PAPERS AND SHORT REPORTS

Desmopressin and bleeding time in patients with cirrhosis

ANDREW K BURROUGHS, KATHERINE MATTHEWS, MOHAMMED QADIRI,
NIGEL THOMAS, PETER KERNOFF, EDWARD TUDDENHAM, NEIL MCINTYRE

Abstract

Desmopressin acetate 0.3 µg/kg was given intravenously to nine patients with chronic liver disease and to a further six such patients in a double blind controlled study versus placebo. Desmopressin acetate significantly shortened the bleeding time compared with basal values in both groups and compared with placebo. There was also a significant decrease in partial thromboplastin time (but not prothrombin time) and significant increases in factor VIII and its components, von Willebrand factor and ristocetin cofactor activity, but not in factors VII, IX, X, XI, or XII. Increased fibrinolysis could be blocked by concomitant administration of tranexamic acid. No important side effects were seen.

The multimer pattern of von Willebrand factor was studied for the first time in chronic liver disease. It was normal, but after administration of desmopressin acetate the percentage of multimers of higher molecular weight increased significantly. This may be an important mechanism in the shortening of the bleeding time in cirrhosis, as has been shown in uraemia and other conditions after administration of desmopressin acetate.

Desmopressin acetate may be useful in correcting defects in primary haemostasis in chronic liver disease.

Introduction

Desmopressin acetate is used to correct the bleeding time in von Willebrand's disease because it increases concentrations of von Willebrand factor and in mild haemophilia A because it increases concentrations of factor VIII.¹ Factor VIII and von Willebrand factor occur in plasma as a non-covalent complex of two proteins. Factor VIII is the procoagulant component and is a cofactor with calcium in the conversion of factor X to Xa. The plasma concentration is reduced in haemophilia A. Von Willebrand factor circulates as a series of multimers² and interacts with platelets to promote adhesion to subendothelium. A multimer consists of at least four subunits each having a molecular weight of roughly 220 000 daltons.² The multimers of von Willebrand factor with higher molecular weights may account for the ristocetin cofactor activity of plasma, which is related to bleeding time.³ Von Willebrand factor is either reduced or abnormal in von Willebrand's disease.

Desmopressin acetate, with or without cryoprecipitate, has also been used in patients with uraemia, who may have normal or increased factor VIII concentrations, either to improve haemostasis before surgery or to treat bleeding,^{4,5} and more recently without use of blood products in patients with a variety of bleeding disorders.⁶ Desmopressin acetate releases von Willebrand factor from storage sites (measured as von Willebrand factor antigen, formerly factor VIII related antigen), but it is the release of multimers of von Willebrand factor with higher molecular weights that seems to be the key feature in the mode of action of desmopressin acetate.^{5,7} Desmopressin acetate also releases plasminogen activator, and antifibrinolytics are given routinely before desmopressin acetate to prevent enhanced fibrinolysis.⁸

In chronic liver disease factor VIII concentrations are in the high normal range or increased,^{9,12} as in uraemia.⁴ There is only one report on the use of desmopressin acetate in chronic liver disease.¹³ In this uncontrolled study desmopressin acetate corrected the prothrombin time in eight patients with well compensated cirrhosis—an unexpected effect as factor VIII acts outside the extrinsic pathway. A significant rise of factor XII concentration was also noted.

The aim of our study was to assess the haemostatic effects of desmopressin acetate in chronic liver disease and to study changes in the multimeric forms of von Willebrand factor.

Academic Department of Medicine, Royal Free Hospital School of Medicine, London NW3

ANDREW K BURROUGHS, MB, MRCP, lecturer in medicine

MOHAMMED QADIRI, MB, MRCP, research fellow

NEIL MCINTYRE, MD, FRCP, professor of medicine

Haemophilia and Haemostasis Unit, Royal Free Hospital School of Medicine, London NW3

KATHERINE MATTHEWS, FIMLS, chief medical laboratory scientific officer

NIGEL THOMAS, AIMS, medical laboratory officer

PETER KERNOFF, MRCP, MRCPATH, consultant haematologist

EDWARD TUDDENHAM, MRCP, MRCPATH, senior lecturer in haematology

Correspondence to: Dr A K Burroughs, Academic Department of Medicine, Royal Free Hospital, London NW3 2QG.

Patients and methods

PILOT STUDY

Nine patients participated in the pilot study, eight with cirrhosis confirmed by biopsy (one of whom also had cholestasis due to alcoholic pancreatitis) and one with a hepatic abscess. The patients with cirrhosis had prothrombin ratios ≥ 1.3 despite three daily intramuscular injections of 10 mg vitamin K before the study. There had been no episodes of bleeding or transfusion of blood products in the previous three months, and no drugs affecting platelet function had been given during the previous 10 days. The patients had also abstained from alcohol during this period of 10 days. Table I shows the clinical features. Desmopressin acetate (0.3 µg/kg) was infused

TABLE I—Clinical features of nine patients in pilot study of intravenous infusion of desmopressin acetate

Diagnosis	No of cases	Bilirubin (µmol/l)*	Ascites	Prothrombin ratio	Platelets ($\times 10^9/l$)†
Alcoholic hepatitis with cirrhosis	4	42-218	++	1.5-2.3	104-212
Cryptogenic cirrhosis	2	13, 16		1.3, 1.4	104, 347
Cirrhosis due to lupoid chronic active hepatitis	1	16		1.3	65
Alcoholic cirrhosis with pancreatitis	1	77		1.3	210
Hepatic abscess	1	71		1.1	735

*Normal range = 5-17 µmol/l.

†Normal range = 150-400 $\times 10^9/l$.

Conversion: SI to traditional units—Bilirubin: µmol/l $\approx$ 0.06 mg/100 ml.

intravenously over 15 minutes in 50 ml 0.9% saline. Three patients received 1 g tranexamic acid intravenously over 15 minutes just before the desmopressin acetate was administered. Before the administration of desmopressin acetate and one and three hours after the end of the infusion blood samples were collected with minimal venous occlusion into a one tenth volume of 0.106 Molar (31.3 g) trisodium citrate/l. Template bleeding time was measured using a Simplate II device (General Diagnostics).¹⁴ Blood was also taken to test for full blood count, blood film, plasma electrolyte concentrations, and osmolarity at the same time points, and urine output was measured for 24 hours before and 48 hours after infusion.

Platelet poor plasma was centrifuged at 300 g for 10 minutes at 4°C. Plasma samples were kept on ice until tested (coagulation screening tests, factor VIII assays, and fibrinolytic tests) or stored at -40°C until required. Coagulation screening tests were by routine methods.¹⁵⁻¹⁸ Standard methods were used to measure factor VIII coagulant activity,¹⁹ von Willebrand factor antigen,²⁰ factor VIII ristocetin cofactor,²¹ and factors VII,²² X,²³ IX, XI, and XII.¹⁹ Fibrinolytic activator was monitored by euglobulin lysis time and fibrin plate technique.^{24,25}

The multimeric structure of von Willebrand factor was analysed by a modification of the method of Ruggeri and Zimmerman.² Coded samples were diluted to 1000 U von Willebrand factor antigen/l and then electrophoresis (5 mA) was performed through high purity agarose (1.6%) for 18 hours using the discontinuous buffer system of Laemmli.²⁶ Multimer bands were identified by incubation with radiolabelled rabbit antibody to von Willebrand factor antigen followed by autoradiography. Autoradiographs were scanned using a Beckman computing densitometer, which resolved the lowest to the highest molecular weight multimers as a series of peaks. The combined area of all peaks beyond the tenth was expressed as a percentage of the total area, and an increase in this figure was interpreted as an increase in the multimers with the highest molecular weights. The computation was carried out by an operator unaware of the identity of each sample.

DOUBLE BLIND PLACEBO CONTROLLED STUDY

Twelve patients with cirrhosis were randomly allocated using a sealed envelope technique to intravenous administration of either desmopressin acetate (0.3 µg/kg) in 50 ml 0.9% saline or 50 ml 0.9% saline alone. Selection criteria were the same as in the pilot study. Bleeding time, prothrombin and partial thromboplastin times, full blood count, and factor VIII assays were performed blind before and one hour after the end of the infusion with desmopressin acetate (table II). The same methodology was used as in the pilot study. Three patients in each group suffered from alcoholism; three in the placebo group and four in the group given desmopressin acetate had ascites; and two in the placebo group and one in the group given desmopressin acetate had encephalopathy.

TABLE II—Clinical findings (median (range)) in patients in double blind controlled study of infusion of desmopressin acetate versus placebo

	Group given placebo (n=6)	Group given desmopressin acetate (n=6)
Platelet count ($\times 10^9/l$)	99 (40-151)	70 (41-93)
Bilirubin (µmol/l)	49 (20-372)	59 (8-83)
Prothrombin time (s) (normal 11-14)	20 (16-36)	19.5 (16-26)
Activated partial thromboplastin time (s) (normal 30-40)	47 (36-58)	50 (36-60)
Bleeding time (normal <10 min)	12.5 (6-25)	18.5 (9.5-20)

Conversion: SI to traditional units—Bilirubin: 1 µmol/l $\approx$ 0.06 mg/100 ml.

Statistical analysis was performed using two tailed Wilcoxon signed rank tests. All patients gave informed consent, and the studies were approved by the local medical ethics committee.

Results

PILOT STUDY

A significant shortening of bleeding time compared with the baseline value (median 9 (range 5-26) minutes) was seen one hour (median 6 (3-23) minutes, $p < 0.01$) and three hours (median 6 (3-18) minutes, $p < 0.05$) after infusion of desmopressin acetate. Five patients had prolonged bleeding times before the infusion. The bleeding times of three of these patients became normal (less than 10 minutes) while the bleeding times of the two others improved from 25.5 to 18 minutes and 26 to 17.5 minutes at three hours. The bleeding time in the four patients with normal values shortened in three and did not change in one (a patient with chronic active hepatitis). Red cell, white cell, and platelet counts remained unchanged. Partial thromboplastin time was significantly shortened (baseline median 40 (33-60) seconds) at one hour (median 33 (31-46) seconds, $p < 0.01$) but not at three hours (median 37 (32-61) seconds). Prothrombin time did not change (at both times after infusion the median was 17 (14-28) seconds). No patient had evidence of disseminated intravascular coagulation before infusion of desmopressin acetate. Baseline fibrinogen concentrations (median 2.2 (1.7-6.2) g/l) and thrombin time (median 17 (13-26)) remained unchanged, and no fibrin monomers or fibrin degradation products were detected after infusion.

Both factor VIII and von Willebrand factor concentrations were in the high normal range or raised before infusion of desmopressin acetate (normal plasma concentrations 500-2000 U/l). Factor VIII, von Willebrand factor antigen, and ristocetin cofactor all showed significant increases after infusion of desmopressin acetate (table III). The patient with chronic active hepatitis and cirrhosis whose bleeding time did not improve did not show any change in any component of factor VIII. In all the patients factors VII, IX, X, XI, and XII showed no significant changes.

TABLE III—Effects of desmopressin acetate on bleeding, prothrombin, and partial thromboplastin times and components of factor VIII and multimers of von Willebrand factor of higher molecular weight (median (range)) in pilot study (n=9)

	Basal value	One hour after infusion	Three hours after infusion
Bleeding time (min)	11 (5-26)	6 (3-23)*	6 (3-18)**
Prothrombin time (s)	17 (14-28)	17 (14-28)	17 (14-28)
Activated partial thromboplastin time (s)	40 (33-60)	33 (31-46)*	37 (32-61)
Factor VIII (U/l)†	1450 (840-2200)	2000 (1300-3000)*	2150 (1250-3100)*
von Willebrand factor (U/l)†	3800 (1070-7000)	4240 (2000-7800)*	4000 (2200-8000)*
Ristocetin cofactor (U/l)†	3200 (1600-5800)	3500 (2400-5000)*	3550 (2000-7000)*
Higher molecular weight multimers of von Willebrand factor (%)‡	17.2 (9.9-36.5)	24.4 (8.4-49)***	25.4 (8.8-44.4)**

* $p < 0.1$, ** $p < 0.05$, *** $p < 0.02$ compared with basal values (two tailed Wilcoxon rank test).

†Normal values 500-2000 U/l.

‡Represents combined areas of all peaks beyond 10th on densitometer scan of autoradiographs of electrophoresis of von Willebrand factor, expressed as percentage of total area of all peaks.

Electrophoresis of multimers of von Willebrand factor showed a normal triplet pattern in the lowest electrophoretic bands, consisting of a central dense band flanked by two lighter bands in both the basal samples and the samples taken after infusion of desmopressin acetate (figure). After the infusion, however, the electrophoretic pattern showed a pronounced

Autoradiograph (left) and densitometry scan (right) of electrophoretic strip of multimers of von Willebrand factor before and after infusion of desmopressin acetate (at one and three hours) compared with normal plasma (control) in (top) patient with no response and (bottom) patient with representative response.

increase in the proportion of multimeric forms with higher molecular weights. The median (range) proportion of these electrophoretic bands was 17.2% (9.9-36.5%) before infusion, 24.4% (8.4-49%) at one hour ($p<0.02$), and 25.4% (8.8-44.4%) at three hours ($p<0.05$). The coefficient of variation of this assay based on several analyses of a normal plasma sample was 4%. The figure shows a representative response to infusion. In all but one patient, including the patient without shortening in bleeding time, who had a slight reduction in the proportion of the highest multimeric forms, changes in multimeric forms of von Willebrand factor paralleled changes in the results of other haemostatic tests.

In the six patients who did not receive tranexamic acid fibrinolysis increased: the euglobulin lysis time shortened from a median of 65 (range 15-360) minutes to 17.5 (15-120) minutes ($p<0.01$) at one hour and 37.5 (15-180) minutes (NS) at three hours. In the three patients given 1 g tranexamic acid fibrinolysis was blocked and an increase in euglobulin lysis time was seen at three hours (basal median 30 (25-125) minutes; three hour median 75 (20-180) minutes; NS). The results of fibrin plate assays were similar.

There were no significant changes in plasma urea concentrations, electrolyte concentrations, or osmolality. Packed cell volume showed a non-significant decrease from basal values (0.387 (SD 0.021)). Only one patient (taking diuretics) had a decreased urine output (1000 ml in the 24 hours after administration).

CONTROLLED STUDY

The groups given placebo and desmopressin acetate did not differ significantly in their clinical state and results of tests of haemostasis. In the group given placebo there were no significant changes from baseline values in bleeding time, partial thromboplastin time, factor VIII, von Willebrand factor, and ristocetin cofactor (table IV). In the group given desmopressin acetate all these variables had changed significantly ($p<0.01$) one hour after infusion compared with baseline values (table IV). Maximal shortening of

Desmopressin acetate has little effect on bleeding time in types IIa and IIb von Willebrand's disease, in which there is no rise (IIa) or only a minor rise (IIb) in high molecular weight forms of von Willebrand factor and ristocetin cofactor.⁶ The patient in this study whose bleeding time did not change and in whom only minor changes in factor VIII components and multimeric forms occurred may have had an inability to release or synthesise the highest molecular weight forms of von Willebrand factor. At present it is not known if there are some patients with cirrhosis who will not respond to desmopressin acetate.

Although platelet aggregation was not measured in this study, it was probably not modified by desmopressin acetate as previous studies in patients with uraemia⁴ or other bleeding disorders, including platelet defects,⁶ showed no change in aggregation with desmopressin acetate. Packed cell volume was not significantly affected by a single 0.3 µg/kg dose of desmopressin acetate. Reduction in packed cell volume would be expected to increase bleeding time²⁷ at least in patients with platelet counts above $100 \times 10^9/l$.²⁸

Our major findings were significant increases in all components of factor VIII and changes in the proportion of highest molecular weight forms of von Willebrand factor. Factor VIII is the only clotting factor that also has major extra hepatic synthetic sites, and concentrations of its components rise in chronic liver disease^{9,12} and uraemia.⁴ The antigenic activity of factor VIII (factor VIII antigen) and von Willebrand factor (von Willebrand factor antigen) rose more than their biological activity (factor VIII, ristocetin cofactor, respectively).^{9,10,12} Recent work shows that both factor VIII and factor VIII antigen activities are modified by vitamin K.¹² They fall with vitamin K repletion, perhaps because of increases in protein C.¹² Although factor VIII antigen was not measured in this study,

TABLE IV—Effects of placebo and infusions of desmopressin acetate on bleeding and partial thromboplastin times and concentrations of factor VIII (median (range))

	Group given placebo (n=6)		Group given desmopressin acetate (n=6)	
	Basal	After 1 hour	Basal	After 1 hour
Bleeding time (min)	12.5 (6-25)	12 (6-25)	18.5 (8.5-20)	12 (5-15)*
Activated partial thromboplastin time (s)	41 (40-51)	40 (38-50)	49.5 (38-60)	44 (35-51)*
Factor VIII (U/l)†	1850 (1250-3400)	2200 (1250-3600)	1270 (1200-1550)	2400 (1800-3100)*
von Willebrand factor (U/l)†	3380 (1400-7650)	3590 (1500-7500)	2810 (1900-4500)	3370 (2500-5200)*
Ristocetin cofactor (U/l)†	3750 (2400-8150)	4090 (2400-8200)	3790 (1840-4500)	4400 (2900-6800)*

* $p<0.01$, paired two tailed Wilcoxon rank test.

†Normal values 500-2000 U/l.

bleeding times was from 20 to 11.5 minutes, and the smallest decrease was from 18 to 15 minutes. The pattern of increases in factor VIII, von Willebrand factor antigen, and ristocetin cofactor concentrations was similar to that seen in the pilot study, with greater increases in factor VIII than in either von Willebrand factor antigen or ristocetin cofactor. Percentage changes from baseline values ranged from 27 to 132% (median 70%) for factor VIII, from 0 (one patient) to 50% (14.7%) for von Willebrand factor antigen, and from 14.5 to 108% (55%) for VIII ristocetin cofactor. There were no significant changes in packed cell volume, osmolality, urea and electrolyte concentrations, or urine output before and after infusion.

Discussion

This study shows that desmopressin acetate shortens the bleeding time in patients with cirrhosis. The results of the pilot study were confirmed in a double blind placebo controlled study in different patients. Although bleeding time is affected by many variables, such as platelet behaviour, plasma coagulation proteins, vascular contractility, and packed cell volume,^{27,28} the effect in this study on patients with cirrhosis seems to be explained by the temporal relation of the increase in high molecular weight forms of von Willebrand factor in plasma to shortening of the bleeding time. Release from storage sites of higher molecular weight forms of von Willebrand factor by desmopressin acetate has been shown.^{4,7,29}

the increases in factor VIII were independent of vitamin K depletion as all patients were given sufficient vitamin K to replete stores.

This is the first report of the pattern of multimers of von Willebrand factor in chronic liver disease. Previously, two dimensional immunoelectrophoresis has been used to study von Willebrand factor in liver disease, and abnormally fast moving material was found.¹⁰ Our multimeric analysis showed a normal triplet pattern in individual bands and no increase in multimers with lower molecular weights that would correspond to the faster moving material found on two dimensional immunoelectrophoresis.¹⁰ As the usual triplet pattern was seen abnormal polymerisation of von Willebrand factor does not seem to be an explanation for the variation of biological activity of factor VIII and von Willebrand factor in liver disease.¹² In uraemia the multimer pattern of von Willebrand factor and the response to desmopressin acetate is normal, and the increase in multimers of von Willebrand factor with high molecular weights paralleled the shortening of bleeding time and rise in ristocetin cofactor.⁴ As in previous studies, however, we could find no direct correlation between absolute changes in bleeding time and plasma factors.^{4,6} This suggests that there may be other explanations for the action of desmopressin acetate.⁶

Factor VIII and von Willebrand factor concentrations show little, or no rise after administration of fresh frozen plasma, which

contains only 60-70% of normal plasma concentrations of factor VIII, or about 600-700 U/l.³⁰ Large volumes of fresh frozen plasma increase plasma volume and reduce the effective increase in factor VIII components to about 15%. Several litres would not give increases similar to those seen after infusion of desmopressin acetate. Furthermore, fresh frozen plasma may exacerbate salt and water retention in patients with cirrhosis and carries a risk of infection with transmissible agents such as non-A non-B hepatitis and viruses of acquired immune deficiency syndrome.³¹ When fresh frozen plasma (12 ml/kg) was given to patients with chronic liver disease associated with clotting defects there was no increase in concentrations of factor VIII but there were moderate increases in concentrations of factors II, V, VII, IX, and X; coagulation tests, including tests for prothrombin time, yielded normal results only if basal values showed minor abnormalities; and partial thromboplastin time improved more than prothrombin time.³² Similar improvements in partial thromboplastin time to those after administration of fresh frozen plasma were achieved with desmopressin acetate in our study. Significant decreases in prothrombin time in patients with cirrhosis and vitamin K repletion have been achieved only with factor VII rich prothrombin complexes,³³ but these have potentially serious side effects.

We did not confirm the results of Agnelli *et al*, who found shortening of prothrombin time and a significant increase of factors VII, IX, X, and XI after infusion of desmopressin acetate in patients with cirrhosis.¹³ Our findings support the results of Kobrinsky *et al*, who found no change in prothrombin time in patients without cirrhosis.⁶ A single dose of desmopressin acetate did not have important side effects in our study. Increased fibrinolysis did not occur with concomitant administration of tranexamic acid as in other clinical conditions.^{4*} There was no evidence of disseminated intravascular coagulation occurring after administration of desmopressin acetate.

The prothrombin time, which is often used in clinical practice as the principal index of risk of bleeding, was not corrected by desmopressin acetate. It may not, however, be the best index of risk of bleeding, and we believe that desmopressin acetate will prove of value in patients with cirrhosis. Mannucci *et al* suggested that invasive procedures should not be performed in patients with cirrhosis and defective primary haemostasis, as evidenced by prolonged bleeding time, and pointed out that the bleeding time was not corrected by use of blood products.³² Desmopressin acetate given as a single dose is the first agent without major side effects that has been shown to shorten bleeding time significantly in patients with cirrhosis. It may prove a useful addition to treatment for liver disease as it offers the possibility of specific treatment for correction of primary haemostasis. It could be used just before invasive diagnostic or therapeutic procedures such as liver biopsy, placement of central venous or arterial lines, percutaneous transhepatic cholangiography and portography, endoscopic sphincterotomy of the papilla of Vater, elective sclerotherapy of oesophageal varices, minor surgery including dental extractions, and possibly major surgery, including liver transplantation. Future clinical studies are needed to establish if these improvements in haemostasis will occur during the treatment of active bleeding, when repeated doses of desmopressin acetate would be given.^{34 35}

References

- 1 Anonymous. DDAVP in haemophilia and von Willebrand's disease [Editorial]. *Lancet* 1983;ii:774-5.
- 2 Ruggeri ZM, Zimmerman TS. The complex multimeric composition of factor VIII/von Willebrand factor. *Blood* 1981;57:1140-3.
- 3 Martin SE, Marder VJ, Francis CW, Barlow GP. Structural studies on the functional heterogeneity of von Willebrand's protein polymers. *Blood* 1981;57:313-23.
- 4 Mannucci PM, Remuzzi G, Pusineri F, *et al*. Deamino-8-D-arginine vasopressin shortens bleeding time in uremia. *N Engl J Med* 1983;308:8-12.
- 5 Giotti E, Mecca G, Valentino C, Cortinouis E, Bertani T, Remuzzi G. Renal biopsy in patients with acute renal failure and prolonged bleeding time. *Lancet* 1984;ii:878-9.
- 6 Kobrinsky NI, Israels ED, Gerrard JM, *et al*. Shortening of bleeding time by 1-deamino-8-D-arginine vasopressin in various bleeding disorders. *Lancet* 1984;i:1145-8.
- 7 Ruggeri ZM, Mannucci PM, Lombardi R, Federici AB, Zimmerman TS. Multimeric composition of factor VIII von Willebrand factor following administration of DDAVP: implications for pathophysiology and therapy of von Willebrand's disease subtypes. *Blood* 1982;59:1272-8.
- 8 Mannucci PM, Aberg M, Nilsson IM, Robertson B. Mechanism of plasminogen activator and factor VIII increase after vasoactive drugs. *Br J Haematol* 1975;30:81-93.
- 9 Green AJ, Ratnoff OD. Elevated antihemophilic factor (AHF, factor VIII) procoagulant activity and AHF-like antigen in alcoholic cirrhosis of the liver. *J Lab Clin Med* 1974;83:189-97.
- 10 Maisonneuve P, Sultan Y. Modification of factor VIII complex properties in patients with liver disease. *J Clin Pathol* 1977;30:221-7.
- 11 Lombardi R, Mannucci PM, Segnatchian MJ, Vicente Garcia V, Coppola R. Alterations of factor VIII von Willebrand factor in clinical conditions associated with an increase in its plasma concentration. *Br J Haematol* 1981;49:61-8.
- 12 Kelly DA, Brien FO, Hutton RA, Tuddenham EG, Summerfield JA. The effect of liver disease on the factor VIII complex. *Br J Haematol* (in press).
- 13 Agnelli G, Berrettini M, De Cunto M, Nenci GG. Desmopressin-induced improvement of abnormal coagulation in chronic liver disease. *Lancet* 1983;i:645.
- 14 Mielke CH, Kanoshiro MM, Maher IA, *et al*. The standardised Ivy bleeding time and its prolongation by aspirin. *Blood* 1969;34:204-15.
- 15 Quick AJ. *The physiology and pathology of haemostasis*. London: Henry Kimpton, 1951.
- 16 Proctor RR, Rapaport SI. The partial thromboplastin time with kaolin. A simple screening test for first stage plasma clotting factor deficiencies. *Am J Clin Pathol* 1961;36:212-9.
- 17 Ingram GIC. The determination of plasma fibrinogen by the clot weight method. *Biochem J* 1952;51:583-5.
- 18 Lipinski B, Woronski K. Detection of soluble fibrin monomer complexes in blood by means of protamine sulphate test. *Thrombosis et Diathesis Haemorrhagica* 1969;20:44-9.
- 19 Hardisty RM, MacPherson JC. A one stage factor VIII (antihemophilic globulin) assay and its use on venous and capillary plasma. *Thrombosis et Diathesis Haemorrhagica* 1962;7:215-28.
- 20 Laurell CB. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal Biochem* 1966;15:45-52.
- 21 MacFarlane DE, Stille J, Kirby EP, Zucker AB, Grant RA, McPherson J. A method for assaying von Willebrand factor (Ristocetin co-factor). *Thrombosis et Diathesis Haemorrhagica* 1975;34:306-8.
- 22 Garner R, Conning DM. The assay of human factor VII by means of modified factor VII deficient dog plasma. *Br J Haematol* 1970;18:57-66.
- 23 Denson KW. The specific assay of Prower-Stuart factor and factor VII. *Acta Haematol* 1961;25:105-20.
- 24 Vonn Kaulla KN. *Chemistry of thrombolysis: human fibrinolytic enzymes*. Illinois: Thomas, 1963.
- 25 Nilsson IM, Alow B. Fibrinolysis induced by streptokinase in man. *Acta Chir Scand* 1962;123:247-66.
- 26 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
- 27 Livio M, Gotti E, Marchesi D, Mecca G, Remuzzi G, de Gaetoma G. Uraemic bleeding: role of anaemia and beneficial effect of red cell transfusions. *Lancet* 1982;ii:1013-5.
- 28 Anonymous. The bleeding time and haematocrit [Editorial]. *Lancet* 1984;i:997-8.
- 29 Takahashi H. Studies on the pathophysiology and treatment of von Willebrand's disease. V. Properties of factor VIII after DDAVP infusion in variant von Willebrand's disease. *Thromb Res* 1981;21:357-65.
- 30 Rizza CR, Biggs R. Blood products in the management of haemophilia and Christmas disease. In: Foller L, ed. *Recent advances in blood coagulation*. London: J A Churchill, 1969.
- 31 Anonymous. Blood transfusion, haemophilia, and AIDS [Editorial]. *Lancet* 1984;ii:1433-5.
- 32 Mannucci PM, Franchi F, Dioguardi N. Correction of abnormal coagulation in chronic liver disease by combined use of fresh frozen plasma and prothrombin complex concentrates. *Lancet* 1976;ii:542-5.
- 33 Green G, Poller L, Dymock IW, Thomson JM. Use of factor VII rich prothrombin complex concentrate in liver disease. *Lancet* 1975;ii:1311-4.
- 34 Vicente V, Alberca I, Mannucci PM. Reduced effect of exercise and DDAVP on factor VIII-von Willebrand factor and plasminogen activator after sequential application of both the stimuli. *Thromb Haemost* 1984;51:129-30.
- 35 Canavese C, Salomone M, Pacitti A, Mangiarotti G, Calitri V. Reduced response of uraemic bleeding time to repeated doses of desmopressin. *Lancet* 1985;i:867-8.

(Accepted 22 July 1985)