a heparin resistance test, tested his own blood, twice weekly, for 2 years and all results were between 11 and 13 minutes. Similarly, two other healthy persons were tested twice to four times a month for one year and gave results consistently between 12 and 14, and 13 and 16 minutes respectively. Many patients, suffering from cerebral or coronary thrombosis, similarly tested once a month, for periods up to four years, gave results consistently between 6 and 7 minutes. Gormsen in describing these results, points out that although the values show marked dispersion, the same person exhibits extremely constant values under otherwise unchanged conditions.

When a clotting test, obtained from a patient, is evaluated in terms of results obtained from a population of healthy persons, which are characterized by wide variations in clotting factor concentrations and/or activity, it is sometimes difficult to decide whether or not a change is significant. For example the normal range for factor VIII concentration is commonly given as being between 50% and 200%. Thus, if the level of factor VIII in an individual, following some appropriate stimulus, rises from 50% to 150%, the rise in concentration still results in a factor VIII level well within normal limits. If however, the result be examined in terms of the pre-stimulus value of the individual, then the post-stimulus result is likely abnormal for that individual, since the stimulus has pro-

duced a three-fold increase in the level of factor VIII. Dilution of blood samples prior to using the blood in various test systems, will undoubtedly increase the sensitivity of the test, but will also increase the dispersion of results, resulting in an even wider range of values in any population tested. For these reasons, this study has concentrated on following the haemostatic changes that result from surgery, using the patient's own pre-operative values as a baseline. All postoperative results occurring in each individual patient have been compared to the pre-operative baseline.

The results of the phase 1, phase 2, phase 3, autocoagulography, autocoagulography control and heparin resistance tests in the sequentially studied normal males and females (see Tables 4 to 9, pages 45 to 50). In all individuals forming this small group, with the exception of normal female # 2, the tests showed a small degree of variability during testing. The results of normal female #2 were, in all probability, influenced by the cessation of oral contraceptives.

Tables 10 and 11, (pages 51 and 52), show the results of limited sequential testing of patients #1 and #2, who were scheduled for surgery, informed of this, but who did not subsequently undergo surgery. These results were included in order to ascertain whether or not the usual pre-operative apprehension, experienced by most

patients, resulted in significant changes in coagulability. The results were remarkably consistent during the period of testing.

Patients # 3 and #4, underwent minor surgical operations involving limited surgical dissection under general anaesthesia. These limited results (see Tables 12 and 13, pages 53 and 54), suggest that minor surgery under general anaesthesia does not result in significant changes in coagulability in the early post-operative period.

The results of the phase 1, phase 2 and heparin resistance tests in the main group of 25 patients who had major elective surgery, all show increased coagulability, which is apparant very soon after the surgical operation. Similar results are seen in the data obtained from the autocoagulography and autocoagulography control tests. (see Figures 12-13, pages 81 and 82, and Figures 15-17, pages 84 - 86). The similarity of the rise in coagulability demonstrated by all of these tests, during the first few days following surgery, is not suprising, since it is likely that all of the separate tests measure some common clotting activity. The demonstration of increased coagulability by a number of different tests lends support to the concept of a developing hypercoagulable state in the postsurgical patient, if increased levels of clotting factors and/or activity satisfies one criterion for the

Table 39. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 5. (Cholecystectomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED.
Phasel	Five
Phase 2	Five
Heparin Resistance	Five
Autocoagulography	Three
Autocoagulography Control	Five

Table 40. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 6. (Bowel Ressection).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED
Phase 1	Five
Phase 2	Five
Heparin Resistance	Five
Autocoagulography	Three
Autocoagulography Control	Five

Table 41. Showing the postoperative day on which maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 7. (Removal of large pelvic tumour).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED
Phase 1	Four
Phase 2	Four
Heparin Resistance	Four
Autocoagulography	Four
Autocoagulography Control	Four

Table 42. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 8. (Gastrectomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED
Phase 1	Three
Phase 2	Three
Heparin Resistance	Six
Autocoagulography	Three
Autocoagulography Control	Three

Table 43. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control test for Patient # 9. (Vagotomy and Pyloroplasty).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED
Phase 1	Eleven
Phase 2	Ten
Heparin Resistance	Eleven
Autocoagulography	Nine
Autocoagulography Control	Eleven

Table 44. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 10. (Thoracotomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED.
Phase 1	Ten
Phase 2	Four
Heparin Resistance	Eight
Autocoagulography	Four
Autocoagulography Control	Four

Table 45. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 11. (Gastrectomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED.
Phase 1	Eight
Phase 2	Eight
Heparin Resistance	One
Autocoagulography	Six
Autocoagulography Control	No increase demonstrated

Table 46. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 12. (Right Nephrectomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED.
Phase 1	Eight
Phase 2	Eight
Heparin Resistance	Eight
Autocoagulography	Four
Autocoagulography Control	Eight

Table 47. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 13. (Cholecystectomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED
Phase 1	Four
Phase 2	Four
Heparin Resistance	Four
Autocoagulography	Four
Autocoagulography Control	Four

Table 48. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 14. (Laparotomy with removal of intestinal Polyps and Adhesions).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED.
Phase 1	Five
Phase 2	Six
Heparin Resistance	Six
Autocoagulography	Five
Autocoagulography Control	Five

Table 49. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 15. (Splenectomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED
Phase 1	One
Phase 2	Three and Seven
Heparin Resistance	Three
Autocoagulography	One
Autocoagulography Control	Three.

Table 50. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 16. (Cholecystectomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED
Phase 1	One
Phase 2	Five
Heparin Resistance	Three
Autocoagulography	One
Autocoagulography Control	Three

Table 51. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 17. (Sub-total Gastrectomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED.
Phase 1	Three
Phase 2	Three
Heparin Resistance	Four
Autocoagulography	Three and Eight
Autocoagulography Control	Three

Table 52. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 18. (Vagotomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED
Phase 1	Ten
Phase 2	Three, Five and Nine
Heparin Resistance	Five and Ten
Autocoagulography	Three and Nine
Autocoagulography Control	Ten

Table 53. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 19. (Exploratory Laparotomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED
Phase 1	Eight
Phase 2	Four
Heparin Resistance	One
Autocoagulography	Eight
Autocoagulography Control	Four

Table 54. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 20. (Cholecystectomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED.
Phase 1	Four
Phase 2	Four
Heparin Resistance	Four
Autocoagulography	Eight
Autocoagulography Control	Four

Table 55. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 21. (Removal of Abdominal Tumour).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED
Phase 1	Three
Phase 2	Three
Heparin Resistance	Three
Autocoagulography	Three
Autocoagulography Control	Two

Table 56. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 22. (Splenectomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED.
Phase 1	Three
Phase 2	Two
Heparin Resistance	Three
Autocoagulography	One and Seven
Autocoagulography Control	Three and Nine

Table 57. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 23. (Exploratory Laparotomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED.
Phase 1	Five
Phase 2	Five
Heparin Resistance	Five
Autocoagulography	Three and Five
Autocoagulography Control	Five

Table 58. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 24. (Ruptured Appendix).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED.
Phase 1	Three
Phase 2	Three
Heparin Resistance	Five
Autocoagulography	Three
Autocoagulography Control	Three

Table 59. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 25. (Laparotomy and Gastrectomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED.
Phase 1	Seven
Phase 2	Three
Heparin Resistance	Three
Autocoagulography	Three
Autocoagulography Control	Three

Table 60. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 26. (Splenectomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED.
Phase 1	One
Phase 2	Three and Seven
Heparin Resistance	Three
Autocoagulography	One
Autocoagulography Control	Three

Table 61. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 27, (Gastrectomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED.
Phase 1	Three
Phase 2	Three and Five
Heparin Resistance	Seven
Autocoagulography	Three
Autocoagulography Control	Three and Seven

Table 62. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 28. (Splenectomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED
Phase 1	Four
Phase 2	Four
Heparin Resistance	Two
Autocoagulography	Two
Autocoagulography Control	Two

Table 63. Showing the postoperative day on which a maximum increase in coagulability was observed in each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests for Patient # 29. (Gastrectomy).

TEST	POSTOPERATIVE DAY ON WHICH PEAK COAGULABILITY WAS OBSERVED.
Phase 1	Six
Phase 2	Three
Heparin Resistance	Three
Autocoagulography	Three
Autocoagulography Control	Six

definition of such a state. If, for all patients, the day on which the peak increase in coagulability is found, for each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests (see Tables 39 to 63, pages 96 to 120). one may examine the time period in which increased coagulability reaches a peak for the group of 25 postsurgical patients. Figure 22 (page 122), illustrates the result of this procedure, and it is readily apparant that the peak increase in coagulability occurs between days 3 to 5 postoperative, with an indication of a second smaller peak occurring around the 8th postoperative day (in some tests). A summary of the data presented in Figure 22, is shown in Figure 23 (page 123). These figures are of interest since the maximum incidence of pulmonary embolism has been claimed to occur on the seventh postoperative day (Evoy 1949). In addition Kakkar (1972), has shown that about 50% of venous thrombi in surgical patients form within the first 24 hours following surgery, and the remaining thrombi form within the third to seventh day after operation. Browse and Negus (1970), and Williams (1971), present evidence that many patients who develope venous thrombosis will do so within the first three postoperative days. These findings suggest that many venous thrombi are formed during the period, when, as shown by the phase 1, phase 2, heparin resistance,autocoagulography and autocoagulography control tests, increased blood coagulability can be demonstrated.

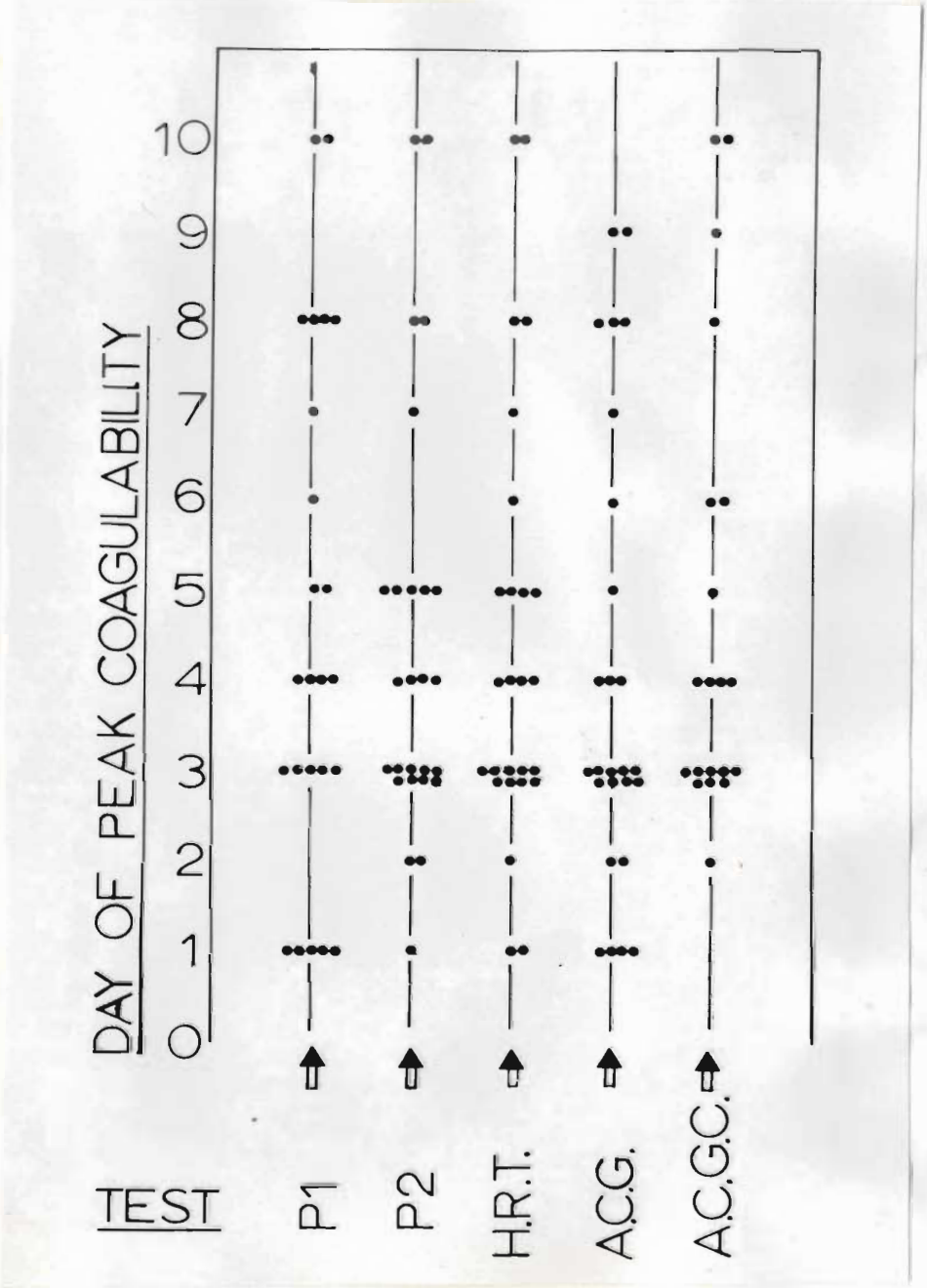

Fig. 22. Showing the number of each of the phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests which demonstrated maximum increased coagulability on different postoperative days for the group of 25 patients who had major elective surgery.

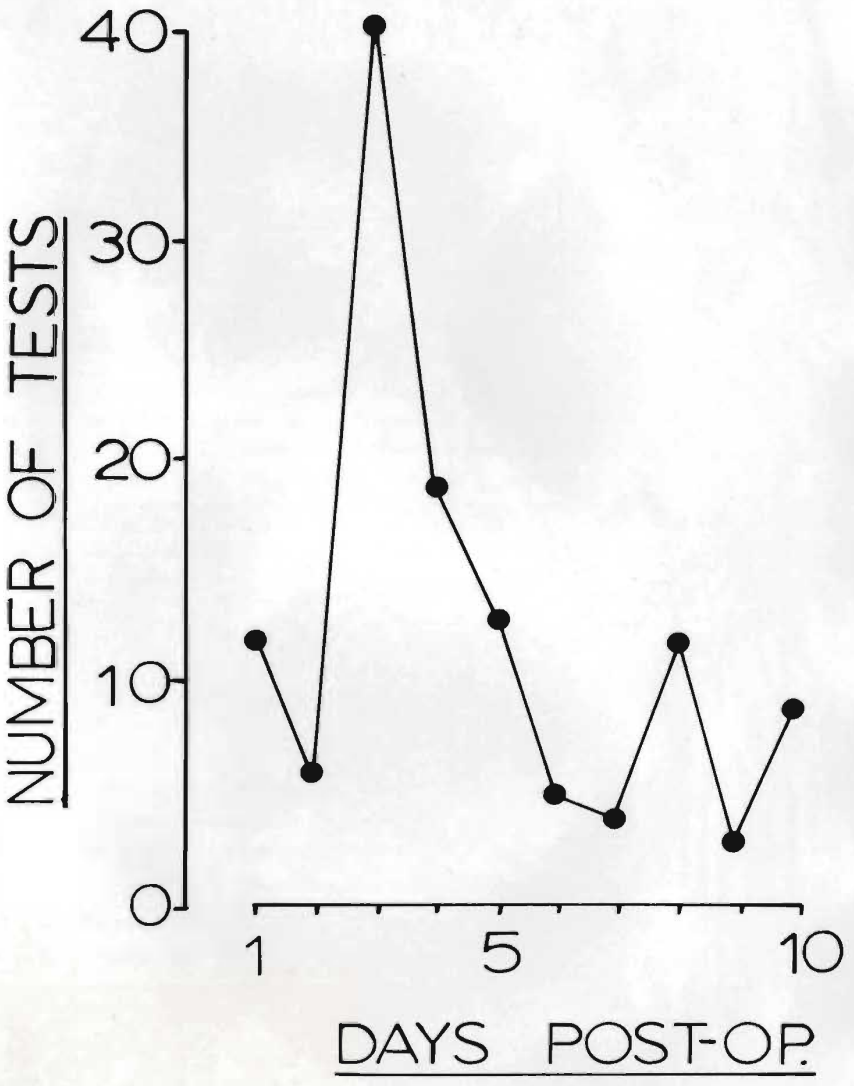

Fig. 23. The figure represents a summary of the data seen in Fig.22 (page 122), and shows the peak increases in coagulability found by obtaining the total number of all tests (phase 1, phase 2, heparin resistance, autocoagulography and autocoagulography control tests), showing peak increases in coagulability on various post-operative days.

The results of the phase 3 test show considerable dispersion and although many patients demonstrated an increased coagulability, again having a peak increase around days 3 to 5 postoperative, others showed a decrease in coagulability, which was sometimes very striking (see Figure 14, page 83). These results however, are not to be unexpected, since the results of clotting tests which utilize tissue thromboplastin (e.g. the prothrombin time), are variable in postoperative patients, some showing a shortening of the prothrombin time, that is, increased coagulability (Sandrock et al 1948), others show an increased prothrombin time (decreased coagulability), as demonstrated by Egan et al (1974), and still others showing no change in coagulability (Kimche and Eisenkraft 1971).

Postsurgically, in most patients, an increase in coagulability was demonstrated with the heparin resistance test as used in this study. The test has been used with many modifications to study patients in whom a hypercoagulable state is thought to exist. Ogura (1946) found accelerated heparin sensitized clotting tests in 21 of 27 patients with recent coronary thrombosis. Rosenthal (1952), found similar acceleration in 14 of 19 cases of recent coronary thrombosis and Poller (1953), found acceleration in 37 of 50 cases of recent thromboembolic disease, findings supported by the observa-

tions of Peel (1953). Gormsen (1959), found accelerated heparin sensitized clotting times in 148 patients during the first 24 hours after acute cardiac infarction, in 95 cases of cerebral thrombosis, confirmed by arteriography and all cases of venous thrombosis. Such reports lend support to the concept that accelerated clotting tests, sensitized with heparin, may have value in the detection of a hypercoagulable state.

In this study, the increased *in vitro* coagulability, found in most patients during the first postoperative week, as demonstrated with the heparin resistance test, confirms the findings of Waugh and Ruddick (1944), Silverman (1948), Holger-Madsen and Schiolar (1960), Gormsen and Haxholdt (1960), of a similar postoperative increase.

Both of the standard clotting tests employed in this study, namely the kaolin-cephalin clotting time (kaolin activated partial thromboplastin time) and the one stage prothrombin time, were comparatively poor indicators of postsurgical increases in coagulability. Egan *et al* (1974), in a series of 39 postsurgical patients, found that 48% of the patients had a shortened partial thromboplastin time immediately following surgery, 8% showed shortening on the first postsurgical day, 12% between the third and fourth, and 25% between the fifth and sixth days. Apart from those results which showed acceleration, most of the patients in this study had results which fell within the normal limits.

It has been shown by Ygge (1970b), that postsurgically there is a significant increase in the levels of factor VIII and fibrinogen. It is well known that decreases in the concentration of clotting factors, participating in the intrinsic pathway of coagulation (see Figure 24, page 127), results in prolongation of the kaolin-cephalin clotting time (partial thromboplastin time). Thus a deficiency of factor VIII and/or fibrinogen, would be expected to lengthen the result , while increases in the levels of the same factors might be expected to shorten the result. In this study however, most of the kaolin-cephalin clotting times remained within normal limits, althogh the majority of results lie beneath the mean of the normal range and the mean daily value for patient results has an oscillating characteristic (see Figure 19, page 88). These results would suggest, that the test, while being sensitive to deficiencies in one or more clotting factors, is not very sensitive to increases in clotting factors or their activity, and may be unsuitable, in an unmodified form for use in the study of the hypercoagulable state.

Postsurgically, the alterations in the numbers of circulating platelets, namely an initial decrease, followed by a steady increase during the first postsurgical week, has confirmed the findings of Dawbarn et al (1928), Potts and Pearl (1941), Adams (1944), Ham and Slack (1967), Fogliano et al (1973) and Egan et al (1974).

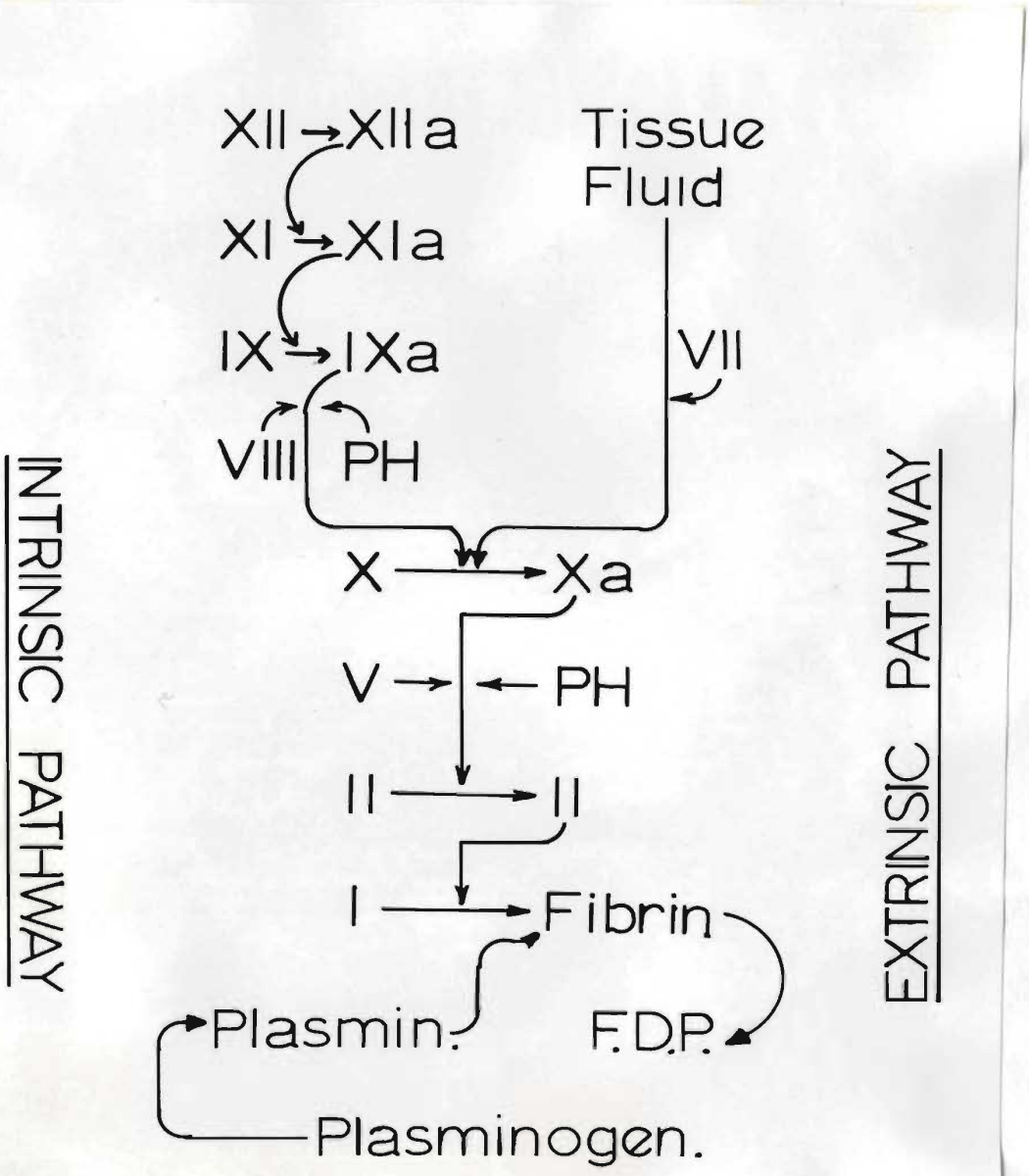

Fig. 24. Illustrating a modern concept of blood coagulation.
"a" = the activated form of the clotting factor, PH = phospholipid, F.D.P. = fibrinogen degradation products.

Similarly, the change in fibrinogen levels, namely that of a steady and in most cases, an immediate increase, is a confirmation of the findings of Warren et al (1950), Atkins and Hawkins (1969), Ygge(1970a,b), Hammer et al (1973) and Egan et al (1974).

The majority of patients, at some time during the first postsurgical week, were characterised by the presence of low levels of serum fibrin(ogen) degradation products. Of the group of 25 patients who had major elective surgery, 21 patients (84%) at some time during the first seven postsurgical days, had small increases in the concentration of fibrin(ogen) degradation products. Only 4 patients, (16%) gave completely negative assays. The results of these assays are seen in Table 64, page 129). It is of interest that, of the patients who produce the fibrin(ogen) degradation products, more patients had increased levels between days 1 and 4 postsurgically. These findings are in accord with the recently published work of Egan et al (1974), who found an increase in the levels of serum fibrin(ogen) degradation products in all of 39 postsurgical patients, with more positive findings being found on the third and fourth postsurgical days.

The changes in clot retraction were studied with an original technique. It would be expected that the changes in clot retraction will be influenced largely by the following factors: 1) the numbers of circulating

Table 64. Showing the relationship between serum fibrin(ogen) degradation products and the postoperative period in which these products were found in the group of 25 patients who had major elective surgery.

Post-op Day	Level of Fibrin(ogen) Degradation Products.		
	10-20ug/ml	20-40ug/ml	40-80ug/ml
	# of patients	# of patients	# of patients
1	7	0	0
2	3	0	0
3	8	2	0
4	4	1	1
5	1	0	0
6	3	1	0
7	0	0	0
8	2	0	0
9	0	0	0
10	0	0	0

platelets, 2) the functional activity of platelets, 3) the concentration of fibrinogen and 4) the numbers of erythrocytes (and to a lesser extent, leucocytes). In this study, no patient had a change in the packed cell volume of greater than $\pm$ 9% during the first 5 postsurgical days. It is therefore highly unlikely that the changes in the numbers of circulating red cells would have any appreciable effect on the clot retraction studies.

Budtz-Olsen (1951) in his admirable monograph, records the results of his experiments to determine the role of various factors in clot retraction. He demonstrated that, with a constant number of platelets, clot retraction becomes progressively less with increasing concentrations of fibrinogen, and if the concentration of fibrinogen reaches very high levels, retraction may be inhibited. With a constant fibrinogen level, clot retraction becomes progressively less as the numbers of platelets decrease. It is therefore likely that the changes in clot retraction in this study, will reflect the quantitative and qualitative characteristics of the platelets interecting with the changing fibrinogen levels. Budtz-Olsen (1951), also points out that all other factors present in the plasma, play only a minute role in the phenomenon of clot retraction, a suggestion which is fully supported by his experimental observations. The blood

samples used for the clot retraction studies were, as for most of the tests used in this study, diluted with tris buffer. Such dilution has been shown to have no effect on clot retraction (Arthus and Chaperio 1908, Masy Magro 1924, Lampert 1932, and MacFarlane 1938).

The results of the clot retraction studies in the group of 25 patients who had major elective surgery (see Tables 14 to 38, pages 56 to 80), indicate that on the first postsurgical day, when the greater majority of patients were characterized by a slight decrease in the platelet count (see Figure 20, page 89) accompanied by an insignificant change in the fibrinogen level (see Figure 21, page 90), an increase in clot retraction was found in 50% of the patients. If, as results indicate, the platelet count and the fibrinogen level show no real change, then it would seem logical to expect no change in the clot retraction activity. One may speculate that the increase in clot retraction may be related to the release, from the bone marrow, of young platelets with greater clot retractive ability. It is has been demonstrated by Enticknap et al (1970), that the size of the platelet increases postsurgically and that the peak increase in size occurs during the first to third postsurgical days. The peak increase in size occurred before the platelet count showed any significant increase. The change in size was also accompanied by changes in adhesi-

vity and aggregability to adenosine diphosphate. Such evidence suggests that some changes in platelet morphology and physiology are to be expected in the early postsurgical period. Table 65 (page 133), shows the results of the median retractive index for all patients in the group. If the mean median retractive index for the whole group is found for each day in the surgical period, and the data is graphically depicted (see Figure 25, page 134), a change in the index is seen to occur between the first and second postsurgical day.

An examination of the maximum retractive activity time data for the group shows, that during the first three postsurgical days, 44% of the patients had a shortening of the maximum retractive activity time, 40% demonstrated no change from the preoperative value, and 16% showed an increase in this indice. When shortening of the indice occurs, this suggests that the available platelets are capable of effecting retraction of the clot more rapidly, on the other hand, when the maximum retractive activity time increases, the ability of the available platelets to effect retraction, may in some way, be impaired.

This rather limited data, suggests that in addition to changes in the platelet count, platelet adhesiveness and platelet aggregation, changes in the phenomenon of clot retraction may slo occur. It is tempting to speculate that, since the postsurgical patient is at high risk for the development of venous thromboembolism, changes in clot

TABLE 65. Showing the changes in the median retractive index, in the main group of surgical patients.

	Days in Surgical Period.											
Pat #	Pre	0	1	2	3	4	5	6	7	8	9	10
	Median Retractive Index.											
5	58		33		45		43		55			
6	34		52		54		30		26			
7	32			24		25			27		36	
8	58		55		60			52		56		
9	-			-	55		55				47	46
10	-		60			40		35		43		37
11	-		44			30		27		29		
12	-			43		47		60		47		
13	36			30		46		40		35		
14	26		27		30	24	29	27		27		
15	49		45		32		35		28			
16	56		49		46		44		52			
17	46		60		40					41		
18	54		59		57		52		50		45	52
19	-		52			30		26		33		
20	32		36			32		28		43		
21	43		50		40		40		35			
22	43		47		47		35		28			
23	58		56		52		45		45			
24	50		53				43		55			
25	43		48		40		41		36			
26	50		45		32		35		28			
27	15		11		19		25		24			
28	46	40		26		33		44		38		
29	46	50			22			18		20		

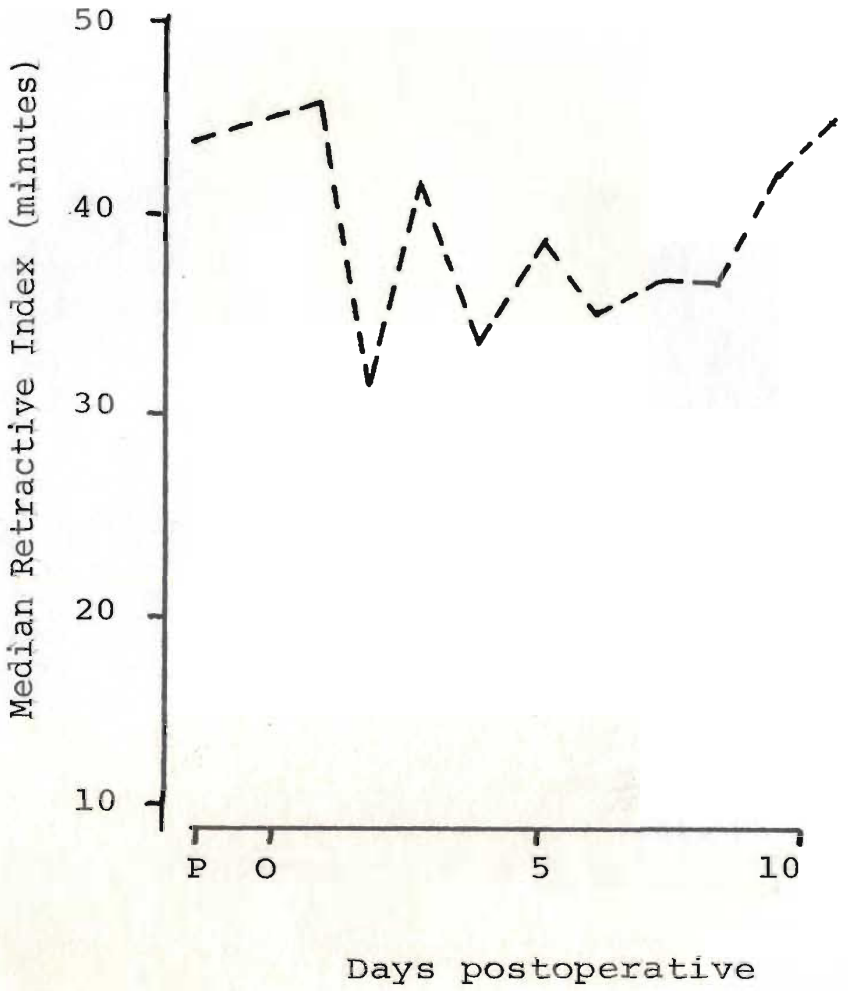

Fig. 25. Showing the graph of the mean values for the median retractive index (obtained from the data presented in Table 65 (page).

P = pre-operative
O = day of surgery

retraction may influence the morphology and size of a recently established venous thrombus, and may also be involved in the birth of an embolus. Clot retraction, by the extrusion of nascent thrombin from a freshly formed venous thrombus, may result in an extension of the thrombus. The process of extension, brought about by the extrusion of nascent thrombin and its coagulant effect on fibrinogen, may result in the entrapment of fresh platelets, eading to further retraction. Under the conditions present in a vein, occluded or partially occluded by a thrombus, freshly extruded thrombin would not readily be rendered innocuous by dilution with the circulating blood, since impairment to blood flow would be expected. This process of repeated retraction, and its influence on fibrinogen and circulating platelets could result in the formation of long slender thrombi, dangerously waving to and fro in the blood stream, the flow of which may be partially restored through clot retraction. It is tempting to speculate, but very difficult to prove, that the more active the process of clot retraction, the more likely the thrombus would be to extend and the greater the danger of it being dislodges to form a potentially dangerous embolus.

The increase in the concentration of fibrinogen, the numbers of circulating platelets and in the level of serum fibrin(ogen) degradation products in the majority of the postsurgical patients indicates that such patients should not have their haemostatic mechanism evaluated in

terms of the usual normal values. It is obvious for example, that a fibrinogen level of 200 mg/100ml. plasma, found in a patient tested on the fifth post-operative day, would likely be abnormal for this patient, in spite of the fact that the value falls within the commonly cited normal range of 200 - 400 mg./100ml. plasma. Similarly, a platelet count of 200,000/c.mm. of blood, found on the same postsurgical day, may very well be abnormal, again in spite of the fact, that as an isolated test result, it falls well within the usual normal range. It is particularly important that these postsurgical changes be kept in mind when attempting to make a diagnosis of disseminated intravascular coagulation in postsurgical patients. The laboratory diagnosis of such a state, rests in part on the demonstration of a fall in the fibrinogen concentration and in the numbers of circulating platelets, together with an increase in the concentration of serum fibrin(ogen) degradation products. It would seem more appropriate that, in order to detect varying degrees of haemostatic failure in the postsurgical patient, evaluation of postsurgical test results be made with pre-surgical values, keeping in mind the changes that result from surgical operations.

SUMMARY AND CONCLUSIONS

The detection of increased coagulability of the blood (hypercoagulability?) in the post-operative patient can be accomplished providing the following laboratory procedures are established; a) that whole blood samples, processed in a standard way, and subjected to dilution be tested, b) that the patient's blood be tested prior to surgery and sequentially during the post-operative period and further, that the post-operative results be compared to those obtained prior to the surgical procedure, thus dispensing with the usual practice of evaluation of post-surgical findings with those obtained from a "normal" population.

This study has demonstrated a peak increase in blood coagulability *in vitro* occurring around post-operative days 3 to 4, and an indication of a lesser increase occurring at the end of the first post-operative week.

It has been shown that in approximately half of the patients undergoing major elective surgery accelerated clot retraction was found. Thus documenting an additional alteration in the physiology of the platelet induced by surgery.

This study has confirmed the increase in fibrinogen, platelets, and serum fibrin(ogen) degradation products in the post-operative patient.

Finally, since the increase in fibrinogen concentration, platelet count, and the concentration of serum fibrin(ogen) degradation products seems to be a characteristic response of most post-surgical patients, it is recommended that such patients, in times of possible haemostatic failure, such as that which occurs in disseminated intravascular coagulation, should have their haemostatic mechanism evaluated in terms of a pre-operative study, rather than an evaluation based on the so-called "normal values". Any post-surgical evaluation of haemostasis must be made bearing in mind the haemostatic changes that result from surgical operations.

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APPENDIX

Abbreviations and Units of Mesurement Used in All Tables.

Abbreviation	Meaning	Units of Mesurement
	Phase 1	Seconds.
	Phase 2	Seconds.
	Phase 3	Seconds.
H.R.T.	Heparin Resistance Test	Seconds.
A.C.G.	Autocoagulography Test	Seconds.
A.C.G.C.	Autocoagulography Control Test	Seconds.
P.T.	Prothrombin Time	Seconds.
K.C.C.T.	Kaolin-cephalin Clotting Time	Seconds.
G.E.F.	Gravimetric Estimation of fibrinogen.	mg./100ml.Plasma.
F.D.P.	Serum Fibrin(ogen) Degradation Products	micro-grams/ml.
M.R.I.	Median Retractive Index	Minutes.
M.R.A.T.	Maximum Retractive Activity Time	Minutes.
Plats.	Platelet Count	/c.mm. Blood.
C.R.	Clot Retraction	Percent.