MMed(Anaesthesia) Part III Dissertation

THE EFFECT OF *IN VITRO* HAEMODILUTION ON COAGULATION

PREPARED BY: Dr T.G.Ruttman MBChB(UCT)FCA(SA)
Department of Anaesthesia
University of Cape Town
Groote Schuur Hospital
Observatory

SUPERVISOR: PROFESSOR J.F.VILJOEN

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PREFACE

My interest in the effect on coagulation of various intravenous solutions was originally aroused by the conflicting recommendations made for the use of the colloids in the resuscitation of patients who needed relatively large amounts of intravenous fluid. Many of the studies which comment on the effects of these fluids on coagulation were done under poorly controlled conditions, where extraneous factors, such as surgery, drugs, stress or physiological states such as malignancy, might have had an effect on the results. The idea for this project was initially suggested to me by Professor M.F.M. James whose key role in arousing my interest in the subject I gratefully acknowledge. Once we started reviewing the available literature, it serendipitously became apparent that saline itself may not be inert as an intravenous infusate, and that several previous studies had been done to establish the effect of saline on coagulation. Again I gratefully acknowledge Professor James for steering me in the right direction.

We decided to use the thrombelastograph (TEG) as a measuring tool, because of its established role in assessing whole blood coagulation. The Department of Anaesthesia at Groote Schuur Hospital has a TEG which was made available for use in this project, as were the disposables needed for the TEG and for venesection. All the studies were done on blood from consenting volunteers, many of them colleagues in the Department, and I would like to thank them for donating their blood so unflinchingly. The results were measured, recorded and tabulated by Ms Gail Neill who works as a research assistant in our departmental laboratory and I am grateful for the support she has lent me during completion of this project. It should be noted that the paper speed of the TEG is 2mm per minute. Where raw data are appended as well as in the reproduced sample TEG tracings, the r and k values are thus reflected in millimetres as measured on the graph paper. They have been converted to time (min) in the interpretation. The statistical analysis of the raw data was performed by Professor James without whose help I would not have been able to complete this project. The processing of this document was my own work. One published paper has to date arisen out of this thesis:

1. Ruttmann TG, James MFM, Viljoen JF. Haemodilution causes a hypercoagulable state. *British Journal of Anaesthesia* 1996; **76:** 412-414.

THE EFFECT OF IN-VITRO HAEMODILUTION ON COAGULATION

INTRODUCTION

Haemodilution with intravenous fluids will decrease the concentration of clotting factors in the blood, and intuitively it would be anticipated that this should induce some degree of impairment of coagulation. It has, however, been suggested that haemodilution may possibly provoke an increase in whole blood coagulation. In 1959 Tocantins[1] and Monkhouse[2] reported that moderate haemodilution with crystalloids could induce a hypercoagulable state. This was later borne out by Janvrin[3] in a clinical trial which investigated the incidence of post-operative deep vein thrombosis related to intra-operative fluid administration. Many of the studies which comment on the effects of colloids on coagulation have used a crystalloid control[4 5 6 7] or have not made allowance for any crystalloid the patient was given in addition to the colloid, *viz*. cardio-pulmonary by-pass pump prime[8 9]. Popov-Cenic[10] et al refer to the effect of surgery on enhancing coagulation and the modification thereof by hydroxyethyl starch. The hydroxyethyl starches are a relatively new group of compounds designed to provide plasma volume expansion during anaesthesia, surgery, trauma resuscitation and endotoxic shock. Whilst these products provide highly effective volume expansion, there is a question against all of the synthetic colloid solutions regarding their effects on haemostasis, although the dextrans are thought to pose a greater risk than either modified gelatins or hydroxyethyl starch[11]. The higher molecular weight hydroxyethyl starch (hetastarch MW 450,000; HES) has been associated with a number of changes in coagulation parameters including small, but significant, prolongations in partial thromboplastin, prothrombin and bleeding times, decreases in fibrinogen, anti-thrombin III and factor VIII concentrations and an increase in clot lysis[12, 13]. Pentastarch (MW 264,000; PES) was found, by Strauss et al[14], to exert significantly

lesser effects on standard tests of blood coagulation than HES despite a greater effect on haemodilution. Various clinical studies have been conducted on the hydroxyethyl starches in this regard with conflicting results. Kuitunen et al showed both types of hydroxyethyl starch solutions (HES and PES) to have adverse effects on coagulation as compared to Ringer's acetate when used as priming solutions for cardiopulmonary bypass[6] . On the other hand, blood loss replacement with HES was reported to have no adverse effects on coagulation compared to the use of 5% albumin in gynaecological- and neuro-surgery[15]. Clinical studies conducted on patients undergoing surgery suffer from numerous disadvantages including the variability of haemodilution, the effect of surgery on coagulation and the possible unpredictable interactions between plasma expanders and substances such as heparin which are independently used to modify coagulation. Vinnazzer and Bergmann[16] in 1975 performed a double-blind study which compared standard tests of coagulation as well as thrombelastographs pre- and post-operatively in two groups viz. one treated with Hydroxyethyl starch and the other with isotonic saline peri-operatively. Their findings show a post-operative hypercoagulable state in the control (saline) group, and an insignificant change in the test (Hydroxyethyl starch) group. As their study was done post-operatively, it does not show whether these changes on coagulability were already present after infusion of the intravenous fluid, before any surgical stress response occurred, which might further have influenced coagulation parameters. Tuman et al[17] conducted an *in vivo* study in which they noted that patients receiving crystalloids demonstrated an increase in coagulability as measured by the Thrombelastograph (TEG), but they did not attribute this directly to the crystalloid infusions.

Coagulation

Tissue injury triggers a complex series of physiological responses aimed at maintaining the integrity of the blood vessel while repair of the damaged vessel occurs. After exposure to agonists such as thromboplastin or collagen, platelets undergo a multitude of morphological and biochemical changes resulting in adherence to areas of damaged endothelium[18]. This primary haemostasis results in the formation of a platelet plug without the generation of fibrin[19]. Clotting occurs when the platelet plug is then stabilised as circulating inactive forms of haemostatic proteins are activated by one or more critical proteolytic cleavage events. This system is designed

to generate thrombin at the site of injury. This thrombin, a serine protease, cleaves soluble fibrinogen to form insoluble fibrin strands which cross-link and form the basis of the final stable clot. In addition the generation of thrombin is integrally involved in the stimulation of the fibrinolytic pathway, which serves to lyse the clot and counterbalances the unchecked generation of thrombus[18].

The cascade[20] / waterfall[21] hypotheses, in 1964, organised the events of haemostasis into intrinsic, extrinsic and common pathways. Accordingly coagulation could be initiated via the "intrinsic pathway", so named because all the components were present in the blood, or by the "extrinsic pathway", in which the sub-endothelial cell membrane protein, thromboplastin, was required in addition to circulating components. Initiation of either pathway resulted in the activation of Factor Xa and eventual generation of a fibrin clot through a common pathway.

(Fig. 1).

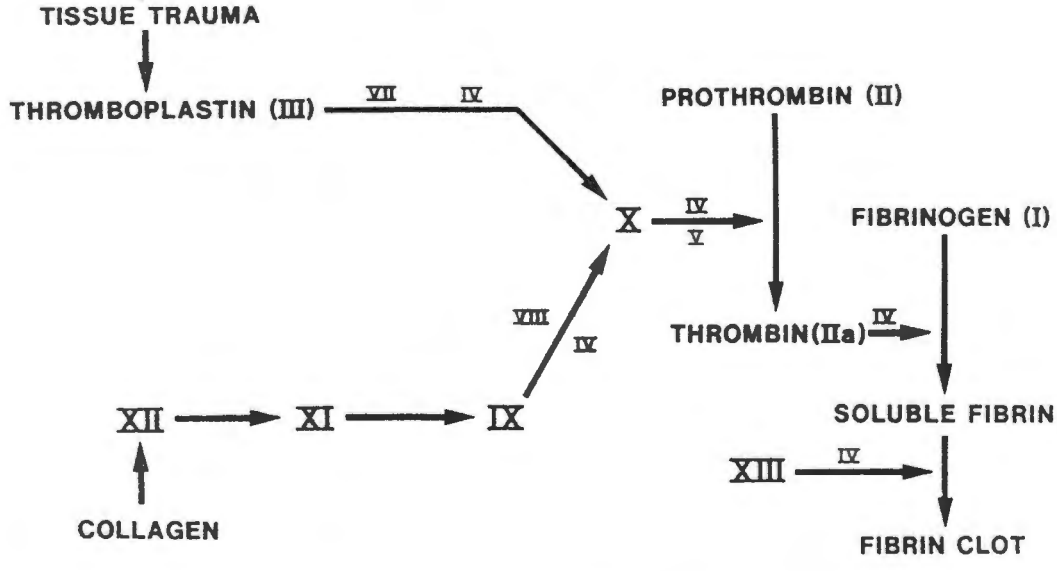

Figure 1 : The waterfall / cascade hypothesis of blood coagulation.

Thrombelastography

Thrombelastography is a technique first developed by Hartert in 1948 [22]. It had little clinical use for many years until it was re-evaluated in the surgical setting in 1974 [23]. Subsequently it was further developed [24], and has been more recently applied and validated in both liver

transplantation [25] and cardiac surgery [26]. The TEG involves two mechanical parts, a cuvette and a piston. Freshly drawn blood (0.36 ml) is measured by automatic pipette and placed in the cuvette which oscillates through 4°45' at 37°C. The piston is suspended in the blood sample by a torsion wire which is transduced mechanically to a heated stylus which moves across heat sensitive graph paper on a chart recorder. When no clot exists, the motion of the cuvette does not affect the piston and the chart records a straight line. As strands of fibrin form they attach to the piston and it becomes coupled to the motion of the cuvette, hence the shearing elasticity of the evolving blood clot is transmitted to the thermal paper (Figure 2).

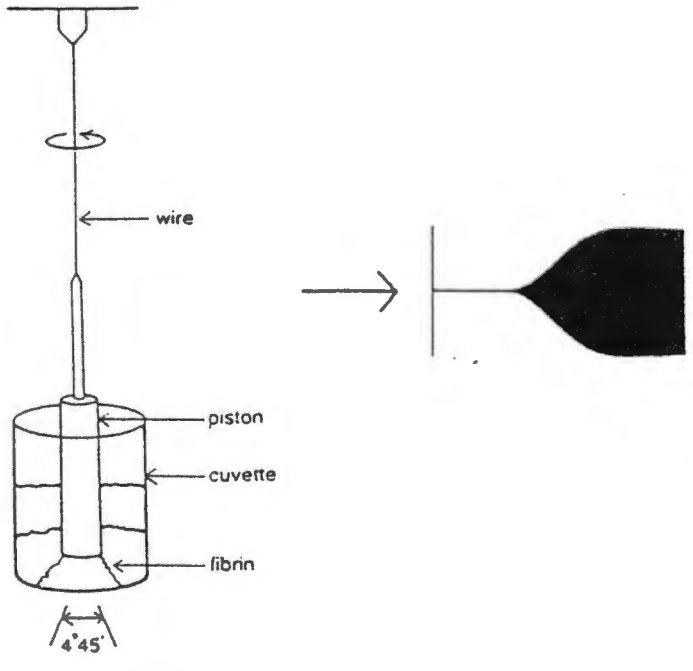

Figure 2 : The mechanism of the thrombelastograph.

The TEG pattern is divided into component variables. It reflects function of the intrinsic clotting pathway. Reaction time (r) is the period of latency from when the blood was placed in the cuvette until initial fibrin formation occurs, and is reflected by time taken from the start of recording until the width of the tracing is 2 mm. Coagulation time (k) is the time required for the width of the tracing to reach a certain arbitrarily predefined level of clot strength (20 mm) and gives information about not only intrinsic factors but also platelet function and fibrinogen, which are also represented by the clot formation rate (α°). Maximum amplitude (MA) is the greatest width achieved on the

thrombelastograph tracing and is a measure of final clot strength and visco-elastic properties, again reflecting the function of platelets and fibrinogen as well as factor XIII.

Thrombelastography is essentially a graphic method of displaying the stages in the formation of a whole blood clot (thrombosis) and as such provides a visual pattern of functional clotting status. Recent advances include computerisation of the TEG, which essentially gives greater accuracy and consistency than manual measurements by removing the human error from the measurements

Although the TEG was developed so long ago, it is only recently that it has acquired widespread acceptance. The main reason for the current interest in the device is the fact that it assesses whole blood coagulation rather than individual components of the coagulation pathway. In addition, the relative rapidity with which results can be obtained has created a role for the TEG in cardiac surgery and in liver transplantation.

The thrombelastograph (TEG) has been widely used as a measure of coagulation in clinical anaesthesia[27]. In a study published in *Anaesthesia and Intensive Care* in 1994, which examined patterns of coagulopathy during orthotopic liver transplantation, the relationship between standard tests of coagulation and the TEG was examined and the TEG found to be an inconsistent correlate of standard laboratory tests of coagulation[28]. However, the authors comment that this is not surprising given that the TEG variables are interdependent, measuring the interaction of the coagulation cascade and platelets in whole blood rather than specific end-points in centrifuged plasma samples[29]. The global, yet overlapping, data that is provided by the TEG allows far more appropriate replacement therapy to be given, as well as providing a useful test of liver function during the immediate post-crossclamp time. The TEG provides in-theatre analysis of coagulation in about 15 minutes while laboratory coagulation tests take about 45 minutes.

Kang *et al* [25] found thrombelastography to be a reliable and rapid monitoring system during liver transplantation. They found the relationship between TEG and standard laboratory tests of coagulation to be similar to that of other researchers[23] [24], with the greatest correlation occurring between *r-time* and *aPTT*.

In general, the TEG measures the coagulation process in whole blood, rather than testing blood coagulability in anticoagulated plasma, as is commonly done to obtain conventional coagulation profiles.

Martin *et al*[30] validate the role of the TEG during paediatric liver surgery, but comment that the role of the TEG in paediatric cardiac surgery is not yet fully defined, in spite of their 100% sensitivity (8/8) and 73% specificity (8/11) in predicting increased post-operative bleeding. In another study comparing the TEG to bleeding time and standard coagulation tests after cardiopulmonary bypass, Essell *et al*[31] found the sensitivity of bleeding time and platelet count to be similar to that of the TEG, but the specificity of the TEG in predicting post-operative bleeding to be greater than that of either bleeding time or platelet count. In their study only 2 out of 27 (7.4%) patients with normal TEG experienced abnormal post-bypass bleeding requiring platelet or FFP transfusion, suggesting that patients with a normal TEG who have a post-bypass bleed should not be given blood products empirically, but that a surgical cause should rather be excluded.

We have conducted two *in vitro* studies, using the TEG as a measure of whole blood coagulation. The first sets out to determine the effect of a 20% haemodilution with either crystalloid (0.9% saline) or a commonly used colloid (gelatin, Haemaccel®) on coagulation compared, with an undiluted control. The second compares the effect on coagulation of a 20% haemodilution with saline and three different colloids (gelatin, Haemaccel®; hetastarch, Plasmasteril®; & pentastarch, Haes-steril®).

All disposables for venesection and the thrombelastograph were provided by the Department of Anaesthesia. Computers and software used, as well as the thrombelastograph belong to the Department of Anaesthesia, and all costs were funded through the department.

HAEMODILUTION INDUCES A HYPERCOAGULABLE STATE

This part of the thesis was designed to investigate the hypothesis that haemodilution of whole blood with either a crystalloid or a colloid diluent altered the coagulability of the blood. The null hypothesis tested by the methodology was that haemodilution was without effect on coagulation as measured by the thrombelastograph.

METHODS

The study was approved by the University of Cape Town Research Ethics committee.

The experiment was conducted in two parts. In the first part, blood samples were obtained from twenty consenting volunteers. Blood was taken from a free-flowing forearm vein into a 10 ml syringe and separated into 2 aliquots of 4 ml each in polypropylene plastic tubes. Isotonic (0.9%) saline 1 ml was added to one sample whilst the other sample was left undiluted and served as a control. Specimen tubes containing each sample were inverted several times to ensure thorough and similar mixing of the blood in each tube. The control samples were inverted in a similar fashion to those of the diluted group to ensure that, as far as possible, each sample was treated in a similar fashion. Specimens from each sample tube (0.360 ml) were then pipetted into the cuvette of the thrombelastograph using an automatic pipette, and simultaneous traces recorded of the normal blood sample and of the saline diluted sample, running concurrently in the two channels of the TEG machine. The samples were randomly alternated (based on the flip of a coin) between the two channels of the TEG to exclude a recurring machine error affecting the result of either group. The TEG trace was recorded for one hour and the r and k times, alpha angle ($\alpha°$), and maximum amplitude (MA) were measured for each sample. Care was taken that less than one minute should elapse between obtaining specimens and commencing the TEG tracing.

In the second limb of the trial the methodology described above was repeated, except that the test samples were diluted with 1 ml of a modified gelatin colloidal solution (Haemaccel®). Again one sample was used for testing the effect of haemodilution, whilst the undiluted sample served as the control.

It should be noted that while there was some overlap between the two groups, they were essentially two different groups of volunteers. Power analysis showed that the size of the groups (20) should be sufficient to demonstrate a difference of 10% in all values between diluted sample and undiluted control within each group (Table 1). Both sets of controls fall within the normal range quoted for the TEG[17 27], although they are statistically different from each other.

	Size of change to be detected	Anticipated S.D.	No.of observations required
r-time	1.5 min	2 min	19
k-time	1 min	1 min	8
α angle	8°	9 °	14
MA	5 mm	6 mm	16

Table 1: Number of observations required to achieve statistical significance as predicted by "Statgraphics" statistical package.

Data from each diluent group were compared to those obtained from the matched controls in each limb of the experiment and any differences noted were analysed for statistical significance using a paired Student's *t*-test. Statistical analysis was performed using Statgraphics version 6 statistical package running on an IBM-compatible 486 microcomputer under MSDOS version 6.

Haemascope, the TEG manufacturers, recommend that a normal range be established for each machine, and this we have done in over 100 samples.(Table 2)

	r-time (min)	k-time (min)	r+k time (min)	α-angle (°)	ma (mm)
Mean	7.3	3.8	11.1	35.6	50.2
Std Dev	1.2	0.9	2.1	6.7	4.7
Maximum	10	5	15	50	60
Minimum	5	2	7	22	40

Table 2: Means, Standard Deviations, Maximum and Minimum normal TEG values for our machine

RESULTS

The results are tabulated in Table 3 & 4. In the first limb of the experiment, there were statistically significant differences between the saline treated samples and the untreated samples in all the parameters measured by the TEG. (Table 3)

	Control	Saline	*p*
r (min)	8.3	6.5	0.0068
(SD)	(2.2)	(1.7)	
k (min)	3.7	2.2	0.00
(SD)	(1.1)	(0.9)	
r+k (min)	12.0	8.8	0.0004
(SD)	(3.2)	(2.4)	
α (°)	38.7	51.0	0.0004
(SD)	(9.6)	(10.1)	
MA (mm)	50.7	55.0	0.0327
(SD)	(6.1)	(6.4)	

Table 3 Mean (SD) TEG values in control and saline diluted samples, together with p values.

There was a highly significant decrease of r and k times, a highly significant increase in the α angle and a more moderate, though still significant, increase in the maximum amplitude. These differences may be demonstrated graphically by reproducing a TEG tracing recorded from one subject in the study.(Fig.2)

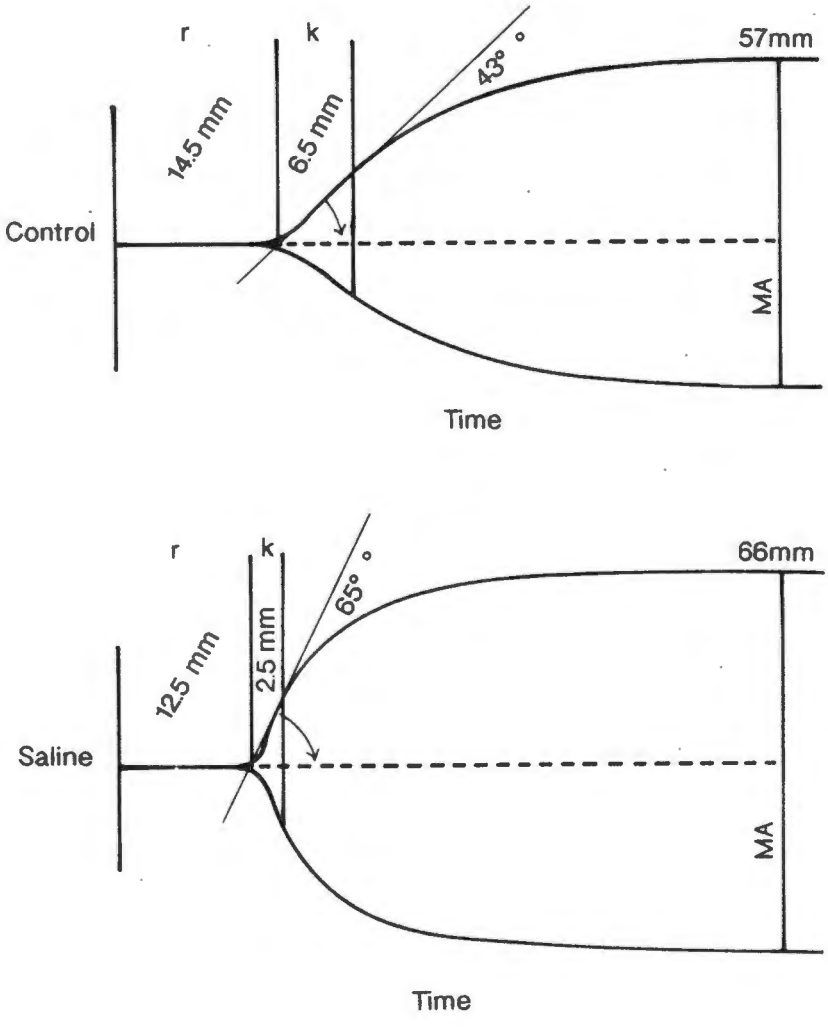

Figure 3: Sample TEG tracings from the control versus saline dilution group.

(r and k values are expressed in millimetres as recorded on the TEG graph paper. Paper speed equals 2 millimetres per minute.)

In the second part of the study, there were statistically significant differences between the Haemaccel treated samples and the untreated samples in all the parameters measured by the TEG, except for maximum amplitude (MA), which was not affected by dilution with Haemaccel. (Table 4)

	Control	Haemaccel	*p*
r (min)	9.1	7.1	0.0001
(SD)	(1.6)	(1.3)	
k (min)	4.3	2.4	0.00
(SD)	(1.0)	(0.8)	
r+k (min)	13.4	9.5	0.0004
(SD)	(2.6)	(2.2)	
α (°)	33.15	49.7	0.00
(SD)	(6.4)	(9.3)	
MA (mm)	46.5	47.2	0.8
(SD)	(6.4)	(6.9)	

Table 4 Mean (SD) TEG values in control and Haemaccel diluted samples with p values.

There was a highly significant decrease of r and k times, and a highly significant increase in the α angle. These differences may be demonstrated graphically by reproducing a TEG tracing recorded from one subject in the study.(Fig.3)

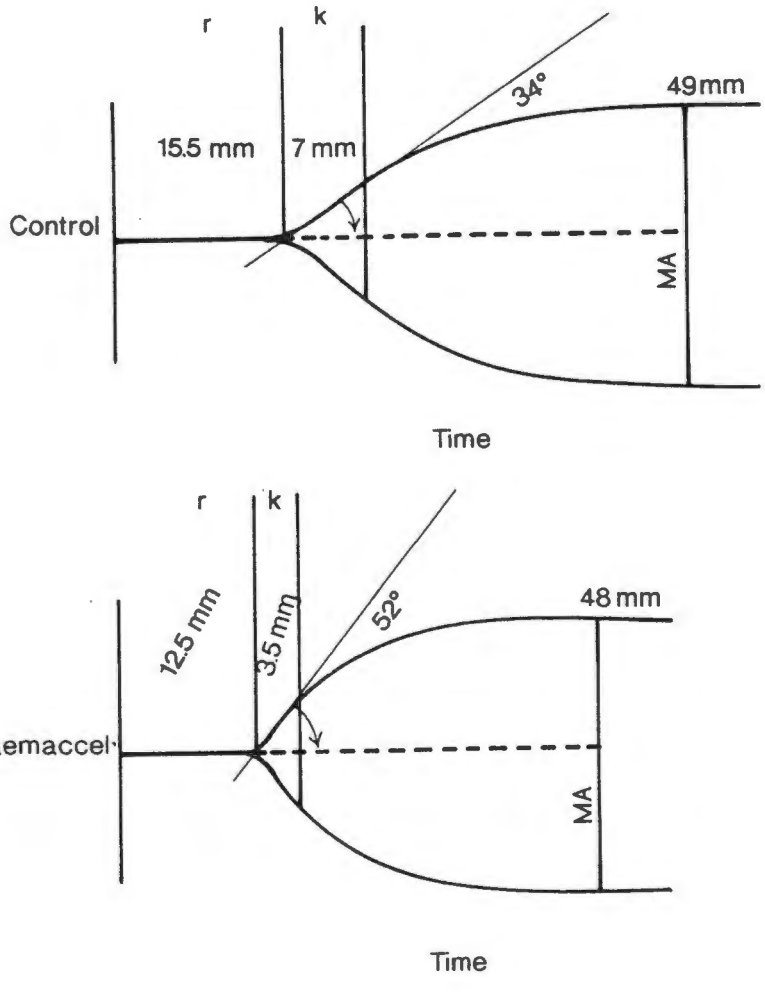

Figure 4: Sample TEG tracings from the control versus Haemaccel dilution group.

(r and k values are expressed in millimetres as recorded on the TEG graph paper.

Paper speed equals 2 millimetres per minute.)

(Lists of the actual data are appended as Annexures 1 & 2.)

DISCUSSION

We performed an *in vitro* study on human blood using the TEG to compare the dynamic coagulation profile of a 20% haemodilution with either crystalloid or colloid to that of an undiluted control taken simultaneously from the same subject. We have shown that haemodilution with either solution causes a decrease in r and k times reflecting increased intrinsic coagulability, and platelet and fibrinogen activity. The α angle, reflecting speed of clot formation, was increased significantly in both test groups. The maximum amplitude showed a moderate increase in the saline group reflecting an increase in clot strength, again indicating increased fibrin and platelet activity, but this effect was not apparent in the colloid diluted group. The mean k times and r+k times for both test groups are shorter than the minimum values quoted by Mallet and Cox [27], although the other measurements fall within their quoted ranges. The changes demonstrated in our study represent an increase of between 20-40% in the initiation of coagulation and the speed of clot formation induced by haemodilution, with a rather lesser effect on the development of clot strength. This implies that moderate haemodilution with either crystalloid or colloid could cause blood to clot more readily, but will have minimal effect on the strength of the final clot formed. What the effect of greater or lesser degrees of haemodilution might be is not addressed by this study, but Tocantins[1] et al suggest that the effect increases with whole blood dilutions of up to 40% and that the changes in the speed and character of thrombus formation were as a result of haemodilution rather than properties of the saline itself. By repeating the study using a colloid solution as diluent we have shown similar decreases in r and k times as well as an increase in α angles, thereby offering further evidence that the documented changes are brought about by dilution rather than the nature of the actual diluent itself.

The mechanism by which haemodilution causes this increase in coagulation is far from clear. Tocantins et al[1] in their study showed that the nature of the crystalloid diluent was not important, and that all crystalloid solutions produced similar effects. Our own unpublished data confirms this impression.(Annexure 3).

By doing an *in vitro* study we have eliminated extraneous factors such as heparinisation, stress response, tissue damage and endothelial injury all of which could affect coagulation in an *in vivo* study. All the changes we have been able to demonstrate have thus been a direct consequence of the addition of either saline or Haemaccel to normal blood.

Our results confirm that the addition of either saline or Haemaccel to a 20% dilution (equivalent to 1000 ml intravenous fluid in an average 70kg person) *in vitro* renders blood hypercoagulable, affecting all aspects of the coagulation cascade from initial fibrin formation (decreased r time), rapidity of fibrin build-up and cross-linkage (decreased k time), and speed of clot formation (increased α angle). Final absolute clot strength (increased MA) was only increased with saline haemodilution and unchanged with Haemaccel haemodilution. A possible explanation for this is supplied by the findings of Mardel *et al* who suggest that gelatin based products may become incorporated into developing clots and reduce the function of fibronectin in forming covalent cross-linkages and normal covalent associations with fibrin, thus interfering with polymerisation of fibrin monomers.[32]

If this has application *in vivo,* as suggested by Janvrin[3], the ramifications are potentially profound with possible implications for the management of stroke with haemodilution, post-operative DVT, peripheral vascular surgery and trauma. In coronary artery bypass graft surgery it is possible that haemodilution could adversely affect graft survival once the action of heparin has been reversed, and normal platelet function restored. The role of heparin in opposing this effect of haemodilution is an interesting area which remains to be explored.

AN *IN-VITRO* INVESTIGATION OF THE EFFECTS OF SYNTHETIC COLLOIDS ON COAGULATION AS MEASURED BY THE TEG.

INTRODUCTION

In the previous *in vitro* study, we have shown, using the TEG , that crystalloid haemodilution to 20% causes a hypercoagulable state[33]. This study was designed to compare the effect on coagulation of a 20% haemodilution with saline and three colloids (Gelatin, Haemaccel®; hetastarch, Plasmasteril®; and pentastarch, Haes-steril®), using the TEG as a measure of whole blood coagulation.

METHODS

The study was approved by the University of Cape Town Research Ethics committee.

Blood samples were obtained from freely consenting volunteers. On each occasion 8 mls of whole blood was sampled from a large free-flowing vein. The 8 mls of blood was divided into two 4 ml samples in polypropylene tubes, and 1 ml of a colloid solution was added to one sample and 1 ml of saline added to the other. This provided a 20 % haemodilution in each sample. (Equivalent to 1 000 ml infusion in a patient with a blood volume of 5 000 mls). Specimen tubes containing each sample were inverted several times to ensure thorough and similar mixing of the blood in each tube. As far as possible, all sample were treated in a similar fashion. Specimens from each sample tube (0.360 ml) were then pipetted into the cuvette of the TEG using an automatic pipette and simultaneous traces recorded of the colloid diluted sample and of the saline diluted sample, running concurrently in the two channels of the TEG machine. The samples were randomly alternated (based on the flip of a coin) between the two channels of the TEG to exclude a recurring machine error affecting the result of either group. The TEG trace was recorded for one hour and the r and k times, alpha angle ($\alpha°$), and maximum amplitude (MA) were measured for each sample. Care was taken that less than one minute should elapse between obtaining specimens and commencing the TEG tracing. Three different colloid solutions viz. Gelatin (Haemaccel®), 6 % Plasmasteril®(Hetastarch MW ±450 000, HES) and 6 % Haes-steril® (Pentastarch MW. ±264 000, PES) were compared against saline. Twenty samples were collected for each of the three colloids tested. Each group was comprised of different volunteers although some of the volunteers were used for more than one of the 3 groups. However, since each subject acted as his own control, it was not necessary to have the same twenty volunteers for each group, since only simultaneously made recordings were compared. Our machine was re-calibrated between the previous study and this one. The differences between values in the two separate studies therefore have should be interpreted with caution. Data from the first study cannot be extrapolated to that obtained in the present study.

Statistical analysis was performed using Statgraphics version 6 statistical package running on an IBM-compatible 486 microcomputer under MSDOS version 6. Within group comparisons between saline and the colloid solution were performed using a paired Student's *t*-test, and between group

comparisons were performed using analysis of variance with the 95% confidence interval multiple range test to identify significantly different results. In order to eliminate individual variations, between group comparisons were performed on the difference in the result between saline and the colloid diluted specimens.

RESULTS

The group of twenty samples to which gelatin was added are shown in Table 5 together with their saline controls.

	MEAN	S.D.	P.
r saline	6.14	1.50	0.41
r gelatin	5.95	1.18	
k saline	2.2	0.70	0.35
k gelatin	2.31	0.69	
α saline	51.5	9.58	0.12
α gelatin	49.5	8.70	
MA saline	54.05	5.22	<0.0001
MA gelatin	48.7	5.06	

r, k, α and MA refer to r-time(min), k-time(min), α-angle(°) and maximum amplitude(mm) of the TEG respectively.

Table 5 :TEG variables for gelatin *v* saline.

There were no significant differences in r-time, k-time or α-angle between gelatin and saline haemodilution The MA, however, was significantly smaller ($p < 0.002$) in the gelatin group.

The samples diluted with HES showed no significant difference in r and k times and no change in α angle as compared to the saline-diluted controls. The MA was, however, significantly smaller ($p < 0.0001$) in this group.(Table 6)

	MEAN	S.D.	P.
r saline	7.46	1.26	
r HES	7.51	1.27	0.82
k saline	2.5	0.53	
k HES	2.57	0.73	0.60
α saline	47.3	6.11	
α HES	44.95	6.07	0.10
ma saline	53.45	4.63	
ma HES	47.5	4.65	<0.0001

r, k, α and MA refer to r-time(min), k-time(min), α-angle(°) and maximum amplitude(mm) of the TEG respectively. HES indicates hetastarch (Plasmasteril®)

Table 6 : TEG variables for HES *v* saline.

In the PES group the k time was significantly longer ($p < 0.0002$), the α angle less ($p < 0.0001$) and the MA significantly smaller ($p < 0.0001$) than in the saline diluted samples.(Table 7)

	MEAN	S.D.	P.
r saline	6.1	1.5	0.01
r PES	6.5	1.22	
k saline	1.78	0.59	0.0002
k PES	2.26	0.8	
α saline	57.1	8.50	0.0001
α PES	50.1	9.32	
MA saline	57.75	5.94	<0.0001
MA PES	49.35	6.26	

r, k, α and MA refer to r-time(min), k-time(min), α-angle(°) and maximum amplitude(mm) of the TEG respectively. PES indicates pentastarch (Haes-steril®).

Table 7 : TEG variables for PES *v* saline.

Results for saline haemodilution were significantly different between the groups for reasons that are not obvious, but presumably simply reflect chance differences in response to haemodilution between the groups of subjects.

In the saline treated samples in the three groups, the r-time with saline in the HES group was significantly longer than that of the other 2 groups, whereas the k-time, α-angle and MA in the saline treated samples were significantly different in the PES group.(Table 8)

	Gelatin	HES	PES
r	6.14	7.46*	6.1
k	2.2	2.5	1.77*
α	51.5	47.3	57.1*
MA	54.0	53.4	57.7*

r, k, α and MA refer to r-time(min), k-time(min), α-angle(°) and maximum amplitude(mm) of the TEG respectively. PES indicates pentastarch, HES indicates hetastarch.

The * indicates values that are significantly different from the other two groups ($p < 0.05$).

Table 8 : TEG variables obtained from blood diluted with saline in the various groups.

The differences between saline and colloid haemodilution are shown in Table 9.

	Saline - Gelatin	Saline - HES	Saline - PES
r1 - r2	0.19	-0.05	-0.400*
k1 - k2	-0.11	-0.07	-0.49‡
α1 - α2	2.00	2.35	7.00‡
MA1 - MA2	5.35	5.95	8.40‡

r, k, α and MA refer to r-time(min), k-time(min), α-angle(°) and maximum amplitude(mm) of the TEG respectively. Denoters 1 and 2 refer to saline dilution or colloid dilution. PES indicates pentastarch, HES indicates hetastarch.

Table 9 : Differences between saline haemodilution and colloid haemodilution in the three groups. r1, k1,α1 and MA1 indicate TEG values obtained with saline haemodilution. r2, k2,α2 and MA2 indicate TEG values obtained with colloid haemodilution.

* - The differences in r-time between saline and colloid dilution were significantly different between the gelatin and PES groups ($p < 0.05$). The HES group was not significantly different from either of the other two.

‡ - The changes in k-time, α-angle and MA were significantly different in the PES group compared to the other two groups.($p < 0.05$)

In all groups the comparative differences from saline were greatest in the PES group.

(The actual data from all three groups are attached as Annexures 4, 5 & 6)

DISCUSSION

It has been thought for some time that the use of various colloid agents as plasma volume expanders has a deleterious effect on haemostasis. Common practice is to limit their use to 2 units (1000 ml) in the average 70 kg adult [5]. Kuitunen et al [6] showed that both high molecular weight (HMW) and low molecular weight (LMW) hetastarch solutions used in cardio-pulmonary bypass pump (CBP) prime had deleterious effects on laboratory indices of haemostasis, while Claes et al [16] found no effect on bleeding. Penner et al [34] looked at specific dilutions (20%) of 10% hydroxyethyl starch and their effect on haemostasis and found no apparent clinical effects.

There appears to be no consensus in the literature as to the effect of various colloid solutions on haemostasis. We felt that previous studies were limited as they were performed in conjunction with surgery where multiple factors such as surgical trauma, heparinisation, protamine reversal, stress response, endothelial exposure, etc. could all play a role in altered haemostasis.

The TEG is a well established device for measuring whole blood clotting times and has been thoroughly assessed for its role in the detection and management of coagulation problems [22 23 24 25 26 27 28 30 31]. We have previously shown that in vitro haemodilution *per se* enhances whole blood coagulation with both crystalloid (saline) and colloid (gelatin) [33]. It has also been stated that the TEG is more appropriate for detecting hypercoagulable states than conventional tests of blood coagulation, since the latter are designed to test for defects in the clotting system rather than increased coagulability [35]. It was appropriate, therefore to conduct an in vitro study into the effects of the synthetic colloids on coagulation profiles and comparing these with the established effects of saline.

We have been able to show that 20% haemodilution with gelatin, HES (HMW) and PES (LMW) caused statistically significantly less increase in MA (maximum amplitude) reflecting reduced clot strength, which is a function of the dynamic properties of fibrin and platelets, as compared with the 20% saline haemodilution control. This difference was greatest in the PES group. In addition, k-times, reflecting intrinsic clotting factor activity, platelet and fibrinogen activity, were significantly greater in the PES group. The α angles, reflecting speed of clot formation, were also significantly

less in the PES group. Both of these indices suggest less procoagulant effect in the PES group than that shown in the saline group

It would appear then that all the synthetic colloids studied had a lesser effect than saline on platelet and fibrinogen activity as measured by MA's, but that PES had a significantly smaller effect compared to saline on the speed of clot formation than did the other two colloids studied. These results are in agreement with our previous results where 20% saline and gelatin diluted samples were compared to undiluted controls from the same subject [33].

We can conclude, therefore, that haemodilution with synthetic colloids affects coagulation similarly to haemodilution with saline as far as initial clot generation is concerned. However, all three colloids had a significantly smaller effect than saline on MA probably reflecting some effect of the colloid on platelet aggregability. This may well be on the basis of their effect on fibronectin in developing clots as shown by Mardel *et al*[32]. The magnitude of this effect is greatest in the PES group.

We have previously demonstrated that haemodilution with saline produces hypercoagulability *in vitro*, and this present study indicates that haemodilution with colloids has a similar effect to haemodilution with saline. We suggest that an *in vivo* study needs to be performed using intravenous infusions of both saline and a colloid to demonstrate whether or not this effect occurs in intact human subjects.

CONCLUSION

We have demonstrated, using the TEG, that haemodilution can induce a procoagulant state *in vitro*. These results bear out findings by Tocantins [1] and Monkhouse [2] as well as the clinical study done by Janvrin in 1980 [3]. Our findings suggest that the mere addition of a diluent to blood somehow accelerates the process of platelet aggregation and fibrin strand formation as measured by the TEG. The mechanism by which this phenomenon takes place remains unclear. Monkhouse speculated on an imbalance between thrombin and anti-thrombin, the generation of thrombin being a function of a procoagulant state. Anti-thrombin, which combines with, and neutralizes, the thrombin, has a much longer plasma half-life and once used would take longer to regenerate, thus setting up an imbalance between the two. Quite why haemodilution should produce such a procoagulant state, however, is not explained. The clinical study by Janvrin [3] highlights this effect again by documenting an increase in post operative deep vein thrombosis (DVT) in patients who received peri-operative intravenous fluids. The TEG is a tool which provides a dynamic measurement of the intrinsic pathway of coagulation, but does not measure the extrinsic pathway, nor the initial platelet-endothelial interface. It is an established measuring tool in dynamic coagulation testing that has been extensively validated [22 23 24 25 26 27 28 30 31]. By following our methodology in performing the trials we describe, we have removed extraneous causes of a procoagulant state such as stress response, disease states, the effect of drugs, inadequate heparin reversal and laboratory errors.

If our findings have application *in vivo,* the ramifications may be potentially profound. One can speculate about possible implications this phenomenon might have in several clinical settings. For example, the management of stroke with haemodilution[36], which is based on hypervolaemic heamodilution to improve perfusion of relatively ischaemic areas of brain, may lead to further risk of clot formation and may be counter-productive if a procoagulant state should ensue as a result of the infusion of intravenous fluids. The risk of post-operative DVT may be increased following routine surgery where an intra-venous infusion was used to supply intra-vascular volume replacement peri-operatively. Indeed most patients undergoing routine surgery are given

intravenous fluids as a matter of course, without it necessarily being warranted or required. This is not only a costly practice, but also a potentially dangerous one according to our findings. In patients undergoing vascular surgery the infusion of large volumes of intra-venous fluid is commonplace, but this practice may indeed put the graft at risk of thrombosis, as well as putting the patient at risk of developing a DVT as discussed previously, and demonstrated by Janvrin[3]. In this country there is an ever-increasing load of trauma victims, and most of these patients receive large volumes of intravenous crystalloid. While many of these patients receive clear fluids well in excess of 40% of circulating blood volume and so have extensive dilution of their clotting factors, with the accompanying risk of bleeding, some however, have only minor injuries with minimal blood loss, yet still receive large amounts of intravascular fluid. These patients may well be at an increased risk of clotting peri-operatively, especially as they may have severe pain, inadequate analgesia and so lie motionless.

One may also speculate on the effect this phenomenon might have on the initiation or intensification of disseminated intravascular coagulopathy (DIC), which is characterised by uncontrolled activation of the coagulation system, with consumption of platelets and procoagulants. The causes of DIC are multiple, and are often associated with massive tissue damage and shock. Even though the clinical diagnosis of DIC relies on haemorrhage from wounds and sites of placement of intravascular catheters, the underlying pathology is initially hypercoagulability. One may speculate that this phenomenon might be modulated by the choice of intravenous fluid used for the treatment of shock, if our findings have relevance in the *in vivo* situation.

Our findings suggest that it would be important to set up an *in vivo* study, which should consist of two limbs. The first should be a saline haemodilution which should be compared to its own control, while the second should be a colloid haemodilution compared to its own control. It would appear from our results that Pentastarch (Haes-steril®) is the ideal colloid, because its *in vitro* effect on coagulation as measured by the TEG seems furthest removed from that of saline. One can postulate, therefore, that haemodilution with pentastarch might result in a lesser degree of procoagulant activity. Furthermore, any *in vivo* study should include standard parameters of

coagulation viz. INR, aPTT, Anti-thrombin III, platelet counts and full blood count, in addition to thrombelastography, as well as objective measures of platelet aggregation.

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ANNEXURES

ANNEXURE 1

Control vs Saline. Values designated 1 are control values, those designated 2 are Saline

No.	r1(mm)	k1(mm)	a1(°)	ma1(mm)	r2(mm)	k2(mm)	a2(°)	ma2(mm)
1	15	8	34	56	14	3	60	64
2	10	4	56	55	9	3	60	55
3	20	8	35	46	16	3	57	58
4	11	4	57	58	8	2	69	64
5	23	11	25	39	18	7	40	45
6	24	12	25	40	19	8	34	43
7	13	6	41	52	11	5	49	55
8	15	7	43	57	13	3	65	66
9	23	8	32	47	17	7	38	45
10	14	4	54	57	12	4	53	57
11	11	5	48	53	9	4	58	57
12	18	8	34	45	13	3	60	53
13	12	6	46	59	10	5	52	63
14	17	8	36	51	14	5	49	55
15	19	8	34	44	9	3	56	53
16	20	8	37	54	18	4	46	56
17	22	11	26	46	16	7	36	50
18	12	7	36	54	9	4	55	55
19	17	6	42	54	12	4	38	56
20	18	8	33	46	15	6	44	50
Means	16.70	7.35	38.70	50.65	13.10	4.50	50.95	55.00
Std Dev	4.43	2.25	9.61	6.05	3.48	1.70	10.13	6.36

ANNEXURE 2

Control vs Haemaccel. Values designated 1 are control values, those designated 2 are Haemaccel

No.	r1(mm)	k1(mm)	a1(°)	ma1(mm)	r2(mm)	k2(mm)	a2(°)	ma2(mm)
1	24	9	29	50	18	5	44	48
2	22	10	28	45	19	7	38	42
3	16	10	29	44	12	6	41	42
4	16	6	39	51	12	3	64	58
5	20	11	27	34	14	5	48	45
6	18	7	38	46	12	3	58	48
7	15	7	38	51	12	4	52	48
8	19	11	26	37	14	5	50	44
9	21	9	31	51	17	4	56	55
10	13	6	44	55	11	4	52	53
11	13	6	45	58	13	3	64	58
12	16	7	37	49	12	3	58	52
13	16	7	34	49	13	4	52	48
14	18	12	25	34	14	7	38	32
15	16	6	42	48	12	3	59	51
16	16	9	32	47	15	5	50	42
17	22	10	33	50	14	5	45	48
18	21	11	28	41	18	8	33	39
19	23	11	24	41	19	7	35	37
20	20	8	34	49	14	4	57	53
Means	18.25	8.65	33.15	46.50	14.25	4.75	49.70	47.15
Std Dev	3.26	2.03	6.35	6.42	2.57	1.55	9.30	6.88

ANNEXURE 3

Control vs Warm Plasmalyte-B dilution. Cont refers to control and plas to Plasmalyte B

No.	cont-r	cont-k	cont-a	cont-ma	plas-r	plas-k	plas-a	plas-ma
1	13	5.5	45	56	9	4	56	54
2	10	5	50	54	11	4.5	49	56
3	12.5	6.5	38	54	12	4.5	50	57
4	16	7	36	53	9	4	51	56
5	12	6	43	51	10	2	48	48
6	7	2.5	67	62	6.5	2	67	66
7	15	6.5	39	59	11	4	45	58
8	9.5	5	49	55	8.5	4	53	53
9	14.5	5.5	45	57	9.5	3	60	58
10	13.5	5.5	42	61	12	3	57	59
11	13	6.5	41	56	12	5.5	43	59
12	15.5	6.5	39	58	13	3.5	58	60
13	14	6	42	55	12.5	3.5	54	57
14	9	7	39	53	9.5	3.5	58	56
15	14	6.5	42	52	12	6	41	49
16	13.5	4.5	51	64	8.5	2.5	65	66
17	14	5	46	66	9.5	3	60	62
18	15.5	7.5	34	52	13	6	42	53
19	17	6.5	41	54	16	3.5	55	55
20	17.5	7.5	34	57	14	5.5	43	54
Means	13.30	5.93	43.15	56.45	10.93	3.88	52.75	56.80
Std Dev	2.72	1.17	7.39	4.14	2.26	1.19	7.61	4.63

ANNEXURE 4

Saline vs Gelatin. Values designated 1 are saline values, those designated 2 are Gelatin

No.	r1(mm)	k1(mm)	a1(°)	ma1(mm)	r2(mm)	k2(mm)	a2(°)	ma2(mm)
1	9	4	53	52	15	5.5	44	42
2	13	5.5	42	56	13	6	42	48
3	18	7.5	33	45	16	6.5	39	44
4	15	6	45	54	13	4	53	53
5	11	4.5	49	56	11	4.5	49	56
6	15	4.5	53	52	15	5	51	47
7	13	3	61	58	11.5	3	58	51
8	11	5	49	56	11.5	4	53	51
9	14.5	4.5	50	51	12	5	46	45
10	13	4.5	50	46	11	4.5	46	43
11	12	3	58	57	11	4.5	48	49
12	11	5	46	47	13	5	44	41
13	13	5.5	44	51	11	6	43	44
14	13	3	59	58	11	3.5	58	53
15	4	1.5	75	66	5	1.5	73	60
16	13	5	47	53	13	4	48	49
17	11.5	4	52	55	11	4.5	47	51
18	8.5	3	63	62	10	2.5	65	55
19	11	3	62	57	10	5.5	46	46
20	16	6	39	49	14	7.5	37	46
Means	12.28	4.40	51.50	54.05	11.90	4.63	49.50	48.70
Std Dev	2.99	1.39	9.58	5.22	2.35	1.39	8.70	5.06

ANNEXURE 5

Saline vs HES. Values designated 1 are control values, those designated 2 are HES

No.	r1(mm)	k1(mm)	a1(°)	ma1(mm)	r2(mm)	k2(mm)	a2(°)	ma2(mm)
1	13	4.5	48	52	15	4.5	46	48
2	16	5	46	54	16	3.5	50	51
3	17	6	43	51	17	4	48	51
4	15	5	46	51	13	4.5	47	46
5	16	5	47	52	16	5	46	45
6	18	5	45	57	22	5.5	40	53
7	12	4.5	52	56	13.5	6	40	44
8	19	4.5	52	58	20	4.5	48	54
9	12.5	4	49	52	12	4	49	52
10	17.5	5.5	45	50	15	5	46	49
11	13.5	5.5	41	50	16	7	38	42
12	16	7.5	33	44	15	7.5	36	41
13	15.5	5	48	59	15	3.5	55	52
14	15.5	4	52	56	15.5	5.5	44	46
15	9.5	3.5	57	57	12	5	46	46
16	16.5	5	45	47	14	5.5	43	43
17	18	7	38	49	14	9.5	29	38
18	12.5	3.5	58	64	13.5	4	48	53
19	11.5	4	55	53	11.5	5	46	44
20	14	6	46	57	14.5	4	54	52
Mean	14.93	5.00	47.30	53.45	15.03	5.15	44.95	47.50
Std Dev	2.53	1.05	6.11	4.63	2.54	1.47	6.07	4.65

ANNEXURE 6

Saline vs PES. Values designated 1 are control values, those designated 2 are PES

No	r1(mm)	k1(mm)	a1(°)	ma1(mm)	r2(mm)	k2(mm)	a2(°)	ma2(mm)
1	13	5.5	45	49	13.5	6.5	40	45
2	10.5	3.5	57	58	10.5	3	60	56
3	9.5	2.5	69	67	12.5	4	52	54
4	10.5	2.5	66	63	12.5	4	50	53
5	8.5	2.5	67	63	8.5	2.5	61	54
6	11	5	44	53	14	6.5	38	42
7	11	4	53	52	11.5	4	52	45
8	15.5	3	61	61	15.5	4.5	50	49
9	11	3	60	64	11.5	5	46	54
10	12.5	5	48	52	11.5	5.5	44	48
11	13.5	3	59	62	14	3	59	54
12	21.5	5.5	45	50	19.5	6.5	40	40
13	15.5	3.5	57	57	15	4	53	49
14	12.5	5	47	50	12.5	6	41	39
15	15	5	46	50	17	8.5	29	36
16	10.5	2.5	65	63	12	3	60	55
17	13	2.5	63	63	13.5	3	58	57
18	9	2	67	65	11	4.5	48	54
19	11	3	60	58	13	3.5	57	49
20	9.5	2.5	63	55	11	3	64	54
Mean	12.20	3.55	57.10	57.75	13.00	4.53	50.10	49.35
Std Dev	3.01	1.18	8.50	5.94	2.45	1.60	9.32	6.26