Identification of a congenital defect of factor VII in a colony of beagle dogs: the clinical use of the plasma

L. POLLER, JEAN M. THOMSON, C. H. J. SEAR, AND WENDY THOMAS

From the Haematology Department, Withington Hospital, Manchester

SYNOPSIS A severe, pure deficiency of factor VII has been defined in haematological and biochemical terms in the Alderley Park colony of beagle dogs. By modification of the conventional factor VII assay to improve its specificity an apparent low activity of between 1 and 2.5% of the normal beagle has been found.

When this plasma is used as a substrate for factor VII assays it compares well with human factor VII-deficient plasma in the measurement of both raised and depressed factor VII activity. It also appears to be a valuable reagent in the quality control of tissue thromboplastin extracts.

Part I Identification of the clotting defect in a colony of beagles

There is at Alderley Park, near Manchester, a colony of nearly 300 beagle dogs with a hereditary coagulation defect. This abnormality has been investigated to determine whether it was a true congenital deficiency of factor VII and whether the plasma would be of clinical value in laboratory assays.

Human congenital factor VII deficiency occurs rarely and an acquired factor VII-deficient plasma for laboratory assays from coumarin-treated patients is almost as difficult to obtain.

In the first part of the study we present laboratory data on the identification of the congenital coagulation defect present in the beagle dogs. In the second part, clinical studies of the measurement of human factor VII activity are presented, and in the third part data on the characterization of tissue thromboplastins used in controlling oral anticoagulant treatment are described.

Method of Study

The following studies were performed on 25 beagles with the clotting defect and also on 10 normal beagles. Quick's prothrombin time (modified, Poller, 1964) using the British Comparative Thromboplastin (BCT) as the tissue extract; cephalin time (Hjort, Rapaport, and Owren, 1955; Egeberg, 1961); throm-

bin-fibrinogen time; factor V assay (Wolf, 1953, modified); factor VII assay (Poller and Thomson, 1964, modified), using both pooled normal human and normal beagle plasma. Buffered citrate saline, double seitz-filtered beagle plasma and charcoal-filtered ox plasma, respectively, were used as diluents. Results were expressed as percentage factor VII activity from both the human plasma curve and the dog plasma curves; factor VIII assay (Egeberg, 1961); factor IX assay (Egeberg, 1961); factor X assay (Denson, 1961).

Correction of the clotting defect was attempted with stored, normal human, and beagle serum.

The effect of species specificity in the 'extrinsic' clotting system was also studied, eg, by substituting beagle brain tissue extract for the BCT. Dog plasma tends to cause a non-specific acceleration of the assay systems whereas human plasma retards clotting in these tests. The effect was studied in some detail in both factor V and factor VII assays to quantitate the respective percentage levels in the dog.

Thrombelastography (TEG) was performed on six dogs: three normal beagles and three with the coagulation defect.

Platelet aggregation studies were performed with the Chandler's tube (modified, Poller, Thomson, and Priest, 1969). The Chandler's tube method was adapted for the measurement of the 'extrinsic' clotting system by the addition of 1:40,000 human brain thromboplastin (BCT) before recalcification.

The test was performed on three normal beagles and three with the clotting defect.

A comparison was made of the clotting factor activities present in the fractions obtained by gel filtration of the barium sulphate eluates from normal and deficient beagle plasma. The method of Prydz (1963) was modified as follows.

Ten ml platelet-poor plasma yielded 1 ml barium sulphate eluate, to which sodium chloride was added to increase its concentration to 1M. Gel filtration of the eluate was carried out on a 1.5×80 cm column of Sephadex G200¹ using, as eluant, 0.05M imidazole buffer, pH 7.35, containing 0.1M NaCl. Fractions of 5 ml each were collected at approximately 20 ml/hr, estimated for protein by optical density measurements at 280 nm, and concentrated to 1 ml by preweighed amounts of Lyphogel.² Each fraction was assayed for the following clotting factor activities: factor VII, factor X, and prothrombin (Loeliger, Meeuwisse-Braun, Muis, Buytendijk, Veltkamp, and Hemker, 1970).

Results

Coagulation results are summarized in Tables I and II

There is thus a deficiency of the 'extrinsic' system in the group of dogs with the coagulation defect shown by a prolonged one-stage prothrombin time.

¹Pharmacia (GB) Ltd, Paramount House, 75 Uxbridge Road, London W5.

²Gelman-Hawksley, 12 Peter Road, Lancing, Sussex.

In contrast the cephalin time was greatly accelerated and the TEG shows 'hypercoagulable' patterns in both groups of dogs (Fig. 1). Results of specific assays in Table II indicate high levels of clotting factors but the factor VII activity in dogs in the abnormal group, although low, was difficult to express in quantitative terms (see later).

The clotting defect, present in the prothrombin time test, was completely restored by the addition of 1/10 volume stored human serum in the presence of human brain tissue extract and 1/10 volume stored dog serum in the presence of dog brain tissue extract.

It is relevant to note that human brain reacted very quickly with normal beagle blood, giving prothrombin times of 7 to 8 seconds. Dog brain reacted more slowly with dog plasma, giving a normal time of 8.5 to 10 seconds. Human blood showed a marked species-specific delay with dog brain giving a normal of 30 seconds.

Further evidence of the species-specificity difficulties was obtained from the factor V assay. Using dilutions of dog plasma in this assay, the accelerating effect was approximately 10 times the effect of human plasma. The acceleration depends not only on factor V but is also due to the species effect, ie, dog plasma reacts more quickly than human blood in this test system.

Additional evidence of this difficulty was obtained from the factor V level of human blood obtained from the factor V-deficient dog test system. In this test the 5% level of normal human plasma restored the prothrombin time from 28 to 24 seconds but no

	Mean Clotting Time (sec)	
	Normal Control Beagle Plasma	Plasma from Beagles with Coagulation Defect
Quick test prothrombin time, Using BCT as tissue extract	7	28.5
Using dog tissue extract	8.5	31.5
Cephalin time	20	20
Thrombin-fibrinogen time	Accelerated	Accelerated
Thrombelastogram (TEG)	Hypercoagulable pattern	Hypercoagulable pattern

Table I Screening procedure

	Mean Clotting Time (sec)	
	Normal Control Beagle Plasma	Beagle Plasma with Coagulation Defect
Factor V	Raised levels	Raised levels
Factor VII	Raised levels	Severe defect (see text)
Factor VIII	Raised levels	Raised levels
Factor IX	Raised levels	Raised levels
Factor X	Raised levels	Raised levels

Table II Specific assays

Fig. 1 Thrombelastograph patterns

further shortening was obtained by increasing the concentration of plasma, the undiluted human plasma giving a prothrombin time of 31 seconds. The specific clotting factor assay results given in Table II are therefore merely recorded as raised levels because of the species difficulties.

The same difficulty was apparent in attempting quantitative factor VII assays. Percentage readings expressed in the normal way from a human plasma dilution curve in citrate buffer gave a range of factor VII activity of 17 to 22% for the dogs with the clotting defect. Because this seemed an incorrect result, some modifications of the factor VII assay system were introduced to overcome the effect of

species-specificity. The factor VII activity was interpreted in terms of the normal dog 'pool' and modified diluents were used to try to make the test system less artificial by the use of adsorbed dog plasma and charcoal filtered ox plasma which makes the assay more specific for factor VII. The results are given in Table III.

Diluent	Range of VII Activity as Percentage of Normal Beagle 'Pool'
Buffered citrate saline	7-9
Adsorbed dog plasma	5-8.5
Charcoal-filtered ox plasma	1-2.5

Table III Factor VII levels in beagles with the clotting defect

Chandler's tube platelet aggregation on a normal dog and a deficient dog gave similar results (6 minutes). When the Chandler's tube platelet aggregation technique was modified by the addition of tissue thromboplastin the result in the deficient dog was not accelerated to the same degree as the control, indicating an 'extrinsic' clotting defect only, ie, normal dog shortened to 1 min 20 sec and dog with clotting defect only shortened to 4 minutes.

Figures 2a and 2b illustrate a typical gel filtration experiment. Normal (Fig. 2a) and deficient (Fig. 2b) beagle plasma eluates gave similar protein elution patterns. Fractions 9 to 13 from the normal beagle plasma eluate restored the clotting defect of human factor VII-deficient plasma, whereas only a slight restoration of the clotting defect could be detected in the fractions from the deficient beagle plasma eluate in fraction 9. Factor X was present in both sets of fractions as shown in Figures 2a and 2b. Prothrombin was detected in fractions 9 to 11 from the normal beagle plasma eluate, and in fractions 8 to 10 from the deficient beagle plasma eluate.

The small amount of factor VII activity detected in fraction 9 from the deficient beagle plasma eluate may be caused by a species specificity effect.

Discussion

Results in the colony of dogs with the clotting defect indicate that the 'extrinsic' system alone is abnormal and this, on haematological and biochemical evidence, is a pure severe defect of factor VII. Preliminary incomplete haematological observations of this colony by Garner, Hermoso-Perez, and Conning (1967) and Garner and Conning (1970) indicated an 'extrinsic' clotting defect but results were equivocal, particularly as the prothrombin time was not corrected to normal by stored dog or human serum.

Fig. 2a Gel filtration of the barium sulphate eluate prepared from normal beagle plasma.

○—○ Factor X activity
●—● Factor VII activity
The histogram represents the protein distribution.

Fig. 2b Gel filtration of the barium sulphate eluate prepared from deficient beagle plasma.

○—○ Factor X activity
●—● Factor VII activity
The histogram represents the protein distribution.

The clotting deficiency now fulfils all the conventional haematological criteria for a severe factor VII defect. We have been able to add to this confirmatory evidence from platelet aggregation studies by modifying the technique of the Chandler's tube test. The results indicate that in the presence of a minute amount of tissue thromboplastin the normal dog's platelets aggregated more rapidly than the platelets of the dog with the clotting defect. This exemplifies the defect in 'extrinsic' clotting and is of considerable interest in demonstrating the importance of factor VII in the thrombin phase of platelet aggregation when a trace amount of tissue extract is present (as in our modification of the Chandler's tube technique).

The gel filtration experiments have shown that factor VII is grossly deficient. We have been unable to determine, however, whether the slight restoration of the clotting defect of human factor VII-deficient plasma is due to a very small concentration of factor VII, contact activation product, or a species specificity effect.

It is perhaps of considerable clinical interest that in spite of the apparent severity of the clotting defect

present in the 'extrinsic' system, these dogs do not suffer abnormal bleeding and, despite the defect, haemostasis after venepuncture appears more rapid than in man. This may perhaps be due to the markedly accelerated 'intrinsic' clotting system noted both in the normal and the factor VII-deficient dogs. The 'hypercoagulable' patterns present in the thrombelastograms in both factor VII-deficient dogs and controls are seen in Figure 1.

It will be noted also that the cephalin time was very rapid in both groups, ie, 20 seconds, as opposed to a human plasma result of 60 to 70 seconds with the same test system. Considerably raised levels of the other 'intrinsic' factors—V, VIII, IX, and X—were also recorded in control and factor VII-deficient dogs. As in the case of the factor V assay, no attempt has been made to interpret these levels as percentages of human activity because of the species difference. The dog plasma caused a non-specific acceleration in the assay system for both factors V and VII, causing an apparent exaggeration of the respective levels and making it difficult to quantitate precisely activity of dog factors V and VII in human terms. It may also be complicated by 'contact

product' which simulates factor VII activity in the assay. The net overall activity, whether due to a species effect, or a very small concentration of factor VII, is, however, so low that it does not invalidate its use as a substrate for the human assay as is seen in part II of this study.

Conclusion

From haematological and biochemical assays the

clotting abnormality in the deficient dogs is a pure factor VII defect. In absolute terms the factor VII level has been found to be between 1 and 2.5% of the level in the normal beagle. These results indicate that there is a unique source of congenital factor VII-deficient blood available in considerable volume which may be used as a substrate for human factor VII assays or as a reference standard plasma in the quality control of prothrombin times and tissue thromboplastin extracts.

Part II Clinical applications

In the second part of this report the method of obtaining a reproducible factor VII-deficient plasma is described and preliminary data on lyophilization and storage are presented. The use of the resulting lyophilized material as substrate for factor VII assays and for measuring the sensitivity of various tissue thromboplastin extracts to the factor VII defect is also described.

If the plasma is to be used widely in hospital practice, it must be shown that it can be kept in a stable form. We have prepared a large freeze-dried batch of the material in small volumes. Four hundred samples for degradation studies have been laid down at the following temperatures: 56°C, 37°C, room temperature, 4°C, and -20°C. The effect on stability of buffering the plasma by collection into buffered citrate is being studied in parallel. Results at the one year stage indicate that only those stored at 56°C and 37°C have shown appreciable deterioration.

Factor VII Assay

MEASUREMENT OF HIGH LEVELS

A parallel study has been performed in various clinical states using a coumarin-induced factor VII-deficient substrate obtained as previously (Poller and Thomson, 1964) and the beagle factor VII-deficient substrate.

Raised levels of factor VII activity occur in women taking conventional oral contraceptives and during the third trimester of pregnancy (Poller and Thomson, 1966; Poller, Tabiwo, and Thomson, 1968) when tested with human coumarin-induced factor VII-deficient plasma. Studies were therefore performed in parallel using this substrate and the beagle factor VII-deficient plasma in parallel. The groups studied were as follows:

Group	Total
1 Normal females	23
2 On estrogen-progestogen oral contraception	23
3 On progestogen oral contraception only	27
4 In third trimester of normal pregnancy	20

The results indicate that both substrate plasmas registered raised levels of factor VII activity in groups 2 and 4 compared with the normals and group 3.

MEASUREMENT OF LOW LEVELS

Parallel tests were performed on 35 patients stabilized on long-term anticoagulant therapy whose prothrombin ratios were therapeutic or higher using the British Comparative Thromboplastin (BCT). There was a good correlation between the human and beagle factor VII-deficient plasma substrate assays, indicating the reliability of the beagle plasma in the measurement of factor VII deficiency at clinically significant levels of depression. There was no significant difference between the methods using the *t* test modified for paired comparisons.

Comment

The above results indicate the reliability of unmodified factor VII-deficient beagle plasma in the assay of both high and low human factor VII levels. There appears to be no need for the plasma to be pretreated to reduce the 'high levels of factor V' as suggested by Garner and Conning (1970). Such manipulation appears undesirable as artefactual changes are introduced and the level of factor V and other clotting factors cannot be rigidly controlled. The degree of elevation of factor V is in any case doubtful as previously mentioned in part 1, because of species specificity.

Part III Quality control of tissue thromboplastin extracts by factor VII-deficient plasma

In Britain, the availability of a national reference thromboplastin (British Comparative Thromboplastin) provides the basis of a uniform system of reporting prothrombin times and providing anticoagulant dosage. The provision of a uniform thromboplastin extract, reproducible between batches, is achieved by the strict system of quality control at the production centre and at the independent monitoring laboratories (Poller, 1970; Thomson and Chart, 1970). At the production centre one part of the quality control depends upon the use of factor VII-deficient plasma to screen new batches for sensitivity to depression of this factor. Unfortunately there is no reproducibility between batches of the VII-deficient plasma. A stable, reproducible factor VII-deficient plasma would be of considerable value in the quality control of the BCT. It would likewise be an invaluable check against deterioration on storage of the several different lyophilized international reference preparations of thromboplastin held on behalf of WHO (Biggs and Bangham, 1971). The general availability of a plasma reference preparation, reproducible between batches, would also assist hospitals which use oral anticoagulants. This would be a quality control of factor VII sensitivity of their commercial or home-made thromboplastin reagent and would be an invaluable check against deterioration. The potential value of lyophilized plasma reference preparations in the quality control of prothrombin time performance at individual hospitals has already been discussed (Poller and Thomson, 1968; Miale and LaFond, 1969).

Method of Study

The lyophilized plasma from the factor VII-deficient 'pool' of 10 beagles was used to characterize a number of available thromboplastin extracts and several of the (Biggs and Bangham, 1971) reference preparations. The reagents were prepared according to the manufacturers' instructions. A normal prothrombin time was first performed on a 'pool' of three healthy normal adults with each of the reagents to ensure that the material had been reconstituted properly and was still active. A prothrombin time was then performed on plasma from a normal beagle dog and then each thromboplastin was tested in turn with the factor VII-deficient plasma and the dog prothrombin ratio determined.

The following reference preparations were tested: British Comparative Thromboplastin; Oxford Re-

agent (ref. 67/40); two saline extracts of human brain supplied from Withington Hospital, Manchester, and lyophilized at Mill Hill (ref. 69/223, 69/179); and a variety of commercial extracts, including Thrombotest. For the sake of interest, a 'home-made' extract of beagle brain was also included. Results are given in Figures 3 and 4. In Fig. 4 the BCT is arbitrarily given a value of unity and the other thromboplastins are expressed in terms of the BCT value of 1.

Comment

The varying sensitivity of the tissue extracts to factor

Fig. 3 Characterization of tissue thromboplastins by beagle factor VII-deficient plasma expressed as prothrombin ratios.

Fig. 4 The relative sensitivity of available tissue thromboplastins to the factor VII deficiency in beagle dogs expressed in terms of the BCT value of 1.

VII deficiency is apparent. Human brains appear the most sensitive, a surprising finding being that dog brain, carefully prepared, is less sensitive to dog factor VII. Therefore the greater sensitivity of human brain is not just due to species-specificity. The least sensitive is the reagent made from human placenta.

If beagle factor VII-deficient plasma becomes available in large volumes for general use, it may help to screen out insensitive tissue thromboplastin reagents used for controlling oral anticoagulant treatment. It should be a valuable check against deterioration of stored preparations.

Special thanks are due to Dr B. J. Leonard, consultant haematologist, ICI Pharmaceuticals, Alderley Park, who suggested this study to us and whose helpful cooperation made it possible.

We are also grateful to Mr W. Hiddlestone, the veterinary surgeon and his staff, for assistance in obtaining blood samples.

References

- Biggs, R., and Bangham, D. R. (1971). Standardisation of the one-stage prothrombin time test for the control of anticoagulant therapy. *Brit. Med. J.*, 3, 441.
- Denson, K. W. (1961). The specific assay of Prower-Stuart factor and factor VII. *Acta haemat. (Basel)*, 25, 105-120.
- Egeberg, O. (1961). Assay of antihemophilic A, B and C factors by one-stage cephalin systems. *Scand. J. clin. Lab. Invest.*, 13, 140-152.
- Garner, R., and Conning, D. M. (1970). The assay of human factor VII by means of modified factor VII deficient dog plasma. *Brit. J. Haemat.*, 18, 57-66.
- Garner, R., Hermoso-Perez, C., and Conning, D. M. (1967). Factor VII deficiency in beagle dog plasma and its use in the assay of human factor VII. *Nature (Lond.)*, 216, 1130-1131.
- Hjort, P., Rapaport, S. I., and Owren, P. A. (1955). A simple, specific one-stage prothrombin assay using Russell's viper venom in cephalin suspension. *J. Lab. clin. Med.*, 46, 89-97.
- Loeliger, E. A., Meeuwisse-Braun, J. B., Muis, H., Buytendijk, F. J. J., Velkamp, J. J., and Hemker, H. C. (1970). Laboratory control of oral anticoagulants: definition of therapeutic range in terms of different thromboplastin preparations. *Thrombos. Diathes. haemorrh. (Stuttg.)*, 23, 569-584.
- Miale, J. B., and LaFond, D. (1969). Prothrombin time standardisation. *Amer. J. clin. Path.*, 52, 154-160.
- Poller, L. (1964). Standardisation of anticoagulant treatment: the Manchester regional thromboplastin scheme. *Brit. med. J.*, 2, 565-566.
- Poller, L. (1970). The British system for laboratory control and reporting of prothrombin results. *Pakistan med. Forum*, 5, 19-24.
- Poller, L., Tabiwo, A., and Thomson, J. M. (1968). Effects of low-dose oral contraceptives on blood coagulation. *Brit. med. J.*, 3, 218-219.
- Poller, L., and Thomson, J. M. (1964). Evidence for 'rebound' hypercoagulability after stopping anticoagulants. *Lancet*, 2, 62-64.
- Poller, L., and Thomson, J. M. (1966). Clotting factors during oral contraception: further report. *Brit. med. J.*, 2, 23-25.
- Poller, L., and Thomson, J. M. (1968). National survey of value of plasma standards for anticoagulant therapy. *Brit. med. J.*, 2, 88-90.
- Poller, L., Thomson, J. M., and Priest, C. M. (1969). Coumarin therapy and platelet aggregation. *Brit. med. J.*, 1, 474-476.
- Prydz, H. (1963). Studies on proconvertin. I. Gel filtration. *Scand. J. clin. Lab. Invest.*, 15, 450-456.
- Thomson, J., and Chart, I. S. (1970). The system for laboratory control of oral anticoagulant therapy using the British Comparative Thromboplastin. *J. med. Lab. Technol.*, 27, 207-212.
- Wolf, P. (1953). A modification for routine laboratory use of Stefani's method of estimating factor V activity in human oxalated plasma. *J. clin. Path.*, 6, 34-38.