but only slit-lamp examination revealed a slight increase in stromal thickness. The corneas remained translucent and the pigment floating in the anterior chambers became visible.

In spite of agitation of the containers for periods up to 10 hours the eyes remained suspended in their glass bells, and the iris diaphragm and anterior lens surface did not come in contact with the corneal endothelium.

Conclusions

Temperatures between -2°C . and $+8^{\circ}\text{C}$. are recommended for the storage of red blood cells (Guest, 1932). Daniel and Droz (1960) reported that from $+3^{\circ}\text{C}$. to $+6^{\circ}\text{C}$. is the optimum range for the storage of bone-marrow and cultured cell strains.

Temperatures above $+10^{\circ}\text{C}$. reduce storage time, and temperatures below $+2^{\circ}\text{C}$. damage stored cells (Thistle, Gibbons, Cook, and Stewart, 1941).

There is as yet no evidence that corneal cells, and, in particular, the endothelial cells, should be kept at any other temperatures in short-term storage, and temperatures between $+2^{\circ}\text{C}$. and $+8^{\circ}\text{C}$. (with the optimum temperature of $+4^{\circ}\text{C}$.) are generally accepted for the storage of fresh donor eyes for periods up to three days (Rycroft, 1959; Peña-Carrillo and Polack, 1964).

Whereas in box A the specimen jars are completely surrounded by ice, in box B only a quarter of each specimen jar is in contact with the ice, the remaining three-quarters being enclosed by the insulating material. While the storage temperature of eyes in box A (and similar containers, such as a Dewar flask) will be very near the ice temperature and always below $+1^{\circ}\text{C}$., that of the eyes in box B will, for all practical purposes, remain within the accepted range of temperatures.

The temperature of ice taken from domestic refrigerators varies, and the assumption that ice of a lower initial temperature will prolong the storage time is not only erroneous but dangerous, as cooling of the eyes to temperatures far below 0°C . will take place.

The storage time for box A is short; its mechanical strength and watertightness are both good, but really irrelevant. Foamed polystyrene is a good insulating material and in the thickness

chosen for box B guarantees not only the necessary storage time but also mechanical strength. The weight and transportation cost of box B are low, and its price, about 6s. (box A costs about £30), has the particular advantage that it can be treated as "disposable" if the eyes are being sent to distant places.

Summary

A container for transporting donor eyes, made of foamed polystyrene, is described; and its performance compared with that of a metal box in current use.

The new polystyrene container maintains an interior temperature of between $+2.6^{\circ}\text{C}$. and $+6^{\circ}\text{C}$. for periods up to five days, whereas the metal container of earlier design is found to maintain the stored eyes below the accepted storage temperatures of between $+1^{\circ}\text{C}$. and -1.6°C ., and to retain a satisfactory above-zero temperature for insufficient periods.

A glass bell is described that will secure the eye within its jar and effectively prevent damage to the cornea during transport.

We are grateful for the co-operation of John Weiss and Son, London, who manufactured the glass bells. We would like to thank Baxter Laboratories Ltd., High Wycombe, for supplying the latex-rubber caps. We are indebted to Jablo Group Sales Ltd., of Croydon, who advised us on the design of the box and supplied us with the many containers tested during the experiments. We also thank Dr. P. Hansell and his medical illustration department at Westminster Hospital Medical School for the illustrations, and both the secretary and nursing staff of the ophthalmic department for their help and co-operation. Finally, we are grateful to the Medical Research Council for personal grants to two of us (F. O. M. and P. O'N.) towards this research.

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Oral Contraceptive Hormones and Blood Coagulability

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Thrombophlebitis often occurs in women during pregnancy and the puerperium. During these periods there is a rise in the concentration of several different coagulation factors (Koller *et al.*, 1952; Alexander *et al.*, 1954). Oral contraceptive hormones simulate pregnancy to some extent and their administration has been associated with reports of the occurrence of thromboembolic episodes (Jordan, 1961; Zilkha, 1964). Egeberg and Owren (1963) suggested that one preparation, Enavid, caused a shortening of the cephalin time, a rise in antihæmophilic globulin (factor VIII), and a slight but significant increase in factor VII levels during the first and second weeks of therapy.

In the present investigation we have studied a large group of normal women taking a variety of oral contraceptive prepara-

tions and have compared the results with those of a parallel group of normal female controls. In addition, we have studied the changes in coagulability which occurred in normal young females during moderate and severe physical exertion previously shown to shorten clotting-times and raise factor VIII levels in normal adults (Schneider and Zangari, 1951; Rizza, 1961). We also thought it important to include in the study the results obtained in patients with recent deep-vein thrombosis who were not on oral contraceptives and who presented during the period of this trial of oral contraceptives. This was to determine whether any changes in the treated women were similar to those which have been shown to occur in thrombosis (Poller, 1957). They were tested using the same technique and reagents.

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Method of Study

Where possible the women were tested before the start of their course of oral contraception and were asked to attend at monthly intervals for three months, then at three-monthly intervals. Where patients were already on treatment they were included in the study and the duration of their course was noted. Studies were performed on 111 occasions in 40 patients who were receiving the following preparations: Enavid (Conovid) 17, Anovlar 7, Ovulen (ethynodiol) 9, Gynovlar 3, and Volidan (megestrol) 4. The following tests were performed at each visit: prothrombin activity (Quick), cephalin time, factor VII assay, and heparin plasma clotting-time.

Technique

Prothrombin Activity.—The Quick technique (Poller, 1962, modified). A saline extract of human brain was used as a source of thromboplastin. This was phenolized for storage at 4° C. The results are expressed as a percentage of prothrombin activity from a saline dilution curve.

Heparin Plasma Clotting-time (Poller, 1954, modified).—Sodium citrate 3.8% was used in place of oxalate; blood was spun for five minutes at 3,000 r.p.m.; and the strength of the heparin was adjusted to give shorter control times.

Factor VII Assay.—Plasma from patients receiving nicoumalone (Sinthrome) anticoagulant was used as a source of factor-VII-deficient plasma. Blood was obtained from patients whose prothrombin activity within the first 48 hours of treatment was less than 10% but whose thromboplastin generation test was

During the first part of the trial thromboplastin generation tests were performed on 56 occasions. As the results did not reveal any significant changes specific factor VIII (A.H.G.) assays were performed on later patients. Factor VII activity assays and heparin clotting-times were performed because they had previously been shown to be accelerated in patients with thrombosis (Poller, 1954, 1957).

Normal females of a similar age group were studied at each visit in parallel with a batch of patients. The group undergoing physical exertion consisted of 12 healthy females, six of whom undertook moderate exercise (10 minutes' continuous physical training) and six others who undertook more violent exercise, including 10 minutes' energetic netball, followed by a half-mile (800 metres) road race. Tests were performed in the exercise group before and after exertion. As any changes might have been due to simple haemodilution following exercise, haemoglobin levels and packed cell volumes were determined before and after exertion.

Thrombotic Group.—This group consisted of 10 patients with deep-vein thrombosis tested within 24 hours of clinical onset of the condition. Prothrombin activity, factor VII assays, heparin clotting-times, and cephalin times were performed in this group. The results were compared with normal controls tested in parallel. The patients consisted of nine females and one male whose average age was 46 years.

Results

The results of the group on oral contraceptives are given in Table I.

TABLE I.—Results in Women on Oral Contraceptives and Normal Female Controls

Group	Prothrombin Activity %	Factor VII Assay 20% Dilution (sec.)		Cephalin Time (sec.)		Heparin Plasma Clotting-time (sec.)		A.H.G. Assay (Time sec.)					
								10%		20%		30%	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Treated	100	18.5	5.5	62.7	5.3	7.1	2.6	18.1	3.1	15.0	2.4	14.6	1.6
Control	100	18.9	4.4	63.4	4.7	6.9	2.0	18.5	2.7	15.0	1.8	14.5	0.3

normal. The deficiency was corrected by stored normal serum. Factor VII activity was assessed by the ability of the test plasma to restore the one-stage prothrombin time of the deficient plasma compared with control.

A.H.G. Assay (Biggs and Macfarlane, 1957, modified).—Fresh citrated plasma was obtained from a severe case of haemophilia. This was freeze-dried in small volumes within two hours of collection. A.H.G.-deficient plasma was mixed with the patient's plasma to give final dilutions of 10%, 20%, and 30% immediately before testing. This was then diluted 1:5 with saline and the next with equal volumes of 1:10 normal serum. Platelet substitute (Bell and Alton, 1954) and M/40 calcium were used. The technique was that of a standard thromboplastin generation test. Statistical analysis was carried out on the results in seconds.

Cephalin Time (Hjort *et al.*, 1955, modified).—The only modification of the method was that the cephalin was dispensed into small volumes and freeze-dried so that the same reagent might be used throughout the investigation.

Thromboplastin Generation Test Method (Biggs and Douglas, 1953).—With platelet substitute (Bell and Alton, 1954). All tests were performed as soon as possible after collection.

The cephalin time particularly was found to be very sensitive to contact. If the tests were done within two to three hours of collection no marked deterioration was found. All specimens were collected with plastic syringes and stored in plastic containers. Blood samples were spun in plastic tubes at 3,000 r.p.m. for five minutes.

Prothrombin Activity.—The results of the Quick prothrombin activity showed no significant difference in the treated group from the controls.

Cephalin Times.—There was no significant difference between the total cephalin times of the treated and control groups. When these were analysed separately in chronological order, according to the duration of the course, there was no significant difference at any time from the normal controls. The mean duration of those whose course exceeded 12 months was 16 months.

Thromboplastin Generation Test.—There was no significance in the rate or concentration generated in the thromboplastin generation test in the treated group or controls.

Factor VII Assay (see Table II).—The results in the treated group showed a slight increase of factor VII; this was not significant as totals, but when analysed chronologically there was a significant increase in factor VII levels from the third month.

Factor VIII Assay.—There was no significant difference in antihaemophilic globulin (factor VIII) levels in the two groups.

Heparin Plasma Clotting-times.—These showed no significant difference in the two groups.

TABLE II.—Factor VII Assays During Course of Therapy

Before Treatment		1 Month		2 Months		3 Months		3-6 Months		6-12 Months	
Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
20.1	2.23	18.9	1.78	19.8	1.4	18.4	1.58	18.5	2.2	17.8	1.34

Results in Exercise Groups

The results of this part of the study are given in Table III. Both moderate and severe exercise resulted in significant shortening of cephalin times and rise in A.H.G. levels (factor VIII). The effect was enhanced by more strenuous exercise in the second group. There was no significant change in factor

of the known clinical association of such raised levels in established thrombosis and states that predispose to it. It will be important to determine in future investigation whether all the preparations available are equally liable to produce this side-effect.

Egeberg and Owren (1963) reported shortening of the cephalin time in five women taking Enavid tested in the first

TABLE III.—Results in Women Before and After Exercise

				No.	Haemoglobin g./100 ml.		P.C.V. %		Cephalin Time (sec.)		Factor VII Assay 20% Dilution (Sec.)		A.G.H. Assay (Time sec.)					
					Mean S.D.		Mean S.D.		Mean S.D.		Mean S.D.		10%		20%		30%	
													Mean	S.D.	Mean	S.D.	Mean	S.D.
Group I. Moderate Exercise																		
Before	..	..	6	14.0	0.2	43.0	0.24	68.3	1.1	19.7	0.87	17	1.1	14.5	0.5	13.4	0.6	
After	..	..	6	14.0	0.2	46.6	1.53	64	1.9	19.1	0.7	14.1	0.9	13.0	0.4	11.8	0.4	
Group II.—Severe Exercise																		
Before	..	..	6	13.6	1.1	41.4	2.3	66.5	2.4	17.7	1.2	19.5	0.7	17.1	0.9	15.0	0.8	
After	..	..	6	13.9	1.0	41.8	1.8	60	2.2	18.0	1.2	15.7	1.2	14.1	1.2	12.0	0.9	

VII levels. The haemoglobin and packed-cell-volume results indicated that the changes after exercise were not due simply to haemoconcentration.

Results in Thrombotic Group

The dramatic finding in this group (see Table IV) is the shortening of heparin clotting-times, which confirm previous

TABLE IV.—Results in Patients with Deep-vein Thrombosis and Parallel Controls

	Cephalin Time (sec.)		Factor VII Assay 20% Dilution (sec.)		Heparin Plasma Clotting-time (sec.)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Patients	59.7	10.0	17.4	1.94	4.0	1.1
Controls	61.5	5.0	19.4	1.5	7.7	2.2

observations. This was significant when compared with parallel controls, the women on oral contraceptives, and also their parallel controls. Factor VII levels were also significantly increased in this group compared with parallel controls, confirming previous findings (Poller, 1957).

Comment

The important finding in this study is the presence of significantly increased levels of factor VII detected from the third month of therapy, although the level had increased as early as the first month.

Increases of factor VII activity have been reported in normal pregnancy and the puerperium (Koller *et al.*, 1962; Ciulla and Santoni, 1954) and after thrombosis (Poller, 1957). Egeberg and Owren (1963) reported slight but significant increases of factor VII in their five treated women on Enavid followed for one month. Using our own reagents and technique, we have found increased factor VII levels during normal pregnancy and the puerperium (unpublished data). The results of the concurrent study on deep-vein thrombosis also show a marked increase in factor VII levels, although these appear to be even higher than in the oral contraceptive group. The precise role of factor VII in relation to intravascular clotting and thrombosis remains to be established. It is by no means certain that increased factor VII levels predispose to thrombosis, as they may be a secondary effect in patients with established intravascular clotting and result from the release of serum products into the circulation. Factor VII is concerned in the extrinsic (tissue) clotting system, but is generally regarded as having a role in normal haemostasis and thrombosis.

The presence, after a period of oral contraception, of raised levels of factor VII seems an undesirable side-effect in view

month of their course. We have been unable to confirm this in a larger group of women tested at this period nor were we able to demonstrate any significant shortening in subsequent months (see Chart). The results were compared with the women's previous levels before the start of their course and with a group of parallel controls tested with the same technique and reagents. In contrast we have been able to demonstrate marked shortening of the cephalin time after exercise in healthy controls, confirming the observations of Egeberg (1963). This indicates that the technique employed was reasonably sensitive to physiological changes and therefore might be expected to be adequately sensitive for the detection of pathological changes. In a separate study we have detected, with the same technique, shortened cephalin times after the abrupt withdrawal of oral anticoagulants (Poller and Thomson, 1964). No woman in our study had been on oral contraceptives for more than two and a half years, but there was no indication of a trend towards shortened cephalin times with increased period of administration (see Chart). There has previously been no evidence to

Mean cephalin times in treated group (111 subjects) plotted against months of course. The shaded area represents one standard deviation from the mean in the control group of 67 subjects.

indicate that shortened cephalin times should be expected in thrombosis, and our findings in the thrombotic group do not suggest that shortened cephalin times are a constant finding in thrombosis.

Egeberg and Owren (1963) found increased A.H.G. (factor VIII) levels in five women followed for one menstrual cycle on Enavid. We did not find any evidence of a rise of A.H.G. (factor VIII) at this stage or at any of the subsequent months of their course. The normal female controls who undertook exercise showed a dramatic rise in factor VIII, suggesting that the method used was sensitive enough to measure pathological changes. This is confirmed by the fact that in two recent studies using the same technique we have detected increased levels of factor VIII (A.H.G.) in patients who have been treated on a long-term basis with coumarin anticoagulant (Poller and Thomson, 1964, 1965).

It is of interest that physical exertion in normal females produced marked shortening of the cephalin time and a raised

factor VIII level—in other words, acceleration of the intrinsic clotting system—but no changes were detected in the extrinsic system involving factor VII. This is the reverse of the finding in the women on oral contraceptives and the patients with established deep-vein thrombosis.

Heparin plasma clotting-times showed a dramatic shortening in the patients with deep-vein thrombosis, confirming our previous studies. This showed no acceleration, however, after a course of oral contraception, and there was no tendency to shortened clotting-times with increased periods of administration. This is an important point of difference from the thrombotic patients, who, in addition to raised factor VII levels, showed markedly reduced heparin clotting-times.

Summary

A study has been made on the effects of oral contraception on blood coagulability in women being treated with a variety of commercial preparations. The results were compared with those in a group of normal female controls. The results were also contrasted with the effects of exercise on coagulability in normal women using the same techniques and with results in patients with deep-vein thrombosis who presented during the period of the trial.

We found that oral contraceptives caused a rise in factor VII levels significant from the third month onwards. No significant changes in cephalin times or A.H.G. levels were recorded in this group, unlike the physiological changes found after physical exercise in normal women. Increases in factor VII levels were detected also in a concurrent study of patients with deep-vein thrombosis, but these were also associated with shortened heparin clotting-times not found in the women on oral contra-

ceptives. It is considered that this increase in factor VII levels, which is also found in normal pregnancy and the post-partum state as well as in venous thrombosis, is an undesirable side-effect of oral contraception. No woman in this study had been on treatment for more than two and a half years, and further study is necessary to determine whether different changes occur at a later date. It will be important to determine also in future investigations whether all the preparations available are equally liable to produce raised factor VII levels.

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The Plastibell Technique for Circumcision

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We wish to draw attention to a method of circumcision introduced in the United States by Kariher and Smith (1956), but little known in other countries. The technique has many advantages over other procedures, and in our view is the method of choice in the newborn period. Without wishing to enter into the arguments for or against routine circumcision, some parents demand to have the operation performed, and refusal to do so merely results in transferring the onus elsewhere. This report is based on our combined experience with the device in over 150 circumcisions in the past four years.

Patients and Methods

All the operations were performed at the parents' request, mostly six to eight days after birth, but in some as late as at 14 weeks of age. The technique may be used immediately after delivery, as is fashionable in the United States, but it is probably better to wait until it is evident that the baby is making normal progress. Premedication is unnecessary, but newborn infants who have not already received prophylactic vitamin K at birth are given 1 mg. intramuscularly. Normal

feeds are allowed before the operation, but the period immediately after a feed is avoided to lessen the possibility of regurgitation.

The sterile setting consists of two fine artery forceps, a probe, fine-toothed dissecting forceps, a pair of scissors, a towel, and swabs. The Plastibell¹ (Fig. 1) and ligature, pre-sterilized in individual envelopes, is made in three sizes: small (11 mm.), regular (13 mm.), and large (15 mm.), but the 13-mm. bell is suitable in most cases.

Anaesthesia is unnecessary, and the baby is readily restrained by an assistant or by bandaging on to a firm pillow. The lower abdomen, penis, and perineum are cleaned with an antiseptic solution, such as 1% cetrimide or 0.2% chlorhexidine (surgical spirit may cause smarting and should be avoided), and draped with an O towel.

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¹ The Plastibell disposable circumcision device (with illustrated instructions) is made by Hollister Inc., 833 North Orleans Street, Chicago 10, Illinois, and distributed in the U.K. by Chas. F. Thackray Ltd., 38 Welbeck Street, London W.1.