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The Use of Factor IX Concentrates in Man: a 9-Year Experience of Scottish Concentrates in the South-East of Scotland

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Summary. During the last 9 years, two factor IX concentrates produced in Scotland, PPSB and DEFIX, have been used for the treatment of haemophilia B and acquired coagulation disorders, including those due to liver disease, coumarin therapy, neonatal immaturity and post-operative bleeding. During the period of study 112 batches of DEFIX and 40 batches of PPSB were used in the Edinburgh and South-East Scotland Region. Data were analysed from 575 non-haemophilic patients, receiving 968 treatment episodes, as well as 24 haemophiliacs. Serial coagulation studies and analysis of retrospective data showed that both concentrates corrected the coagulation deficiencies in all the above patient groups; in no case was there any evidence of intravascular coagulation resulting from concentrate infusion.

Human factor IX concentrates have been available for clinical use for more than 20 years. Although originally produced for the management of haemophilia B patients there has been an increasing tendency to use them in certain acquired coagulation disorders such as in the neonate (Waltl *et al*, 1973), acute reversal of oral anticoagulant therapy (Tullis *et al*, 1965) and in liver disease (Menaché *et al*, 1959). These developments, plus the introduction of home therapy for the haemophilia B patient, have resulted in at least a 3–5-fold increase in the use of factor IX concentrates in many parts of the world over the last 10 years.

In the early 1970s several reports of thromboembolic side-effects associated with specific batches of concentrates from certain sources were published (reviewed by Kasper, 1975). Some reports indicated that patients with liver disease were likely to be particularly at risk (see Kasper, 1975; Gazzard *et al*, 1974). As a consequence efforts were made to develop *in vitro* assays and animal test models which sought to define the cause(s) of this clinical phenomenon and screen out those batches which were potentially harmful (Kingdon *et al*, 1975; Sas *et al*, 1975; Cash *et al*, 1975; Hedner *et al*, 1976, 1979; Giles *et al*, 1980).

Factor IX concentrates are produced in Scotland by the Scottish National Blood Transfusion Service from plasma collected at the five regional centres and fractionated at the Protein Fractionation Centre (PFC), Edinburgh. A factor II, VII, IX and X concentrate (PPSB: Blatrix

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& Soulier, 1959) was introduced in 1968. This material is prepared from blood collected into EDTA anticoagulant. The separated red cells cannot be used for routine transfusion, nor can factor VIII be prepared from EDTA plasma. A more economical method of preparation of factor IX concentrate, utilizing ACD or CPD plasma, was introduced in 1971. It is a concentrate of factors II, IX and X in which factor VII is present in only trace quantities (DEFIX, Middleton *et al*, 1973). Both types of concentrate, PPSB and DEFIX, have continued to be prepared as it was assumed that some non-haemophilia B patients might require factor VII in addition to II, IX and X.

Although these Scottish products have been extensively characterized *in vitro* (Middleton *et al*, 1973; Sas *et al*, 1975; Pepper *et al*, 1977; Prowse *et al*, 1977) and in animals (Cash *et al*, 1975, 1978; Prowse & Williams, 1979), little data have been published on their effects in man. This report reviews the clinical experience of the last 9 years in one region of Scotland, during which period over one million units of factor IX, in the form of concentrates (either PPSB or DEFIX), exclusively derived from the Scottish Protein Fractionation Centre, have been used clinically. The studies reported include retrospective analysis and prospective serial investigations, designed to detect laboratory evidence of *in vivo* activation of the coagulation mechanism.

MATERIALS AND METHODS

Concentrates

PPSB was produced from blood collected in EDTA anticoagulant. The concentrate was made by adsorption of plasma with calcium phosphate with subsequent elution of the coagulation factors (Blatrix & Soulier, 1959). The final product was dispensed in vials containing a nominal 200 units of factors II, VII, IX and X, and 100 u of heparin (Pularin: Duncan, Flockhart and Co. Ltd, London), as a freeze-dried powder.

DEFIX was made by DEAE–cellulose ion-exchange fractionation of cryo-supernatant plasma derived from blood collected in ACD or CPD anticoagulant (Middleton *et al*, 1973). It was supplied as a freeze-dried powder in vials containing a nominal 300 u of factors II, IX and X. It contained no heparin and negligible amounts (less than 5 u) of factor VII.

In addition to the assay of individual coagulation factors and standard tests for sterility, acute toxicity and pyrogenicity (European Pharmacopoeia, 1971) both types of concentrate were subjected to *in vitro* tests for potential thrombogenicity. Prior to 1975 these included the plasma recalcification time and fibrinogen clotting time tests (Middleton *et al*, 1973) and in addition, after 1975, NAPTT test (Kingdon *et al*, 1975) and TGt50 test (Sas *et al*, 1975). Concentrates issued for clinical use did not shorten the recalcification time, contained little, if any, detectable thrombin and passed arbitrary standards for NAPTT and TG50 tests. Since 1977 these have been > 150 s (buffer blank 200–300 s) and > 10 min respectively.

Vial contents were reconstituted in 10 ml of sterile distilled water. Immediately after reconstitution, doses were given by intravenous injection over a period of 10 min.

Patient Groups

For the purpose of analysis, patients were considered in five clinical groups: those with haemophilia B, those with liver disease, those treated for the reversal of coumarin therapy,

those treated during the neonatal period and a miscellaneous group which included patients with post-operative bleeding, renal disease, myeloproliferative disorders and disseminated intravascular coagulation.

In addition to grouping based on clinical diagnosis further analyses were based on patients with laboratory information available prior to infusion, patients with laboratory data pre and post infusion and patients in whom prospective serial studies had been performed. This latter group consisted of 23 patients with liver disease, 20 patients with coumarin 'overdose' and two patients with haemophilia B. All had given informed consent. In a miscellaneous group (see Fig 4) informed consent was not obtained: the serial samples were judged necessary for the continued acute management.

Methods

Thrombin time, prothrombin time ratio (PTR) and kaolin-activated partial thromboplastin time (PTT) were determined by established methods (Hardisty & Ingram, 1965). Platelet count was determined electronically (Coulter Thrombocounter). Fibrinogen was assayed as described by Ellis & Stransky (1961). Fibrin degradation products (FDP) were initially determined by a haemagglutination method (Hoq & Das, 1971) and subsequently (1974) using a latex agglutination technique (Thrombo-Wellcotest, Wellcome Reagents Ltd, U.K.). The ethanol gelation test (EGT) was carried out as described by Kierulf & Godal (1971) and plasma antithrombin determined by the method of Abildgaard *et al* (1970). Beta-thromboglobulin (βTG) was determined by radioimmunoassay (Bolton *et al*, 1976). Whole blood clotting time (WBCT) was determined in siliconized glass tubes at 37°C.

Individual coagulation factors were all assayed by one-stage methods essentially as described by Hardisty & Ingram (1965) using pooled normal plasma from 24 donors as a standard. Factors VII, VIII and IX were assayed using congenitally-deficient plasmas as substrate, whereas factor II, V and X assays used artificially-prepared deficient plasmas. For the factor II assay Tiger snake (*Notechis scutatus*) venom was used as the activator (Jobin & Esnouf, 1966).

All assays were carried out within 1 h of venepuncture except those of individual coagulation factors, antithrombin III, βTG and those FDP samples assayed by the haemagglutination method which were performed on samples frozen as soon as possible after collection.

Statistics

Results are expressed as the mean and standard error of the mean. The significance of differences between pre-infusion and later assay results was assessed using the paired Student *t*-test.

RESULTS

General Observations

Approximately one million units of factor IX, in the form of concentrates, have been used in the South-East of Scotland (population 1·2 million) over the last 9 years (Table I). Almost 75% has been given in the form of II, IX and X concentrate (DEFIX), and of this approximately 90% has been used in the management of haemophilia B. In contrast, II, VII, IX and X concentrate (PPSB) has been preferred for the majority of the acquired coagulation disorders,

TABLE I. Use of factor IX concentrates

	Haemophilia B	*Liver disease*	*Coumarin reversal*	*Neonates*	*Other*	*Total*
DEFIX						
Vials	2762	94	112	18	77	3063
Different batches	110	26	29	7	25	112
Patients	24	29	39	11	32	135
PPSB						
Vials	20	307*	428*	120*	298*	1173*
Different batches	4	35	34	18	36	40
Patients	4	98	176	75	115	468

These figures show the amount of DEFIX and PPSB issued between January 1971 and January 1980. Two severely deficient patients were responsible for the majority of concentrate use by haemophiliacs. DEFIX and PPSB vials contained 300 and 200 u of factor IX respectively.

* Some PPSB batches were issued in vials one-fifth the normal size (i.e. 40 u factor IX) for use in neonates and young children.

largely those associated with coumarin therapy and liver disease. 55% of the PPSB used in neonates was for a clinical trial which will be reported elsewhere (Turner *et al*, 1981). During the period under review 112 different batches of DEFIX and 40 batches of PPSB have been administered. In all there were 968 separate patient exposures in 575 different non-haemophilic patients. 24 patients with haemophilia B were also treated.

The concentrates used were all screened for the presence of potentially thrombogenic material using *in vitro* tests. The range of results obtained are shown in Table II.

TABLE II. *In vitro* activities of concentrates used clinically

Concentrate	*NAPTT (s)*	*TGt50 (min)*	*Recalcification time (s)*	*Thrombin (u/ml)*
DEFIX				
Range	127–204	10–>30	101–>300	<0·001*
Batches tested	58	75	112	112
PPSB				
Range	133–315	19–>30	103–>300	<0·001
Batches tested	22	26	40	40
Buffer	191–326	—	100–232	—

The table contains fewer results for TGt_{50} and NAPTT tests since these were not introduced until 1975. In the NAPTT and recalcification tests various dilutions of concentrate were used; the shortest clotting times found were used to characterize each batch. Results for individual batches may be obtained on request.

* Except for three early batches which contained up to 0·1 u/ml.

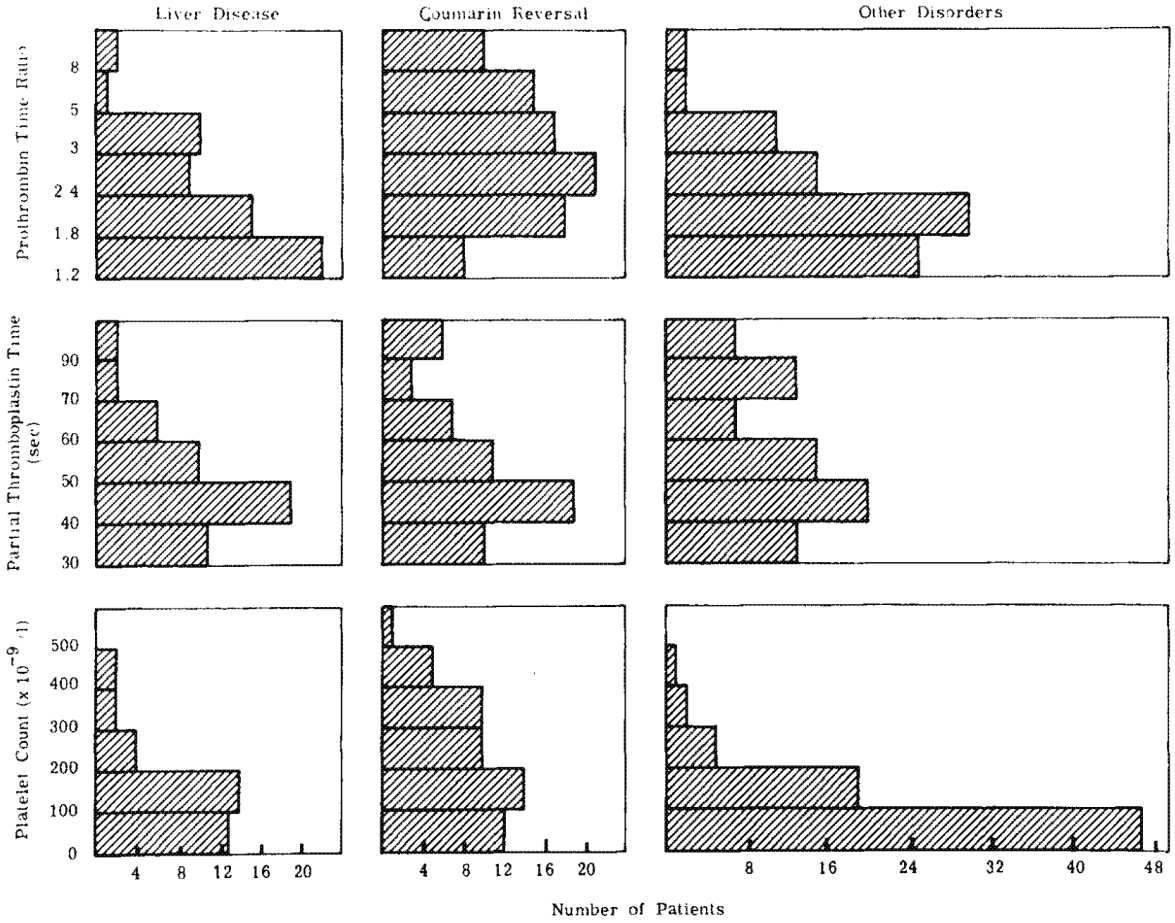


FIG. 1. Coagulation profiles before factor IX concentrate infusion. Prothrombin time ratio, partial thromboplastin time and platelet count prior to infusion of factor IX concentrate in patients with liver disease, patients receiving treatment to reverse coumarin effects and a miscellaneous group of patients with other disorders. Normal values in this laboratory are 1·4 or less for PTR, 30–40 s for PTT and 150–400 × 10^9/l for the platelet count.

Retrospective Studies

Fig 1 summarizes the results (prothrombin time ratio, partial thromboplastin time and platelet count) of those non-haemophilia B patients in whom laboratory results were available prior to the infusion of a factor IX concentrate. The data revealed that, of the patients studied during the 9 year period under review, those with coumarin 'overdose' had the most severe prolongation of the prothrombin time ratio. Patients in the liver and miscellaneous groups had, on the other hand, a more marked disturbance of the platelet count. The partial thromboplastin times were prolonged in all three of these groups, but most severely in the miscellaneous group.

Table III summarizes some of the retrospective laboratory data available in terms of pre and post (less than 2 h) infusion coagulation profiles on the non-haemophilia B patients. There is evidence that both PPSB and DEFIX have some effect, in the doses used, in correcting the prothrombin time ratio. This correction was, however, statistically significant only for PPSB:

TABLE III. Effects of concentrate infusion (retrospective study)

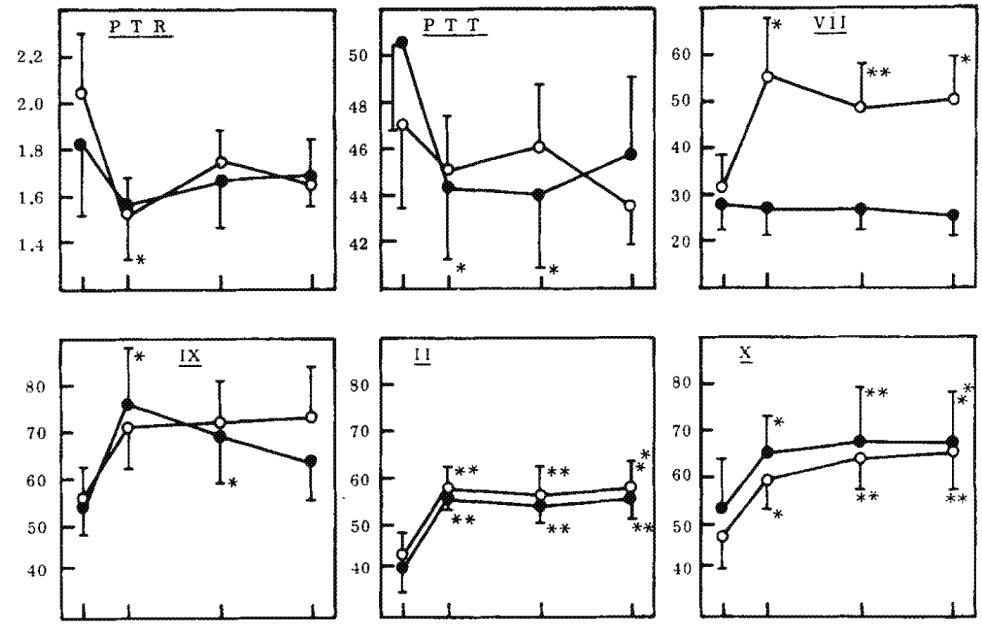
Disorder	Concentrate	Dose (vials)	Prothrombin ratio		PTT (s)		No. of patients
			Pre	Post	Pre	Post	
Liver disease	PPSB	1	2·3±0·3	1·9±0·1*	47±4	43±2	9
		2	2·1±0·2	1·4±0·1**	45±2	42±1	16
		3	3·9±1·2	1·9±0·1*	57±6	46±3*	12
	DEFIX	1	2·7±1·4	2·6±1·5	59±1	53±2	2
		2	1·9±0·3	1·5±0·2	53±3	45±3*	6
		3	1·6±0·1	1·4±0·1	38±8	36±6	3
Coumarin reversal	PPSB	1	3·1±0·5	1·9±0·2**	49±5	39±2	17
		2	5·7±1·0	1·8±0·1**	69±10	42±2*	24
		3	5·2±0·9	2·2±0·6**	45±4	37±4	7
	DEFIX	1	2·9±0·2	2·6±0·1	52±3	44±3*	5
		2	6·2±1·0	3·9±1·4	65±8	52±4	2
		3	11·4±6·8	3·9±1·0	105±58	49±13	4
Other disorders	PPSB	1	2·3±0·2	1·6±0·1**	60±5	46±2*	34
		2	3·0±0·4	1·6±0·1**	62±5	45±3*	12
		3	2·6±0·4	1·7±0·3	71±4	50±3*	2
	DEFIX	1	2·3±0·3	1·7±0·2**	53±5	45±3	7
		2	1·7±0	1·4±0·2	67±29	37±2	2
		3	3·2	1·3	58	47	1

Platelet count, fibrinogen, fibrin degradation products and ethanol gelation tests were also carried out, but did not change significantly in any group. Significance of changes is shown as $*P<0·05$, $**P<0·01$.

a feature which may be due in part to the low numbers of the DEFIX group and its lack of factor VII. Both concentrates corrected the partial thromboplastin time, but neither resulted in a significant change in the platelet count, fibrinogen or serum fibrin/fibrinogen degradation products (data not shown, but available from the authors on request). Three patients gave a positive ethanol gelation test (which was previously negative), but in all three major surgery took place, in addition to the administration of factor IX concentrate, between the pre and post infusion coagulation profiles.

All infusions were performed under the supervision of haematologists and/or medical staff of the Blood Transfusion Service: no clinical evidence of local or systemic thromboembolic

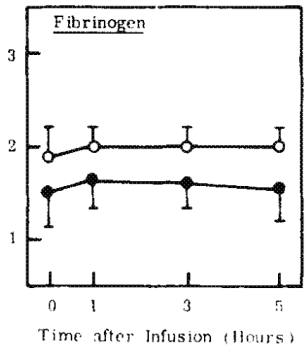
FIG. 2. (a) and (b) Serial coagulation studies in liver disease. Mean (and SEM) results are shown for 16 patients receiving PPSB (○, mean dose 440 u factor IX) and seven patients receiving DEFIX (●, mean dose 690 u factor IX). Assay of PTR, PTT (s), factors II, V, VII, VIII, IX and X (% of normal plasma), fibrinogen (g/l), platelet count ($\times 10^{-9}$/l), FDP (mg/l), thrombin time (s) and β-thromboglobulin (ng/ml) were performed before infusion and 1, 3 and 5 h after infusion. EGT results show the number of positive tests at each time. *In vitro* properties of the concentrates used are also shown. βTG was assayed in only six of the patients receiving DEFIX and four of those receiving PPSB. Factors V and VIII were assayed in all patients receiving DEFIX but only two receiving PPSB. Significance of changes relative to pre-infusion values: $*P<0·05$, $**P<0·01$.

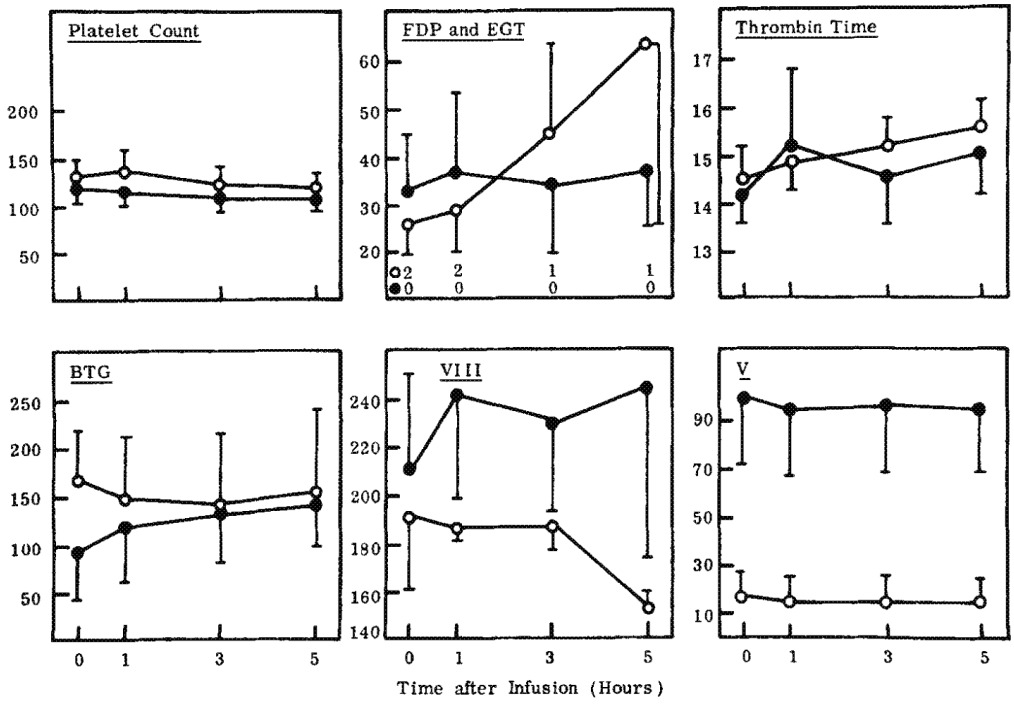


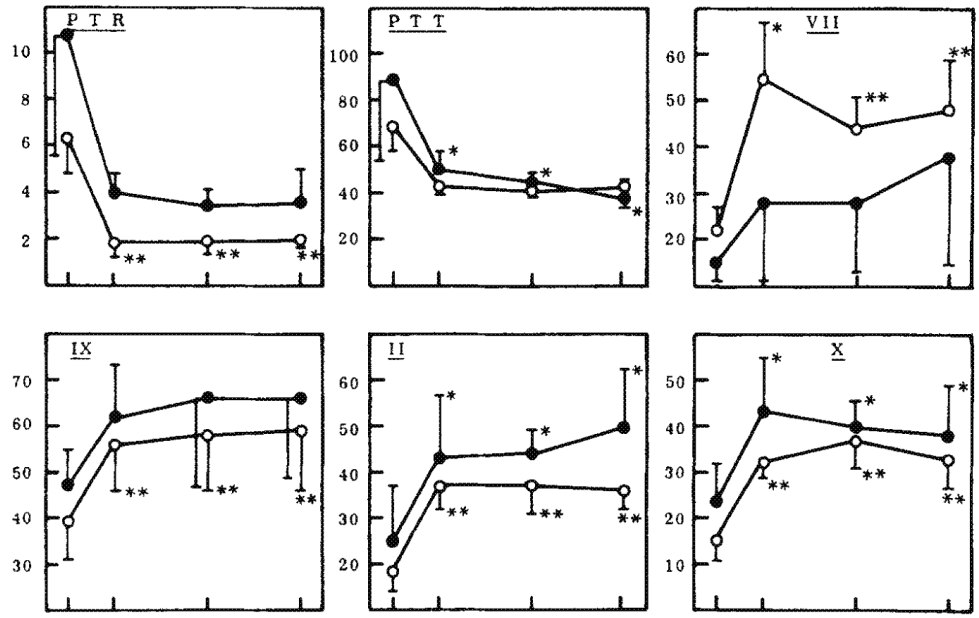
IN VITRO ASSAYS

Concentrate	NAPTT (sec)	TGt_{50} (min)	Recalcification Time (sec)	Thrombin (u/ml)
DEFIX (3 batches)	157-204	16-24	141-165	⩽ 0.001
PPSB (7batches)	180-278†	19->30†	> 300	⩽ 0.001

(†: results not available on all batches)



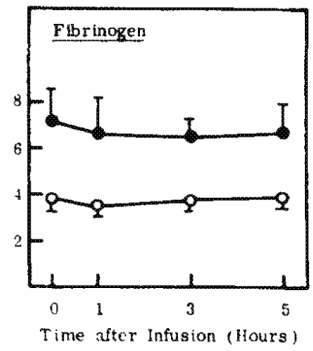


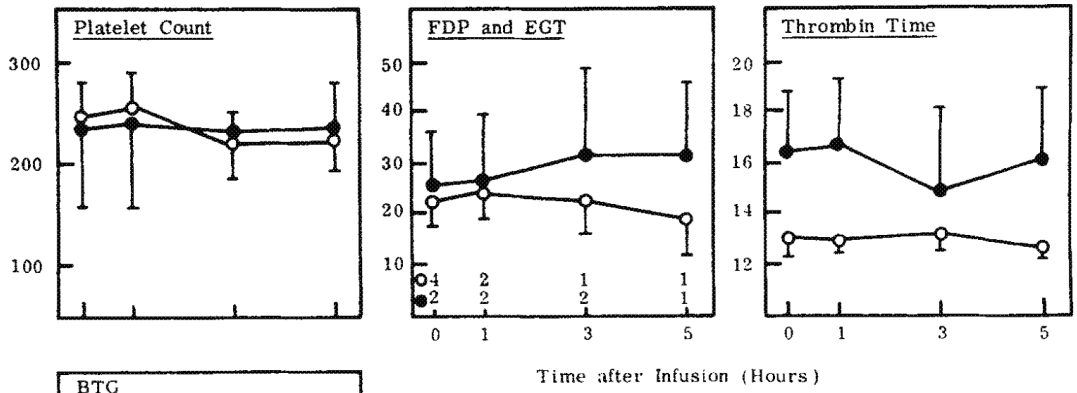


IN VITRO ASSAYS

Concentrate	NAPTT (sec)	TGt_{50} (min)	Recalcification Time (sec)	Thrombin (u/ml)
DEFIX (4 batches)	196†	25†	138–299	⩽ 0.001
PPSB (9 batches)	240–312†	29–>30†	248–>300	⩽ 0.001

(†: results not available on all batches)





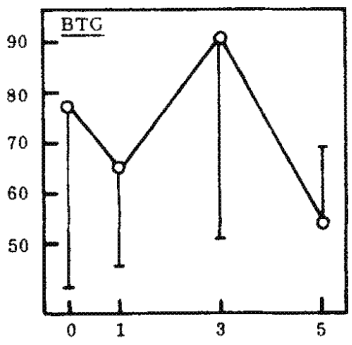
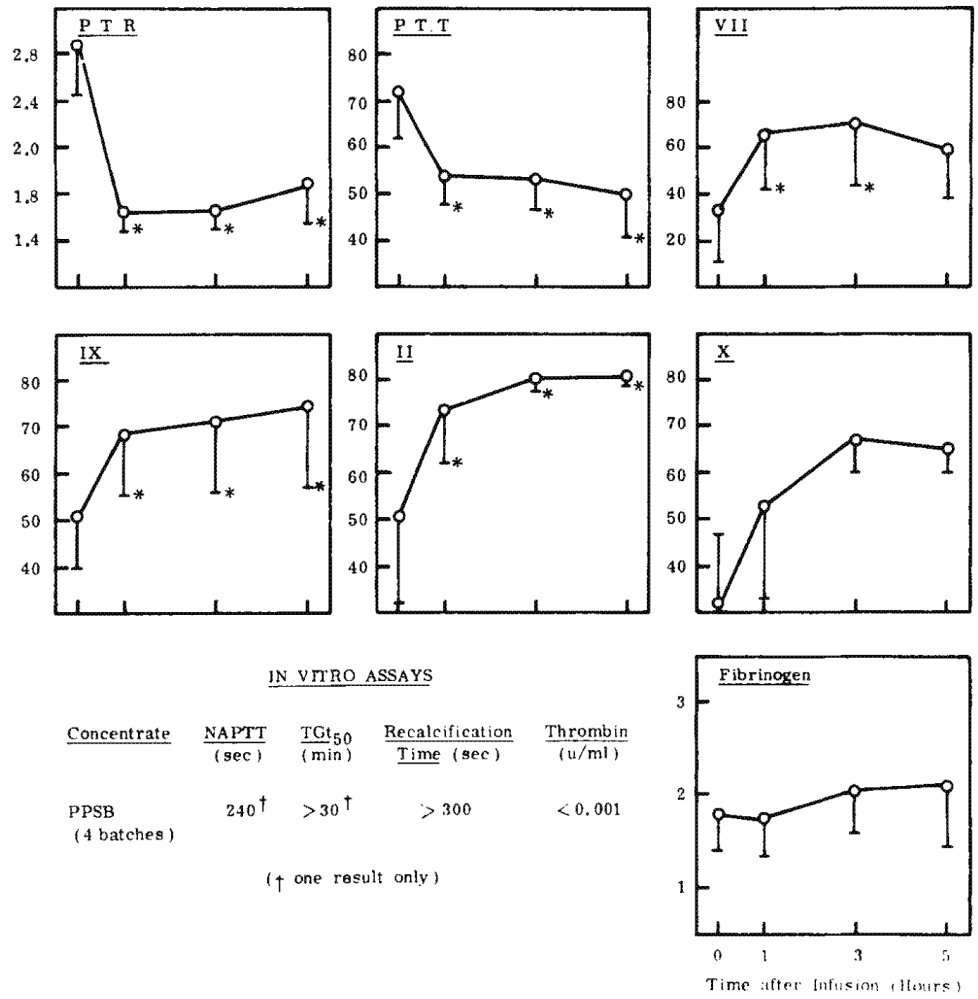


FIG. 3. (a) and (b) Serial coagulation studies in coumarin reversal with factor IX concentrate. Results for 15 patients treated with PPSB (○, mean dose 360 u factor IX) and 5 with DEFIX (●, mean dose 720 units factor IX). Details as in Fig 2. βTG results on only three patients receiving PPSB, and on none receiving DEFIX.



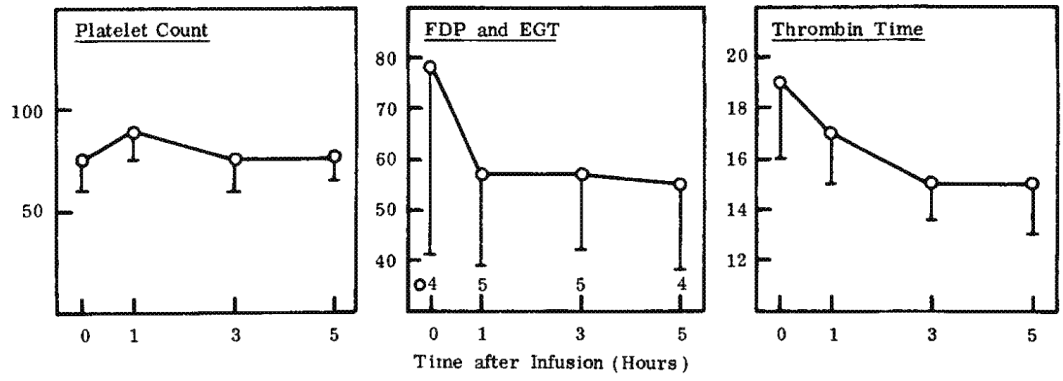
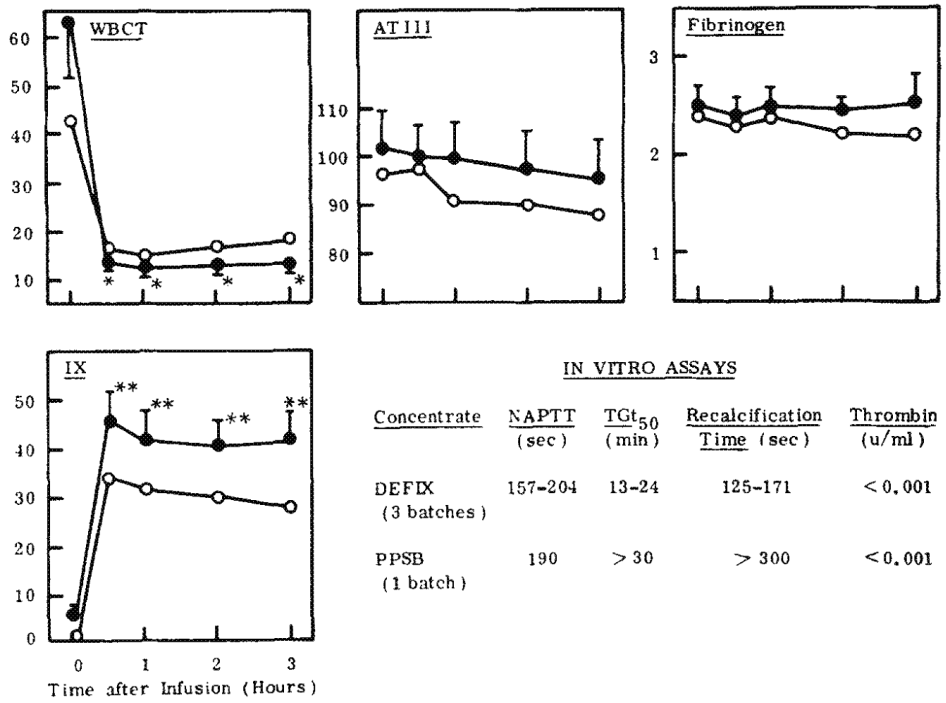


FIG 4. (a) and (b) Serial coagulation studies in other disorders. Results for seven patients treated with PPSB (mean dose 460 u factor IX). Three patients had acute renal failure, two were bleeding post-operatively, one had hepatoma and one disseminated intravascular coagulation associated with septicaemia. Details as in Fig 2.



IN VITRO ASSAYS

Concentrate	NAPTT (sec)	TGt_{50} (min)	Recalcification Time (sec)	Thrombin (u/ml)
DEFIX (3 batches)	157–204	13–24	125–171	< 0.001
PPSB (1 batch)	190	> 30	> 300	< 0.001

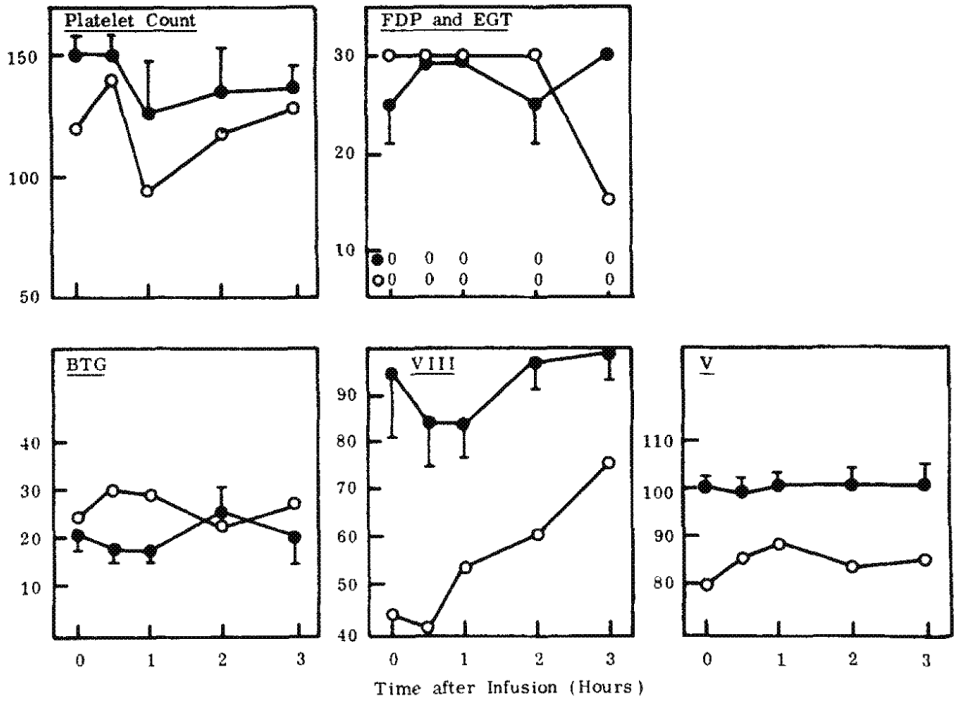


FIG. 5. (a) and (b). Serial coagulation studies in haemophilia B. Results of five infusions (to two patients) of DEFIX (●) at a dose of 2100 u factor IX and for one patient receiving PPSB (○) at a dose of 1400 u. Assays were performed prior to infusion and 0·1, 1, 2 and 3 h after infusion. Whole blood clotting time (WBCT) is given in minutes and antithrombin III (AT III) as a percentage of normal plasma levels. Other details as in Fig 2.

complications associated with the use of these factor IX concentrates were reported during the period under review. Five patients who died within 24 h of receiving factor IX concentrates underwent post-mortem examination. No macroscopic or microscopic evidence of thrombo-embolism was demonstrated.

Prospective Serial Studies

Fig 2 summarizes the results of serial studies on 23 patients with liver disease. PPSB (dose 200–800 u factor IX per patient) gave a shortening of the prothrombin time ratio and partial thromboplastin time, and rises in factors II, VII, IX and X. There was a mean rise in serum FDP, but this was not significant as it was due to one patient whose level rose to 480 mg/l (this isolated value was inexplicable as there was no evidence of a fall in fibrinogen or platelet count). A similar pattern was seen in the patients receiving DEFIX (dose 600–900 u factor IX), although the only statistically significant changes observed were in factors II, IX and X. No laboratory evidence of systemic activation of the coagulation mechanism was observed in any of these patients following the infusion of the factor IX concentrates (10 batches).

Fig 3 summarizes the observations in the group of patients receiving factor IX concentrates for reversal of coumarin effects. The patterns of changes observed in this group were similar to those recorded in the liver disease group. There was, however, stronger evidence suggesting that the efficacy of DEFIX was inferior to PPSB in correcting the prothrombin time ratio to below 1·6. There was no indication, from the laboratory data, of activation of the coagulation mechanism in this group of 20 patients who were exposed to 13 different batches of factor IX concentrates.

The results of the serial studies on seven miscellaneous patients (receiving PPSB: four batches) are summarized in Fig 4. No evidence of thrombogenicity *in vivo* was demonstrated.

Fig 5 records the serial studies performed on patients with haemophilia B. The dose given was calculated to correct the patient's plasma factor IX to approximately 40% of normal. As anticipated, there was a significant rise in factor IX and a marked decrease in the whole blood clotting time. No significant changes were observed in those laboratory parameters used to detect evidence of systemic activation of the coagulation mechanism. The cause of the rise in factor VIII following the one infusion of PPSB was not elucidated.

Although only average results have been reported here it should be emphasized that, while appreciable changes in platelet count, FDP and EGT were noted on single occasions in the occasional patient, in no case did the results indicate evidence of sustained activation of coagulation. At the present time it is assumed that these anomalous results were due to either difficult venepuncture or poor sample preparation, or both.

DISCUSSION

During the last 9 years factor IX concentrate has largely been used in South-East Scotland, as elsewhere, for the treatment of haemophilia B. It has also been used for the treatment of acquired coagulation disorders, including deficiencies resulting from liver disease, coumarin 'overdose', neonatal immaturity, post-operative bleeding, myeloproliferative disorders and disseminated intravascular coagulation. The use of factor IX concentrate in neonates, in this region, has been the subject of a separate clinical trial which will be reported elsewhere (Turner *et al*, 1981).

The two factor IX concentrates produced in Scotland, DEFIX and PPSB, were both found to correct the coagulation deficiencies of patients with haemophilia B and acquired coagulation disorders. In the latter cases PPSB appeared to be more effective than DEFIX, presumably as a result of its therapeutic content of factor VII, although DEFIX also gave an appreciable correction in many cases. As the production of PPSB, from blood collected in EDTA, is wasteful of red blood cells and factor VIII, the production of a separate factor VII concentrate from citrated plasma (Dike *et al*, 1977; Mariani *et al*, 1978; MacLeod & Dickson, 1979), for use in combination with DEFIX, may provide a more economical alternative to PPSB in the future.

The main purpose of this study was to detect possible activation of the coagulation system associated with the use of factor IX concentrates. Such episodes, in some cases fatal, have been associated with the use of certain concentrates (see Kasper, 1975) whereas other concentrates are comparatively safe in this respect (Lane *et al*, 1975), although sub-clinical changes in coagulation parameters may occur (Preston *et al*, 1975; Vigano *et al*, 1979). Such differences in the *in vivo* effects of different concentrates have also been demonstrated in animal models (Cash *et al*, 1975, 1978; Kingdon *et al*, 1975; Hedner *et al*, 1976; Prowse & Williams, 1979; Hedner *et al*, 1979; Giles *et al*, 1980). In addition to these differences in various concentrates, certain groups of patients, for example those with liver disease, may be more susceptible to develop thromboembolic complications following factor IX concentrate infusion (Gazzard *et al*, 1974; Kasper, 1975).

In this study inspection of retrospective data and prospective serial evaluation has failed to demonstrate any changes indicative of the onset of intravascular coagulation or thromboembolism following infusion of PPSB or DEFIX to patients with haemophilia or acquired coagulation disorders. The latter group included patients with liver disease ranging from mild disease, requiring correction of coagulation deficiency to allow liver biopsy, to acute hepatic failure. However, individual doses in these patients did not exceed 25 u factor IX per kg, although in some cases up to five infusions (totalling 2100 u factor IX at most) were given in 24 h.

The occurrence of thromboembolic episodes has been associated with the presence of activated coagulation factors in some factor IX concentrates and as a result *in vitro* tests have been developed for the detection of such factors (Middleton *et al*, 1973; Kingdon *et al*, 1975; Sas *et al*, 1975). Such tests are used to screen PPSB and DEFIX during preparation and this may explain the comparative safety of these products. It has been recommended that heparin should be added to concentrates (Menaché & Roberts, 1975) on the basis of experiments in rabbits (Kingdon *et al*, 1975) and there is some evidence that this additive may be beneficial in man as well (Gazzard *et al*, 1974; Preston *et al*, 1977). As factor IX concentrates contain only low amounts of the heparin cofactor, antithrombin III, only part of which may be functional it is perhaps not surprising that heparin addition has little effect on the *in vitro* activities of concentrates (Hultin, 1979; Prowse *et al*, 1979). In view of the low amounts of heparin added to some concentrates it is therefore surprising that it should be beneficial *in vivo*. We have found no evidence that PPSB (containing heparin) or DEFIX (to which heparin is not added) cause any activation of the coagulation system *in vivo*.

Despite the above evidence that PPSB and DEFIX are comparatively safe products, it should be noted that, in this survey, factor IX concentrates were only rarely used at doses above 40

factor IX units per kg in patients with haemophilia B or 25 u per kg in patients with acquired coagulation disorders. Elsewhere more aggressive therapy may be used, for example in the treatment of haemophilic patients with inhibitors. In animal experiments we have demonstrated that even these comparatively safe concentrates may produce intravascular coagulation at doses above 100 u per kg (Cash *et al*, 1975; Prowse & Williams, 1979). In addition other side effects of factor IX concentrate therapy must be considered. Apart from the perennial problem of hepatitis B, recent findings on the transmission of non-A, non-B hepatitis by factor IX concentrates (Wyke *et al*, 1979) make it probable that many of the patients with milder disorders presented above would now be treated more conservatively (either not at all or with fresh frozen plasma), at least until further data is available on individual concentrates. The incidence of hepatitis following the use of the two concentrates described in this study is currently under investigation.

While we would conclude that the present studies reveal that one manufacturer has, over a period of 9 years, provided two entirely different factor IX concentrates which have had no detectable thrombogenic effects in a wide range of patients, the above comments lead us to suggest that the clinical use of these concentrates should still be subject to further study in the clinical environment and that at all times alternatives should be considered, in particular fresh frozen plasma.

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