Haemophilia and its related conditions: a brief guide to diagnosis and treatment

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MEDICAL RESEARCH COUNCIL

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Preface

This pamphlet supersedes MRC Memorandum No. 32, published in 1955 under the title *The Diagnosis and Treatment of Haemophilia and Its Related Conditions*, and takes account of all the important advances of the last twenty years. The earlier pamphlet was produced by Dr R. G. Macfarlane and Dr Rosemary Biggs (then working on the mechanisms of coagulation and its abnormalities at the Radcliffe Infirmary, Oxford) at the request of the Council's Haemophilia Committee. It was this committee that proposed the creation of Haemophilia Reference Centres to ensure uniformity of diagnostic standards and to coordinate information. The committee also introduced the provision of special medical cards for haemophilic patients and the organisation of a registry of haemophiliacs.

Since 1964 the Health Departments have had the sole responsibility for the reference centres and the Council has ceased to be involved in treatment. Research into haemostatic defects and their treatment continues, however — although the MRC Blood Coagulation Research Unit was disbanded in 1967 on the retirement of Professor Macfarlane as director, Dr Biggs, who had been one of the members of the Unit, is doing part-time research for the Council while directing the Oxford Haemophilia Centre.

The memorandum is designed as an introduction to the diagnosis and treatment of patients with haemostatic defects and is by no means a complete guide. It is intended to help in emergency treatment in consultation with experts, to improve treatment in parts of the world where specialist centres are not in operation and to counteract a modern misconception about the treatment of patients with haemorrhagic states. Some people believe that the greater availability of therapeutic materials nowadays has outdated the need for treatment at specialist centres. The author hopes that this outline will show that there is much more to the treatment of haemophilia than the administration of concentrates.

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Introduction

PATIENTS with congenital haemostatic defects who are prone to bleed excessively, even dangerously, after minor injuries or operations are not uncommon in medical practice. It is important that this abnormality and its significance be recognized, since many deaths have resulted from subjecting such patients to operation without adequate therapy. Such patients may also suffer crippling deformity from bleeding into the musculo-skeletal system that early and effective treatment can prevent. Current policy in Britain is for patients to be treated in special Haemophilia Centres, a list of which appears in Appendix 2. Patients may, however, live far from a Haemophilia Centre or in a country in which such centres have not been developed and it is therefore desirable that there should be a general understanding of patients with congenital haemostatic defects. Even where a special centre exists a tentative diagnosis must be made before a patient can be referred to it and a patient who is suddenly ill or injured may be in no condition to travel. The purpose of this pamphlet is to serve as a guide to the preliminary diagnosis and emergency treatment of patients with congenital haemorrhagic states for those medical practitioners who may encounter such patients but who do not specialize in this field.

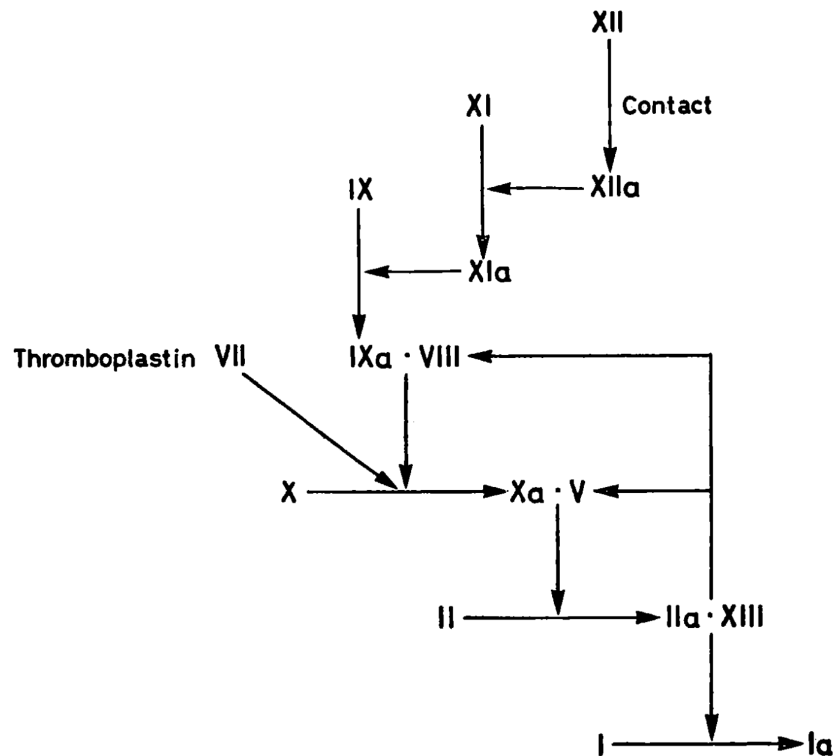

Figure 1. A hypothesis of the blood coagulation mechanism

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Types of excessive bleeding due to a breakdown of the haemostatic mechanism may, for practical purposes, be divided into those resulting from defects of the blood clotting mechanism and those that may be classed as platelet and vascular defects. The latter category is not well defined and includes many patients with minor bleeding disorders.

The blood coagulation system is complex, involving a chain of sequential reactions and alternative pathways. A scheme which may represent these reactions appears in Figure 1. Two main pathways that may lead to clotting are proposed: one involves only blood constituents (the Intrinsic Pathway); the other involves the addition of tissue extract (the Extrinsic Pathway). The pathways have a common termination. The agreed international nomenclature, in which the factors appear as roman numerals has been used in Figure 1. In Table I some common synonyms for the various factors are listed, together with the disease states that arise from lack of one or other of the factors.

Table I

The Roman numerical nomenclature of blood clotting factors, together with some common synonyms and the disease states associated with the coagulation defects

FACTOR	SYNONYMS	DISEASE STATES ASSOCIATED WITH COAGULATION DEFECT
I	Fibrinogen	Afibrinogenaemia; fibrinopenia
II	Prothrombin	Prothrombin deficiency
V	Accelerator globulin; proaccelerin; labile factor	Factor V deficiency
VII	Proconvertin; stable factor; autoprothrombin I	Factor VII deficiency
VIII	Antihaemophilic factor; antihaemophilic globulin; platelet co-factor I; antihaemophilic factor A	Haemophilia A; haemophilia; von Willebrand's disease
IX	Christmas factor; plasma thrombo-plastin component (PTC); platelet co-factor II; autoprothrombin II; antihaemophilic factor B	Haemophilia B; Christmas disease
X	Stuart-Prower factor	Stuart-Prower defect; factor X deficiency
XI	Plasma thromboplastin antecedent (PTA)	PTA deficiency
XII	Hageman factor	Hageman defect
XIII	Fibrin stabilising factor	Factor XIII deficiency

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The various defects in the coagulation mechanism are all rare but some are much rarer than others. The result of an analysis of patients seen at the Oxford Haemophilia Centre emphasizes this difference in frequency (Table II). It may be seen that the coagulation defects most frequently diagnosed and treated are factor VIII deficiency (haemophilia and von Willebrand's disease) and factor IX deficiency (Christmas disease). The other defects have to be borne in mind in making a diagnosis but will not often be encountered. The main clinical features of the more important defects are summarized in Table III.

Table II

Analysis of patients registered at the Oxford Haemophilia Centre on 31.12.72, showing the frequency of the various defects as a percentage of the total number of patients registered at the Centre and the probable incidence of the defects in the population.

DIAGNOSIS	NO. OF PATIENTS	% OF PATIENTS REGISTERED	PROBABLE INCIDENCE IN THE POPULATION PER MILLION
Factor VIII deficiency (Haemophilia)	779	62	30–40
Factor VIII deficiency (von Willebrand's disease)*	200	16	10–20
Factor IX deficiency (Christmas disease)	161	13	6–8
Factor XI deficiency (PTA deficiency)	17	1	Varies with the size of the population of Jewish descent
Platelet defects	44	4	2–4
Circulating anticoagulants	29	2	1–2
Other defects (including factors I, II, V, VII, X and XIII)	24	2	1–2
TOTAL:	1254	100	

*The category of von Willebrand's disease contains many mildly affected patients

The sections that follow describe the clinical and laboratory diagnosis and the treatment of patients in the order in which they should be carried out in practice. The first step is usually to talk with the patient, and to make a clinical study that involves noting personal and family history and observing any signs of present or past bleeding. The next steps are laboratory diagnosis and deciding on treatment.

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Table III

Clinical features of coagulation defects

DEFECT		SYMPTOMS	INHERITANCE	LEVEL OF MISSING FACTOR %
Haemophilia (factor VIII deficiency) / Christmas disease (factor IX deficiency)	Severe	Spontaneous haemarthroses; intramuscular haematomata; disastrous post-operative bleeding	Sex-linked recessive	0–1
	Mild	Haematomata and haemarthroses after injury; severe post-operative bleeding		5–25
von Willebrand's disease (factor VIII deficiency)		Epistaxis; menorrhagia; gastro-intestinal bleeding; post-operative bleeding	Autosomal dominant	0–50
PTA (factor XI) deficiency		Menorrhagia; epistaxis; bruising; gastro-intestinal bleeding; post-operative bleeding	Autosomal and partially dominant	The degree of severity is not related to *in vitro* tests of clotting function
Deficiency of factors I, II, V, VII, X, XIII		Variable symptoms	Autosomal recessive	0–5

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I. Clinical diagnosis

THE RECOGNITION OF A HAEMORRHAGIC STATE

The initial diagnosis of a haemorrhagic state is usually made by the clinician. The patient or his family may consult a doctor because he has excessive bleeding from small injuries, epistaxis, oozing from the gums, menorrhagia, gastro-intestinal haemorrhage, haematuria, easy bruising or purpura. The patient may have a history of undue bleeding after tooth extraction, tonsillectomy or some other operation, even in the absence of other haemorrhagic episodes. He may complain of symptoms that he does not connect with abnormal haemorrhage, for example deep-tissue swellings and painful or swollen joints. The doctor must then determine whether or not these manifestations indicate a generalized haemorrhagic state.

Points tending to confirm the existence of such a state are haemorrhages affecting different parts of the body, a long history of abnormal bleeding, particularly if it extends from early childhood, and a history of a similar abnormality in one or more blood relations. Points somewhat against the diagnosis of a haemorrhagic state are the limitation of bleeding to a particular site and the presence of symptoms suggesting a local cause for haemorrhage. This rule is not absolute since the first haemorrhage in any patient is likely to be localized and since the site of bleeding is to some extent related to the aetiology. Many cases of repeated epistaxis, gastro-intestinal haemorrhage or menorrhagia have been found to be due to an underlying general haemostatic defect. In the absence of a demonstrable organic cause for localized bleeding, therefore, the possibility of the existence of a haemorrhagic state should be investigated.

In assessing the severity of the condition, care must be taken to give proper weight to the patient's description of his tendency to bleed. A complaint of easy bruising may mean little: a large proportion of normal women will state that they bruise easily after trauma. Patients may maintain that they bleed excessively after tooth extraction but the standards by which such bleeding is judged vary greatly. Some normal people may experience more or less continuous oozing of blood from a tooth socket for some time, perhaps for as much as 24 hours, after extraction, but it is probably abnormal for haemorrhage to continue after 48 hours unless a definite local cause, such as acute sepsis, is responsible. Bleeding after throat or nose operations may also persist for several hours, even in the absence of a haemorrhagic state, but, again, oozing that continues for more than 24 hours should be regarded with suspicion. Any patient who requires transfusion after tooth extraction is probably suffering from a haemorrhagic diathesis. Conversely, any patient who has undergone a major surgical operation (including tonsillectomy), or the removal of several molar teeth without undue haemorrhage is unlikely to have been suffering from a severe generalized haemorrhagic disorder at that time. In the absence of severe trauma the occurrence of massive deep-tissue haematoma indicates a haemorrhagic state. Thus a carefully taken history covering all major haemorrhagic episodes and the results of any injuries or surgical operations is an essential basis for diagnosis.

Since many haemorrhagic states are hereditary, compiling the family history also requires care. It is not enough to ask "Does anyone else in your family bleed abnormally?" A family tree should be drawn up going back at least to the patient's grandparents and positive information should be sought for

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each member. In the rarer types of clotting defect and in the case of haemophilic families, the female members of which are said to bleed excessively, attention should be paid to the possibility of consanguinity.

In large families it is fairly easy to distinguish the various types of inheritance, which are illustrated diagrammatically in Figure 2. In autosomal dominant inheritance males and females are equally commonly affected and an affected male or female passes the disease to all or to half the children (see Figure 2*a*), depending on whether the genetic constitution is heterozygous or homozygous. In autosomal recessive inheritance both mother and father appear normal but both must be carriers of the trait if children are affected. One quarter of the children of either sex will be affected. Clearly, consanguineous marriage will increase the probability of autosomal recessive disease (see Figure 2*b*).

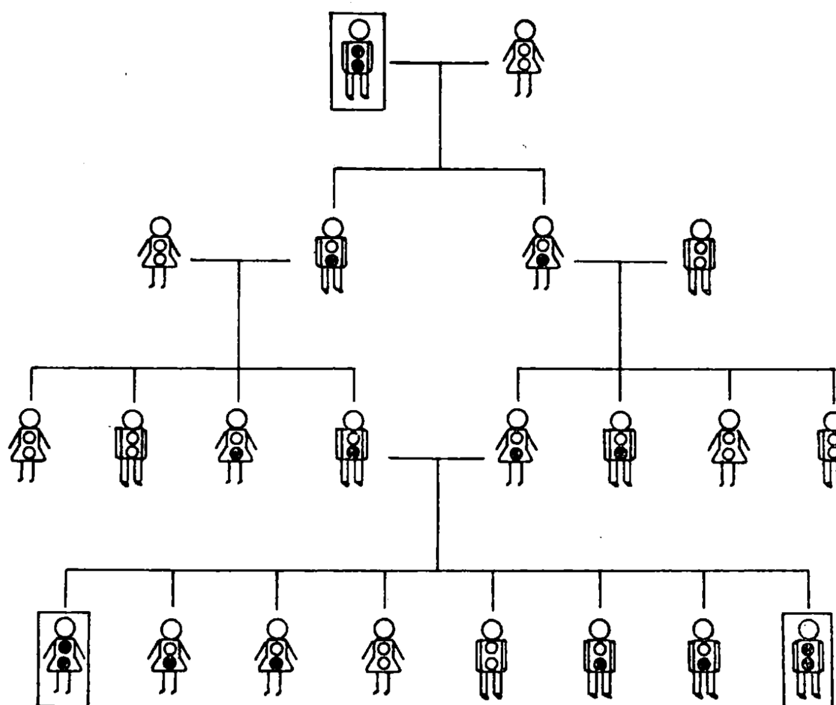

Figure 2. The inheritance of blood clotting disorders:
(*a*) Autosomal dominant inheritance. Individuals enclosed in boxes are homozygous; all individuals with the abnormal gene are clinically affected.

Sex-linked recessive inheritance gives rise to the most complex possibilities (see Figure 2*c*). The disease may pass unnoticed through several generations of carrier females. A normal male, whatever the genetic constitution of his parents, can never pass the disease to his children. An affected male will always have seemingly normal children. All his sons will be free of the disease but all his daughters will be carriers. Thus the evidence of sex-linked inheritance should be sought in the mother's family where uncles, cousins and grandfather may be affected. A father-to-son transmission of haemorrhagic tendency excludes this form of inheritance.

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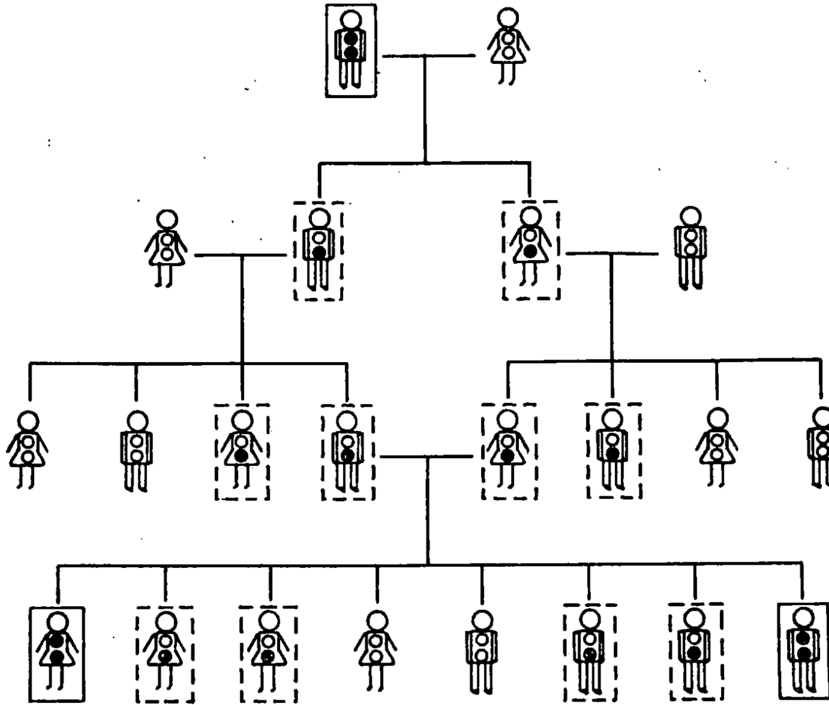
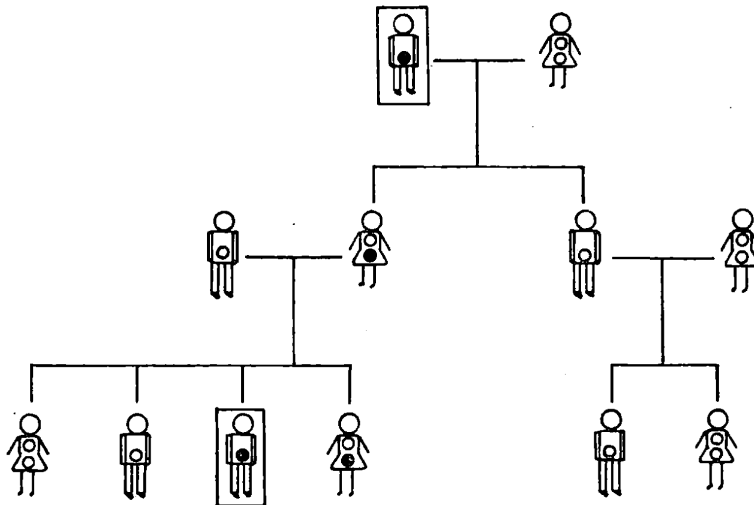


Figure 2. The inheritance of blood clotting disorders:

(*b*) Autosomal recessive inheritance. Individuals enclosed in solid boxes are homozygous and clinically affected. Individuals enclosed in discontinuous boxes are heterozygous and not clinically abnormal.



(*c*) Sex-linked recessive inheritance. Individuals enclosed in solid boxes are clinically affected.

O Normal gene

● Defective gene

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Much emphasis is to be placed on establishing an accurate clinical history. On the clinical findings alone, it is possible to make some separation between patients with coagulation defects and those classed as having capillary and platelet disorders. For example, immediate bleeding after injury, oozing from mucous surfaces (epistaxis and gastro-intestinal bleeding) and a dominant pattern of inheritance are features of the capillary–platelet disorders, while haemarthroses and delayed bleeding after injury are features of coagulation defects. These and other differences are summarized in Table IV.

Table IV

Clinical features of patients with haemostatic defects

NATURE OF ENQUIRY	COAGULATION DEFECT	CAPILLARY DEFECT
Bruising	Large bruises occur	Small bruises common
Haemarthroses	Common in severely affected patients and often the main feature	Very uncommon
Epistaxis	Seldom a predominant symptom	Often a major source of bleeding
Gastro-intestinal bleeding	Seldom a predominant symptom unless peptic ulceration is also present	Often a major source of bleeding
Haematuria	Common	Uncommon
Menorrhagia	Uncommon because most patients are males	Common
Dental bleeding	Starts 1–4 hours postoperatively; lasts 3–40 days and is not controlled by pressure	Starts immediately. Lasts 24–48 hours and is often controlled by pressure
Post-operative bleeding	Late bleeding with wound haematoma formation is characteristic	Bleeding occurs mainly at the time of operation and is less severe than in patients with coagulation defects
Onset of bleeding after trauma	Usually late (1–4 hours after the event)	Usually immediate
Symptoms of the mildly affected patient	Large haematoma following injury. Persistent and often dangerous bleeding after trauma	Epistaxis and menorrhagia
Inheritance	Most are sex-linked and recessive	Mainly dominant

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Capillary–Platelet Disorders

The commonest defect in this group is essential thrombocytopenic purpura, which is not discussed here because it is well described in all general haematological text books. The best defined condition apart from essential thrombocytopenia is von Willebrand's disease, first described in 1931 by von Willebrand as a dominantly inherited bleeding tendency affecting males and females and associated with a long bleeding time. Observers have since found a low plasma concentration of factor VIII in many of these patients. In addition the ability of platelets of these patients to adhere to glass is deficient (Murphy, 1969), the degree of deficiency being related to the length of the bleeding time. Patients severely affected with von Willebrand's disease are easy to categorize but mildly affected patients with varying degrees of clinical severity and a corresponding variability of laboratory findings seem to merge into the normal population. Some may have a long bleeding time and normal plasma concentration of factor VIII, others may have a somewhat low plasma concentration of factor VIII and defective platelet adhesiveness; at the end of the spectrum, merging with the normal population, all laboratory tests may be negative but there may be a history of menorrhagia or post-operative bleeding with a dominant type of inheritance in other members of the family. There is of course no guarantee that all these patients have diseases of the same aetiology, indeed this seems rather unlikely. An understanding of the condition of mildly affected patients is important since they are very commonly encountered.

It is clear that von Willebrand's disease in its more severe forms is partly a coagulation defect (factor VIII deficiency) and partly a capillary defect characterized by a long bleeding time.

Qualitative Platelet Defects

A small group of patients whose symptoms are similar to those of von Willebrand's disease have demonstrably abnormal platelet function. The defect is associated with a long bleeding time, variable plasma factor VIII concentration and certain abnormalities in platelet function. The platelets may not aggregate on the addition of ADP as normal platelets do, they may not accelerate clotting in the presence of kaolin, they may not have normal contact factor activity, clot retraction may be abnormal and the platelets may not adhere normally to glass.

Coagulation Defects

The most important of the coagulation defects are haemophilia and Christmas disease, or factor VIII and factor IX deficiencies (see Table II). The inheritance of both is of the sex-linked recessive type (see Table III). The symptoms of the two diseases are identical and their severity is strongly related to the degree of the factor deficiency shown by *in vitro* test systems. In the severely affected patient in whom the plasma concentration of factor VIII or IX is less than 1 per cent, apparently spontaneous haemorrhages occur into joints and muscles. If not promptly treated, these haemorrhages may give rise to crippling deformities. Post-operative bleeding untreated by specific factor therapy is usually disastrous.

Mildly affected patients usually have 5 to 25 per cent of factor VIII or IX and do not have spontaneous haemarthroses or haematomata. These patients bleed severely after trauma or operation unless adequately treated. In everyday life the patient may appear so normal that the defect may pass unnoticed for

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many years before being disclosed as the result of an accident, dental extraction or major surgery. Patients with 1 to 5 per cent of factor VIII or IX are intermediately affected.

Plasma thromboplastin antecedent (PTA or factor XI) deficiency is usually found in people of Jewish ancestry and the incidence in any one area thus depends on the size of the Jewish population. It is common in Israel and quite uncommon in Britain. The severity of bleeding cannot be predicted from the results of laboratory tests. Patients with less than 1 per cent of the factor as tested may have no symptoms of bleeding. Other patients with 20 per cent as tested may bleed excessively.

The expression of the bleeding tendency in the rarest defects (deficiency of factors I, II, V, VII, X or XIII) is very variable. Haemarthroses are uncommon, though epistaxis, excessive bruising, menorrhagia and post-operative bleeding all occur. Some patients with factor VII deficiency do have haemarthroses but do not bleed excessively after operation.

Two rare states that can be confused with haemophilia and its related conditions can readily be recognized by the clinician. The first is haemorrhagic telangiectasia, which may cause repeated and profuse epistaxis and, more rarely, haemoptysis or haematemesis. In almost every case small telangiectatic lesions may be observed on the lips, tongue and fauces or in the nose and sometimes in the skin, particularly around the finger nails. These lesions can be distinguished from petechial haemorrhages in that they blanch and disappear from view under pressure, which can be conveniently applied with the corner of a glass slide. Haemorrhagic telangiectasia affects both sexes and is inherited as an autosomal dominant. In some instances it may be associated with von Willebrand's disease and in these cases the bleeding is most severe. Bleeding in patients with telangiectasia tends to increase with age, and symptoms may not appear until middle age.

The second rare condition is the Ehlers-Danlos syndrome, which may cause troublesome haemorrhage from wounds and operation sites and may also be associated with epistaxis and menorrhagia. The haemorrhage in this condition is probably due to a fault in the formation of collagen which renders the vessels friable and prevents firm healing. It can be recognized by the hyper-extensibility of the joints, and the stretching of scar tissue. The fingers, for instance, can be bent back to an abnormal degree, the thumbs may often be 'double-jointed', and the elbows can be hyper-extended. In the fully developed condition peculiar stretched scars and pouches of skin around the knees and elbows will nearly always be found. The Ehlers-Danlos syndrome is also inherited as an autosomal dominant.

Patients with factor XII deficiency do not bleed abnormally.

DIFFERENTIAL DIAGNOSIS OF HEREDITARY AND ACQUIRED COAGULATION DEFECTS

This pamphlet is concerned mainly with the congenital haemostatic defects. Acquired defects are mentioned briefly here because they are fairly common and must be distinguished from the hereditary diseases.

If the haemorrhagic state has existed from infancy it is likely to be hereditary, even if there is no history of other members of the family being affected. In about 40 per cent of cases of haemophilia, for instance, the defect has apparently arisen *de novo* either in the patient himself or in his mother, there being no evidence of other affected relatives. It is clear that in the absence of a family

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history no genetic information is available to indicate whether the condition is haemophilia or Christmas disease, or one of the much rarer conditions due to deficiency of factors I, II, V, VII, X, XI or XIII.

If this is the case, and the condition is severe and has arisen in late childhood or in adult life, it is unlikely to be hereditary and an attempt should be made to discover some immediate cause. Circulating anticoagulants, which are antibodies that destroy factor VIII, may have developed and may be giving rise to a haemophilia-like state. They sometimes appear during or after pregnancy and may be due to some foetal–maternal antigenic incompatibility. In other instances the inhibitor is associated with diseases that have an auto-immune element (for example, penicillin sensitivity reactions, pemphigus, Crohn's disease, haemolytic anaemia, etc.).

Excessive intravascular clotting associated with fibrinolysis may occur in association with abruptio placentae, intrauterine foetal death, massive operations, some cases of carcinoma with systemic dissemination, and cases of leukaemia. In the latter condition there may be a reduction in platelet count and low concentrations of factors I, V, VIII and XIII.

Vitamin K deficiency and liver disease may both give rise to haemorrhagic states associated with reduction in the blood of clotting factors II, VII, IX and X. Haematuria, excessive bruising, gastro-intestinal bleeding and oozing from venepuncture or operation sites may all be symptoms of these conditions.

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II. Laboratory diagnosis

The recognition of haemophilia and its differentiation from other related conditions by laboratory tests is a specialist activity. The tests when well standardized will give reliable and specific diagnosis in the majority of patients but some of the tests are complicated and quite uneconomic to set up in laboratories where at most one or two patients are seen in a year. In many laboratories it may not be reasonable to proceed to a complete and specific diagnosis. In that case preliminary tests only should be carried out and, on the results of these and of the clinical study, the patient referred to a specialist. To some people, however, the study of these patients has an interest that outweighs economic considerations and, more important, there are parts of the world where referring a patient to a specialist centre is not easily possible. For these reasons the main steps in specific diagnosis have been given in the ensuing discussion and the simpler laboratory tests are described in detail in Appendix 1.

PRELIMINARY INVESTIGATIONS

The clinical history, discussed in the previous section, will give a good indication of the most valuable laboratory tests in a particular patient. If a capillary type of bleeding disorder is indicated by the history, preliminary investigations should include a platelet count, measurement of bleeding time, a tourniquet test, measurement of whole blood clotting time, a prothrombin consumption test and measurement of the kaolin–cephalin clotting time (KCCT). In the absence of any clinical history suggestive of a severe haemostatic defect, normal results exclude a severe bleeding disorder. Negative results do not, however, necessarily indicate that the patient is haemostatically normal; in expressing an opinion about the presence or absence of impaired haemostasis due weight must always be given to the clinical history of abnormal bleeding. For example, a patient who has required transfusion after dental extraction nearly always has a faulty haemostatic mechanism and, even if laboratory tests are all normal, it would be unwise to assume a normal post-operative course after future operations. Laboratory tests are usually negative in telangiectasia.

If the clinical history suggests that a clotting defect may be present or if the Lee and White clotting time, prothrombin consumption test, one-stage prothrombin time or KCCT is abnormal the problem is to distinguish between the different sorts of clotting defects. These may for convenience be divided into two groups: first, those whose whole plasma gives a long (abnormal) one-stage prothrombin time and second, those whose one-stage prothrombin time is normal. The first group includes deficiencies of fibrinogen, prothrombin, and factors V, VII and X, and may also include certain cases of circulating anticoagulant in patients with lupus erythematosus. The second group includes haemophilia and von Willebrand's disease, factor VIII deficiency, factor IX deficiency (Christmas disease), factor XII deficiency (Hageman factor deficiency) and plasma thromboplastin antecedent (PTA) or factor XI deficiency. Cases with circulating anticoagulants that destroy factor VIII are also included in this category. The one-stage prothrombin time may also be normal in some patients with liver disease who lack prothrombin. Laboratory tests used in diagnosis must distinguish between these types of clotting defects and, when possible, assess the degree of abnormality.

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GROUP WITH ABNORMAL ONE-STAGE PROTHROMBIN TIME

The five conditions in the group with an abnormal one-stage prothrombin time may be characterized as follows:

Fibrinopenia (Factor I Deficiency)

In the one-stage prothrombin time test, the plasma may not clot at all or else only a very small, wispy clot may form. Either of these findings should suggest fibropenia. The diagnosis can be confirmed as follows: if thrombin is added to plasma totally lacking in fibrinogen, no clot is formed. When one volume of saturated $(NH_4)_2SO_4$ is added to two volumes of plasma, giving one-third saturation, a precipitate appears if fibrinogen is present. If no fibrinogen is present no precipitate will form.

In the acute defibrination syndrome fibrinogen is reduced, probably because it has been removed by intravascular coagulation. The defect may be detected by observing poor or absent clotting in the whole blood clotting time or by using the fibrinogen titre test.

Prothrombin Deficiency (Factor II Deficiency)

A deficiency of prothrombin can be confirmed by the two-stage prothrombin test. This test should always be carried out in cases of liver disease.

Factor V Deficiency

When aluminium hydroxide is added to normal plasma it absorbs prothrombin (factor II) and factors VII, IX and X. Thus if the plasma is centrifuged after the addition of aluminium hydroxide, the supernatant contains factor V and factor VIII but not factors II, VII, IX and X. If factor V is deficient from the patient's plasma, the addition to his plasma of 10 per cent of aluminium hydroxide-absorbed normal plasma will appreciably shorten the one-stage prothrombin time. The degree of abnormality may then be assessed by a quantitative assay of factor V.

Deficiency of Factors VII and X

Normal stored serum contains factors VII and X but not factor V. Thus if either factor VII or factor X is deficient the addition of 10 per cent of stored serum will shorten the one-stage prothrombin time. A distinction between the two defects can be made using Russell's viper venom and phospholipid as a source of thromboplastin in the one-stage prothrombin time. In factor VII deficiency the plasma has a normal one-stage prothrombin time in the presence of Russell's viper venom and phospholipid, whereas in factor X deficiency the one-stage prothrombin time is prolonged. Techniques have been devised to improve the sensitivity of the Russell's viper venom method for the assay of factor X. Quantitative assays of factors VII and X will confirm the diagnosis.

Combined Deficiency of Factors II, VII, IX and X and Liver Disease

Factors II, VII, IX and X are all reduced in vitamin K deficiency and after the administration of drugs of the coumarin type. Very rarely a similar defect may be congenital.

In liver disease all four factors are often reduced together. Following the administration of vitamin K, factors VII, IX and X may return to normal but prothrombin (factor II) remains decreased. In these circumstances the one-stage prothrombin time may be normal and diagnosis is established using the two-stage prothrombin test or the assay of prothrombin using the Taipan venom.

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Inhibitors that Affect the One-stage Prothrombin Time

The presence of heparin will lengthen the one-stage prothrombin time. Naturally occurring inhibitors of this type are extremely rare and patients who have received heparin therapeutically should present no problem in diagnosis. The plasma of some patients with disseminated lupus erythematosus may have a long one-stage prothrombin time associated with the presence of a circulating anticoagulant. Usually the kaolin–cephalin clotting time and the Lee and White clotting time are also prolonged and a mixture of equal parts of the patient's plasma with normal plasma gives a long kaolin–cephalin clotting time. Assays for specific clotting factors, with the exception of prothrombin, which is often reduced, are usually normal. The inhibitor does not destroy any known clotting factor but interferes with various clotting reactions in a manner not yet completely elucidated.

GROUP WITH NORMAL ONE-STAGE PROTHROMBIN TIME

In the group with a normal one-stage prothrombin time are included deficiencies of factor VIII (haemophilia and von Willebrand's disease), factor IX (Christmas disease), factor XI (PTA deficiency) and factor XII (Hageman factor deficiency). Distinction between these defects in severely affected patients can often be made using the thromboplastin generation test. However this test only records levels of factors VIII and IX that are higher than 10 per cent of normal, thus mildly affected patients may be missed. Moreover, the test does not record a consistent abnormality for patients with Hageman factor or PTA deficiency. In addition, diagnosis in all these patients should involve an assessment of the degree of abnormality, so that assay methods for specific factors are to be preferred for diagnosis.

Haemophilia

In haemophilia, by far the commonest of the hereditary haemorrhagic states, factor VIII is lacking. Quantitative assay of factor VIII may be based either on a one-stage method using the kaolin–cephalin clotting time or on the two-stage method, which is a modification of the thromboplastin generation test.

Von Willebrand's Disease

Some patients severely affected with von Willebrand's disease have a low plasma concentration of factor VIII. When this diagnosis is possible the factor VIII concentration should always be measured in addition to the bleeding time test and platelet count.

Patients with Antibody to Factor VIII

About 5 per cent of severely affected haemophilic patients develop circulating antibodies that destroy factor VIII. Patients with these circulating anticoagulants will not respond in the expected manner to treatment. No surgical operation should be undertaken in a haemophilic patient unless a test for factor VIII antibody has been carried out. When an antibody is present quantitative tests should be made of the potency of the antibody to both human and animal factor VIII concentrates, since the antibody will very often destroy human factor VIII at a much higher dilution much faster and more thoroughly than factor VIII of animal origin and in emergency the animal material may prove clinically effective. Occasionally very similar antibodies may appear in the blood of previously normal people (see p.11).

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Christmas Disease
In this condition factor IX is lacking. Specific assay of this factor is based either on the one-stage method using the kaolin-cephalin clotting time or on a two-stage method using a modification of the thromboplastin generation test. This assay should be done if a coagulation defect seems likely and if assay of factor VIII has given normal results. Very rarely, patients with Christmas disease may develop circulating anticoagulants that destroy factor IX.

Plasma Thromboplastin Antecedent (Factor XI) Deficiency and Deficiency of Hageman factor (Factor XII)
Presumptive diagnosis of factor XI deficiency can be made using a variety of test systems. The Lee and White clotting time and kaolin–cephalin clotting time are both prolonged. Prothrombin consumption is also usually abnormal. If assays for factor VIII and IX are normal the patient probably has a defect of the contact system (factor XI or factor XII).

Exact diagnosis depends on the use of plasma samples with known deficiencies. If these are not available it is best to send a plasma sample for testing to a reference laboratory at which diagnosis may be established.

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III. Treatment

Haemophilic patients (and those with other coagulation defects) are at greater risk than normal people from the trauma associated with everyday life. Since the disease is rare it is important for the patient to have ready access to a specialist who understands the defect and who is available day and night to give advice. The disease affects every aspect of life from infant care through schooling to marriage and the nature of employment undertaken. It is thus important for the patient to be registered at a Haemophilia Centre. If treatment is undertaken elsewhere the Director of the Haemophilia Centre should be consulted. The following notes are on practical issues that concern all patients and about which questions are often asked.

GENERAL MANAGEMENT OF PATIENTS WITH HAEMOPHILIA AND OTHER SERIOUS COAGULATION DEFECTS

Patients with haemorrhagic states can receive most ordinary drugs safely but must *never* be given them by intra-muscular injection. Immunization by intradermal or subcutaneous injection and vaccination is safe but the site should be inspected daily until healing is complete. Drugs affecting platelet function—for instance aspirin—should never be used since these may promote serious bleeding in patients with coagulation defects.

Modern treatment aims to give the patient as nearly normal a life as possible, but a good deal of understanding and administration will be required to allow a severely affected child to attend a normal school and take normal holidays. The teacher must understand the defect. The ambulance service must be ready to take the child direct to a doctor who understands the treatment. For holidays parents and doctor must have made pre-arrangements for treatment at the holiday centre. This social work is a normal aspect of Haemophilia Centre activity and cannot be neglected.

Many haemophilic patients have unnecessarily decayed teeth. Conservative measures such as filling and gentle scaling are quite safe for these patients and they should have more frequent routine visits to the dentist than normal people. We recommend four visits a year.

Common types of bleeding requiring urgent treatment are haemarthroses, deep haematamata and any severe intra-abdominal pain. Accidents involving a blow on the head followed by headache may presage intra-cranial bleeding and should be treated without delay. Haematuria does not have the same serious implication in the haemophilic patient as in a haemostatically normal person, but repeated or prolonged haematuria may damage the kidneys and should be treated at once.

The patient should be instructed clearly on the indications for hospital treatment. A pocket booklet, *Notes on the care of patients with hereditary haemorrhagic disorders*, giving these instructions is issued in Britain by the Department of Health and should be given to all severely affected patients.

THE GENERAL PRINCIPLES OF REPLACEMENT THERAPY

Rational treatment aims to correct the haemostatic defect by replacing the missing factor or factors. No other form of treatment has proved effective. In recent years much experience has accumulated and the general circumstances affecting the success of replacement therapy are known. At present prophylactic

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treatment of most patients with coagulation defects is not practicable since the supply of materials for treatment is inadequate, but treatment for the control of existing bleeding is possible. The four considerations that most importantly influence the effectiveness of treatment regardless of the nature of the abnormality are:

1) the amount of the relevant factor contained in the material to be administered to the patient;
2) the concentration of the factor in the patient's circulation that is required to control bleeding in any particular circumstance;
3) the proportion of the administered activity retained in the patient's circulation;
4) the duration for which the activity remains in the circulation, or its half-life.

THE TREATMENT OF HAEMOPHILIA

In haemophilia, which is due to the lack of factor VIII in the blood, bleeding is controlled by the administration of material containing this factor.

Therapeutic Materials for the Treatment of Haemophilia

(*a*) *Whole blood* It is seldom possible to achieve effective plasma concentrations of factor VIII using whole blood. Whole blood or packed cells are thus used to correct anaemia but must not be relied on to control bleeding.

(*b*) *Plasma* Plasma prepared from freshly collected blood by centrifugation should contain all the factor VIII activity originally present. The activity can be preserved by storing the plasma frozen solid at –30°C. Unfortunately factor VIII is a labile substance and care has to be taken in the preparing, storing and handling plasma if it is to have a reasonable activity (Preston, 1967). Well-prepared plasma may record 50 to 120 per cent of the factor VIII activity of average normal plasma. It should be given in a dosage of 15 to 20 ml/kg and may raise the patient's plasma concentration of factor VIII by 15 to 20 per cent of normal.

Some patients given frequent plasma infusions may produce antibodies to plasma constituents such as IgA and may then develop serious and even dangerous reactions. In all circumstances more purified preparations are always to be preferred to plasma.

(*c*) *Human concentrates containing factor VIII* This material is presented to the physician for use in two general forms. One is the frozen cryoprecipitate made by the method of Pool and Shannon (1965). This material can be made without special apparatus in any Transfusion Service. It is effective and can be potent enough for most purposes. It is, however, very variable in quality from batch to batch and from centre to centre. It is presented as a frozen material in plastic bags, each bag containing the product of one donation. Since a single dose may require material from 20 to 30 donations, making up a dose is time-consuming and liable to involve loss of much valuable material. The second type of preparation is freeze-dried; a fairly complex fractionation procedure is normally used for its preparation. The loss of activity during preparation is comparable to that of cryoprecipitate. The freeze-dried preparation is much more convenient to use and much more consistent in activity than the cryoprecipitate. The ready availability of cryoprecipitate has enormously improved the treatment of

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haemophilic patients but it is to be hoped that this preparation will ultimately be replaced by freeze-dried preparations.

The activity of concentrates is expressed in units, 1 unit being the activity equivalent of 1 ml of average normal human plasma. The material should be given in doses of 20 to 40 u/kg.

(*d*) *Animal antihaemophilic globulin* (AHG) is available commercially from Maws Pharmacy Supplies Ltd, Aldersgate House, Barnet, Herts, England.

A guide to effective treatment for various lesions in haemophilic patients is given in Table V. Wherever there is a choice between the use of human AHG and plasma the decision will depend on the lesion to be treated and on the availability of concentrates. For example, human AHG might be used for the treatment of haematuria if plasma treatment has always failed in past episodes for that particular patient. Human AHG is always preferred to plasma in the treatment of injuries to children. It is hoped that before long freeze-dried concentrates will replace all other forms of therapy.

The Proportion of Factor VIII Activity Retained in the Patient's Circulation and its Half-life

The proportion of transfused factor VIII activity retained in the circulation immediately after infusion varies from one patient to another and is affected by the type of therapeutic material used. On average 85 per cent of the activity of plasma is retained in the circulation: for human concentrate the figure is 66 per cent and for animal concentrate 44 per cent.

The half-life of factor VIII activity in the circulation is short for all types of therapeutic material, averaging about 12 hours. Thus frequent dosage is usually required to achieve haemostasis.

The Plasma Concentration of Factor VIII Required to Control Bleeding

Episodes of spontaneous bleeding, such as haemarthroses and muscle haematomata, are controlled by relatively low plasma concentrations of factor VIII. Thus fresh or fresh frozen plasma is usually effective. Freeze-dried human concentrate or cryoprecipitate are of course more effective if available.

Some traumatic lesions are more dangerous than others. For example, bleeding after dental extraction is tiresome to both doctor and patient but nowadays seldom very dangerous. Thus for the extraction of one or two teeth plasma will often prove very effective even though the plasma concentration of factor VIII is seldom above 15 to 20 per cent of normal. If plasma is the only therapeutic material available, not more than two teeth should be extracted at one time. Administration of human concentrate is a very effective form of treatment. Using daily doses of 20 to 40 u/kg all dental operations can be undertaken safely, provided treatment is continued until healing is complete (10 to 14 days) and provided the patient does not have an antibody to factor VIII. In our experience a combination of epsilon aminocaproic acid (EACA) administration with factor VIII therapy is most effective for dental surgery (Walsh *et al*, 1971). Under this regime most patients do not require more than a single large pre-operative dose of factor VIII (40 u/kg), provided that the EACA is continued for 10 days. It is possible that EACA would improve the haemostatic effect of factor VIII therapy after other operations. In our opinion EACA is contraindicated in patients with recent or existing haematuria.

Bleeding after abdominal surgery or bone surgery may be very dangerous

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Table V

Amounts of human blood products required daily to treat patients with haemophilia

LESION	DOSE MATERIAL	ML/KG	U/KG	EXPECTED RESPONSE (RISE IN LEVEL)%
Spontaneous bleeding	Plasma	10–15 (4 donors)	7–10	10–20
	Cryoprecipitate (10 u/ml approx. 20 x concentrated)	1–2 (6–12 donors)	10–20	15–30
Dangerous bleeding Dental extractions	Cryoprecipitate	2–4 (12–24 donors)	20–40	30–60
	Human AHG (4 u/ml)	5–10 (12–24 donors)	20–40	30–60
Major surgery	Cryoprecipitate	6–12* (36–72 donors)	30–60	45–90
	Human AHG	15–20* (36–72 donors)	30–60	45–90

*Given divided into two doses, twelve hours apart

The figures in brackets in column three give an assessment of the number of donors required to prepare the dose for an adult patient weighing 60 kg

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and high dosage of factor VIII concentrates must be used. If the human concentrate is not obtainable the animal AHG, which is effective and commercially available, may be used. Since this material is antigenic it may not be possible to use it more than once or twice in the same patient. However the indications for such surgery in haemophilic patients are few and AHG is rarely required for the same patient on more than one occasion. In Great Britain the animal AHG should not be used without consultation with a Haemophilia Centre.

The Duration and Frequency of Treatment

No special rules can be laid down about these. For spontaneous bleeding one or two doses of plasma given 24 hours apart usually suffice (Rainsford & Hall, 1973). Many patients may be treated with single infusions as outpatients, provided that they come to the Haemophilia Centre within 4 hours of the onset of bleeding. It is important for the doctor to establish a close, friendly relationship with the patient and his family and to work out the best method for him to reach hospital. Obviously, experienced staff must be available throughout the 24-hour period to treat the patient when he arrives. It is not desirable for the patient to see a different doctor every time he comes to hospital, or for major delays in treatment to be caused by inexperienced doctors trying to find out what treatment should be given.

For traumatic bleeding the only general rule is that treatment should continue until healing is well advanced.

In dental extractions the tooth sockets take 7 to 10 days to heal. The use of EACA has greatly improved the safety of tooth extraction and reduced the amount of material required.

For abdominal surgery daily treatment with concentrate sufficient to keep the patient's plasma factor VIII concentration above 50 per cent of normal for the duration (about 7 days) is required.

For bone or muscle surgery healing may take 6 weeks to 2 months and healing is encouraged by complete immobilization of the site. If immobilization is good treatment with concentrate for 10 to 14 days may be enough. When the joint is completely immobilized post-operatively, we have found in three cases that the use of EACA greatly reduces the amount and duration of factor VIII treatment required to maintain normal haemostasis.

The Presence of Antibodies to Factor VIII

About 6 per cent of severely affected haemophilic patients develop antibodies that neutralize factor VIII. In these patients treatment may be ineffective and the patient's plasma should always be tested for antibodies before any sort of surgery is undertaken.

Mildly Affected Patients

Patients with more than 5 per cent of factor VIII in their plasma do not suffer from spontaneous bleeding but may bleed disastrously after surgery. It is often mistakenly supposed that these patients are easier to treat than those who are severely affected and that surgery can be undertaken after an infusion of plasma. In fact the control of traumatic bleeding is just as difficult as for the severely affected patient; the same precautions must be taken and the same amount of concentrated factor VIII will be needed.

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Home Therapy

The usual procedure for the treatment of haemophilic patients is for the patient to attend hospital when a bleed occurs; there a suitable dose of therapeutic material is administered to him by skilled staff. This classical medical practice has disadvantages: the patient may not wish to attend hospital, he will lose time from school or work; the episode of bleeding will not be treated at once; medical staff will have to spend time making up and administering doses day and night. In recent years it has been found that many patients can safely administer factor VIII to themselves or that parents or relatives can administer the dose. This treatment undoubtedly has advantages in selected cases. It permits more freedom for the patient, ensures early treatment and relieves the physicians of much routine work. When this home therapy is instituted a very close link between the treatment centre and the patient must be maintained. This form of treatment is at present limited by shortage of freeze-dried factor VIII preparations, which are undoubtedly the best for use in the home.

Local measures

In old monographs on the treatment of haemophilia much emphasis was placed on the control of bleeding with local measures. In small cuts a dressing soaked in coagulant (Russell's viper venom) may be helpful but it is wise to remember that haemophilic bleeding is not controlled by pressure and suturing unless adequate infusions have been given. With adequate infusion therapy the local measures taken to control bleeding should be the same as those appropriate to a normal person. After dental extraction acrylic plates may be used to cover the tooth sockets—these promote healing by protecting the sockets from unnecessary injury. The plates should not be used to bring pressure to bear on the injured tissue. Recently we have found that plates are not essential if the infusion therapy is well controlled and at an adequate level. Local application of mild pressure using a special inflatable rubber bag may assist in controlling epistaxis.

When adequate infusion therapy is not available all the precepts about local treatment apply. The wound should not be tightly sutured and bleeding should not be controlled by tight bandaging. Blood loss should be replaced by transfusion and healing should be encouraged by minimum interference with the traumatized area.

THE TREATMENT OF VON WILLEBRAND'S DISEASE

The factor VIII deficiency in von Willebrand's disease responds to replacement therapy quite differently from that in haemophilia. Charts giving the results of daily treatment in the two conditions are shown in Figure 3. Whereas in haemophilia the factor VIII level in the plasma begins to fall immediately after the dose has been given, in von Willebrand's disease the plasma concentration is maintained and even rises a little for 24 hours. Thus treating von Willebrand's disease is easier than treating haemophilia. Plasma, human AHG and cryoprecipitate are good therapeutic agents in von Willebrand's disease. It must be remembered however that in von Willebrand's disease the bleeding time is prolonged and that it is not always reduced to normal after infusions. Bleeding under these circumstances is only controlled by pressure. Thus operative procedures on patients require very careful attention to local haemostasis.

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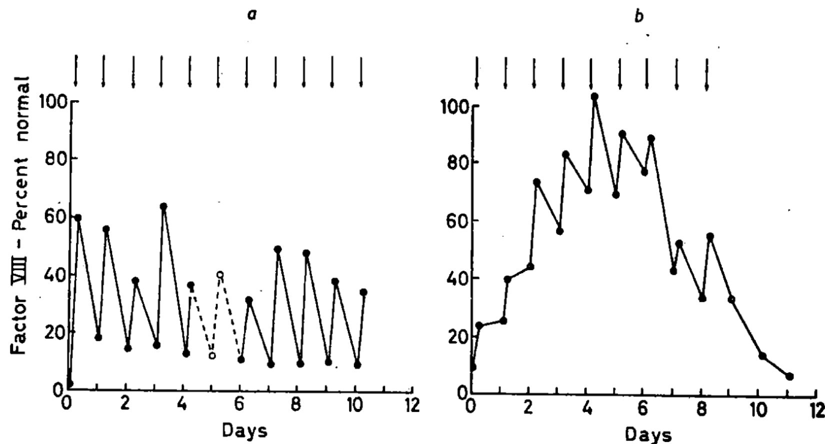

Figure 3. Records of treatment of factor VIII-deficient patients with factor VIII.
(*a*) Haemophilia
(*b*) Von Willebrand's disease
The arrow indicates the administration of a dose.

Where it is not possible to use pressure (for example in menorrhagia and gastro-intestinal bleeding) bleeding may continue despite high plasma factor VIII concentrations.

THE TREATMENT OF CHRISTMAS DISEASE (FACTOR IX DEFICIENCY)

The general principles of treatment outlined for haemophilic patients apply to those with Christmas disease. The materials for treatment are plasma and human concentrates containing factor IX. Since these concentrates usually contain factors II, VII and X as well, they can also be used to treat patients who lack these other factors. Factor IX is much more stable than factor VIII and plasma separated from blood less than 1 week old will contain good concentrations of it. Unfortunately it is common for as little as 25 to 30 per cent of the original amounts of active factor assayed in the dose material to be found in the patient's circulation after infusion. Thus after plasma infusion it is usual for blood concentrations of factor IX to be less than 10 per cent of normal. For this reason plasma infusions are often surprisingly ineffective in the control of bleeding in patients with Christmas disease. In the United Kingdom there is now enough concentrated freeze-dried material to treat all patients. Plasma should no longer be used.

When high plasma concentrations of factor IX are achieved the activity remains in the circulation rather longer than in factor VIII in haemophilic patients. The half-life of the factor IX activity ranges from 14 to 24 hours.

THE TREATMENT OF PATIENTS WITH RARE COAGULATION DEFECTS

The rarer coagulation defects due to lack of fibrinogen and factors II, V, VII, X, XI and XIII are relatively easy to treat. Fibrinogen is available in concentrated form for the treatment of afibrinogenaemia and, since this substance has a half-life in the circulation of about 5 days, safe treatment is readily achieved.

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The maintenance of factor V at levels of 20 to 25 per cent of normal appears to suffice for haemostasis. Such concentrations can be obtained after plasma infusions so concentrated material is unlikely to be required. Plasma infusion treatment of patients with factor XI or factor XIII deficiency has proved effective even for protection during major surgery.

Patients with defects of factor VII or X or prothrombin may be treated either with the 'factor IX' concentrate containing these factors or with plasma. Mercifully, since patients with factor VII deficiency are hard to treat owing to the exceptionally short half-life of the factor in the circulation (about 5 hours), factor VII deficiency as a single defect is very rare. One patient known to us did not bleed excessively after major surgery (tonsillectomy) even without treatment. Patients with qualitative platelet defects may need infusions with platelet concentrates.

THE TREATMENT OF ACQUIRED COAGULATION DEFECTS

Two types of acquired coagulation defects are important. The first is that secondary to vitamin K deficiency or liver disease. The second is acute defibrination secondary to intravascular clotting or fibrinolysis.

Vitamin K deficiency and liver disease cause reduction in factors II, VII, IX and X. Diagnosis is determined by the clinical history and the results of the one- and two-stage prothrombin tests. If prothrombin deficiency is the main abnormality, as is often the case in liver disease, it may be detected only with the two-stage test, which is therefore an essential diagnostic method. Treatment is by administration of vitamin K. If the prothrombin concentration remains below 40 per cent of normal after vitamin K and operation is required, concentrated material (the factor IX concentrate) may be needed.

The acute defibrination syndrome is characterized clinically by shock, severe bruising and abnormal bleeding. Fibrinogen, platelets, and to a lesser extent factors V, VIII and XIII are reduced in amount and an inhibitor (antithrombin VI) that interferes with fibrinogen polymerization appears in the blood. The condition may be the result of intravascular coagulation or of excessive fibrinolysis. In most cases intravascular coagulation is probably the primary cause and excessive fibrinolysis follows. It is claimed that heparin should be used to suppress the excessive coagulation in most cases. Some patients, however, are dangerously ill and bleeding catastrophically and much judgement must be exercised in the choice of cases suitable for heparin therapy. It is probable that heparin is most effective in the more chronically affected patients. Whole blood transfusion and concentrated fibrinogen preparations are valuable forms of therapy in most cases.

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Appendix 1: Technical Methods

In an introductory pamphlet it would be inappropriate to give detailed instructions for all techniques carried out in a specialist laboratory. A laboratory worker who wants to set up all these tests should refer to Biggs and Macfarlane (1966) or Biggs (1972). All the methods included here are simple ones that could be used in any laboratory even where the staff have no special experience of coagulation techniques. This is not to imply that skill, judgement and experience are not required for the carrying out and interpretation of these tests but in emergency or where Specialist Centres are not available these tests will be helpful.

Reagents

1. *Saline Extract of Brain (Thromboplastin)*

A human brain is freed from blood vessels and meninges and washed. The whole brain is macerated for about 2 minutes with warm 0·85 per cent saline in a Waring blender, using 1500 ml of saline heated to 37° C. The emulsion is centrifuged for 25 minutes at 2000 rpm and the sediment discarded. The supernatant is tested by the one-stage prothrombin time test both undiluted and diluted 1 in 2, 1 in 3 and 1 in 5 with 0·85 per cent saline. The dilution giving the minimum clotting time is selected. The whole mixture is diluted appropriately and 10 per cent of Owren's buffer added. The final fluid is dispensed into containers, each of which will provide a day's supply, and stored frozen solid at –20° C.

Many commercial thromboplastin preparations are available. These should be used according to the maker's instructions. Some commercial preparations have added factor V and fibrinogen and will not record deficiency in these factors.

2. *Aluminium Hydroxide Suspension*

This suspension is obtainable from British Drug Houses, Poole, Dorset.

3. *Citrated Plasma*

Nine parts of blood are mixed with 1 part of 3·8% (w/v) sodium citrate in a polystyrene tube and the plasma separated by centrifuging. If plasma rich in platelets is required, the blood is centrifuged at 1000 rpm for 10 minutes. For other purposes the blood may be centrifuged at 3000 rpm for 15 minutes to obtain a platelet-poor plasma.

4. *Absorbed Citrated Plasma*

0·1 ml of aluminium hydroxide suspension is added to 1 ml of platelet-poor plasma (see above). The mixture is stored for 3 minutes at 37° C and the $Al(OH)_3$ removed by centrifuging. The supernatant plasma contains factors V and VIII but most of the prothrombin and factors VII, IX and X will have been removed.

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5. *Serum*

3 ml of whole blood is incubated at 37°C for at least 2 hours; the tube is allowed to stand overnight in the refrigerator at 4°C to permit complete inactivation of thrombin and other coagulants. The serum is then separated by centrifuging. For use in the factor VIII assay larger volumes of serum are similarly prepared and freeze dried. The dried powder is weighed and the amount corresponding to 1 ml of serum is dissolved in 10 ml of glyoxaline buffer and stored overnight at 4°C before being used at 37°C.

6. *Fibrinogen (Alcohol Method)*

Absorbed oxalated bovine plasma is prepared by mixing 1 ml of 1·34 per cent sodium oxalate with 9 ml blood and centrifuging to obtain plasma. The plasma is absorbed by adding 1 g of washed barium sulphate ($BaSO_4$) to every 10 ml plasma and after incubation for ten minutes at 37°C removing the $BaSO_4$ by centrifugation. To 1000 ml of this plasma is added 1·0 ml of Cohn buffer (108·8 g sodium acetate trihydrate and 240 g acetic acid are made up to 1 litre with distilled water). The plasma is cooled to —0·5°C until crystals of ice form. 177 ml 50% (w/v) alcohol cooled to —20°C is then added with mixing. The final temperature should be approximately —3°C. The mixture is then centrifuged and the temperature kept below —1°C. The deposit is washed with 300 ml of glycine/citrate/buffer mixture at —1°C and centrifuged. The deposit is dissolved in 250 ml of a mixture of 25 ml of 3·8 per cent sodium citrate and 225 ml of saline. The resulting fibrinogen solution is deep frozen in 5 ml amounts. For use it is diluted with two volumes of saline and warmed to 37°C.

7. *Chloroform Extract of Brain, Platelet Substitute (Bell and Alton, 1954)*

1·0 g of acetone-dried brain is extracted with 20 ml of chloroform with occasional mixing for 2 hours. The mixture is filtered and the filtrate, which should be clear, is evaporated at 60 to 70° C with a stream of nitrogen. The solid residue is suspended in 10 ml of saline and stored deep frozen. For use it is diluted 1 in 100 to 1 in 200; the optimum dilution must be determined by trial. Two satisfactory commercial preparations are available: Thrombofax (Ortho Pharmaceutical Ltd., Sanderton, Bucks.) and Platelet substitute (Diagnostic Reagents Ltd., Thame, Oxon).

8. *Factor V-Deficient Substrate Plasma*

9 parts of blood are added to 1 part of sodium oxalate. The plasma is separated by centrifuging and incubated at 37°C for 24 to 36 hours. During incubation, the factor V activity is progressively reduced and incubation should be continued until the one-stage prothrombin time of the plasma is at least 90 seconds.

9. *Taipan venom*

Taipan venom can be obtained from Sigma (London) Chemical Co. (Norbiton Station Yard, Kingston-upon-Thames, Surrey, KT2 7BH), or from the Australian Reptile Farm (Wyoming, Gosford, New South Wales, Australia).

10. *$CaCl_2$*

Solutions of the required concentration are made from molar $CaCl_2$, which is commercially available (British Drug Houses, Poole, Dorset).

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Methods

A. METHODS USING WHOLE BLOOD

1. *Whole Blood Clotting Time* (*Lee and White*)

1 ml of whole venous blood is placed in each of four tubes ($2\frac{1}{2}$ x $\frac{3}{8}$ inches) and these are incubated at 37°C. The tubes are all tilted through 90° at $\frac{1}{4}$ to $\frac{1}{2}$-minute intervals until they can be inverted without spilling any blood. The time is recorded from the moment blood begins to enter the syringe until the tubes can safely be inverted, and the clotting time is expressed as an average for the four tubes. The normal range varies from $3\frac{1}{2}$ to 6 minutes, the results being slightly different in different laboratories. For this test it is important to use syringes and needles of constant size and to accept only samples in which no technical difficulties were encountered in obtaining the blood. Preferably new, not washed, tubes should be used.

2. *Prothrombin Consumption Index*

Collection of Serum The blood collected for the Lee and White coagulation time test is used. When coagulation has occurred the blood in the four Lee and White clotting tubes is allowed to stand at 37°C for 50 minutes. This time is measured from the completion of coagulation and not from the time at which blood is withdrawn from the patient. The clot is then gently freed from the sides of the tubes with a wooden applicator stick and the tubes are centrifuged for 5 minutes to obtain serum. The serum from the four tubes is collected with a Pasteur pipette and pooled in one tube. Exactly 1 hour after coagulation has occurred a prothrombin consumption test is carried out.

Technique 0·4 ml amounts of fibrinogen are placed in each of four tubes (measuring 3 x $\frac{3}{8}$ inches) and these are placed in a water bath at 37°C. Another small tube of the same size is placed in the water bath and 0·2 ml of the pooled serum is placed in this tube. 0·2 ml of 0·85 per cent NaCl and 0·2 ml of brain thromboplastin are added. As rapidly as possible thereafter 0·2 ml M/40 $CaCl_2$ is added, a stop-watch being started at this instant. The contents of the tube are thoroughly mixed and exactly 30 seconds after adding the $CaCl_2$ 0·1 ml of the mixture is removed with a graduated Pasteur pipette and rapidly blown out into one of the tubes containing fibrinogen. As this addition is made a second stop-watch is started and the coagulation time of the fibrinogen is recorded. At 60 seconds a further 0·1 ml of the mixture is added to a second fibrinogen tube and the clotting time recorded. The test is then carried out in exactly the same way using the patient's citrated plasma instead of serum. In this test the mixture coagulates because the plasma contains fibrinogen. The coagulum which forms after about 20 seconds can be conveniently removed by inserting the pipette to be used for subsampling and rotating it continuously for 15 to 20 seconds. The fibrin which adheres to the outside of the pipette may then be quickly removed with a paper tissue and the pipette used for subsampling. At exactly 30 and 60 seconds after the addition of $CaCl_2$, 0·1 ml of the defibrinated mixture is added to a tube containing fibrinogen and the clotting times of the fibrinogen recorded as before.

Expression of Results The minimum clotting times for the plasma and serum are used to calculate the index. Four clotting times are obtained. A method of expressing results can best be understood from an example.

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In a sample of normal blood minimum clotting times shown below were obtained. From the clotting times of the fibrinogen a Prothrombin Consumption

Table VI

Prothrombin Consumption Test: typical result in a normal individual

Incubation time	60 seconds
Plasma time	16 seconds
Serum time	160 seconds

Index is calculated (see Table VI), by dividing the shortest time obtained with plasma by that with serum and multiplying the result by 100. In the example quoted the index would be:

$$\frac{16}{160} \times 100 = 10 \text{ per cent}$$

Notes In carrying out the prothrombin consumption test, it is important that the blood should be collected with a syringe and needles of standard size and that exactly 1 ml of blood should have been placed in each of the four small tubes used for collected blood. Deviations from this procedure may have a surprisingly great effect on the results. For example, if the blood is collected into one large container the results of the test may be much too high; if a needle of small gauge is used the results of the test may be too low.

Even with these precautions the results with normals are not constant and it is wise, as with the Lee and White clotting time, to carry out the test once a week on normal blood; the normal range is thus always known with certainty. The test has never been known to record an index of more than 40 per cent in normal blood and in our laboratory none of the normal results for the last 4 years has exceeded 10 per cent.

B. METHODS USING THE ONE-STAGE PROTHROMBIN TIME TEST (EXTRINSIC CLOTTING SYSTEM)

1. *One-Stage Prothrombin Time*

Reagents (*a*) Saline brain extract.
(*b*) 0·025 M $CaCl_2$.
(*c*) Citrated plasma.

Method 0·1 ml of citrated plasma is mixed with 0·1 ml of brain extract in a tube ($2\frac{1}{2} \times \frac{3}{8}$ inches) and warmed to 37°C in a water bath; 0·1 ml of $CaCl_2$ is added and a stop-watch started. The tube is gently tilted at intervals until clotting occurs. The test is carried out in triplicate on normal plasma and on the patient's plasma and the average clotting times of the two samples compared. The brain suspension used in this test should clot normal plasma in 12 to 20 seconds. The patient's clotting time is abnormal if it exceeds that of the normal by more than 2 or 3 seconds.

2. *Taipan Assay of Prothrombin* (Denson *et al.*, 1971)

Taipan venom is made up at a concentration of 1 in 200 000 (w/v) 0·1 ml fibrinogen, 0·1 ml platelet substitute 1 in 100, 0·1 ml of the plasma dilution to be tested are put into a tube and warmed to 37°C. 0·2 ml of a mixture in equal parts of 1 in 200,000 Taipan venom and 0·025 M $CaCl_2$ is added and the clotting time recorded.

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Normal plasma is tested at dilutions 1 in 10, 1 in 30, 1 in 60 and 1 in 100. The dilutions of abnormal plasma are adjusted according to the degree of abnormality. The clotting times are plotted against the reciprocal of plasma concentration for normal and abnormal and the result expressed as a percentage. The line for the abnormal must meet that for the normal at the vertical axis if the test system is to be interpreted validly. Double logarithmic plot cannot be used since the lines are not parallel.

3. *Methods Based on the One-Stage Prothrombin Time*

In the presence of brain extract, factor X is activated rapidly to factor Xa provided that factors V and VII are present in optimal amounts. Thus the one-stage prothrombin time may be modified to measure factors V and VII using substrate plasma samples that lack factors V and VII. Factor V-deficient plasma may be made in the laboratory (see reagents) but for the factor VII-deficient sample plasma from a patient naturally deficient in this factor must be used. The technique in each case involves using 0·1 ml of the deficient substrate, 0·1 ml of a dilution of test or normal plasma, 0·1 ml of brain extract and 0·1 ml of $CaCl_2$. The ability of the test plasma to shorten the clotting time is compared with that of normal plasma. Factor X may be measured in a similar way by replacing the brain extract with Russell's viper venom and using a substrate deficient in factors VII and X. For details of these methods see Biggs and Macfarlane (1966).

C. TWO-STAGE PROTHROMBIN TIME (Biggs and Douglas, 1953)

Reagents (*a*) Normal plasma.
(*b*) Patient's plasma.
(*c*) Saline extract of brain.
(*d*) Fibrinogen.
(*e*) 0·025 M $CaCl_2$.

Method A saline extract of brain is diluted 1 in 5 to 1 in 20 with 0·85 per cent NaCl. The dilutions of brain extract are tested by the one-stage prothrombin test using normal plasma and the dilution of brain that gives a clotting time of 25 to 30 seconds should be used for the two-stage prothrombin test.

0·4 ml amounts of fibrinogen are pipetted into 7 or 8 clotting tubes, which are placed in a water bath at 37°C. 0·4 ml normal plasma and 0·4 ml diluted brain are pipetted into a tube and placed in a water bath at 37°C. 0·4 ml 0·025 M $CaCl_2$ is added to the mixture, and a stop-watch started. The mixture is agitated with a 0·1 ml pipette to remove any clot that may form; it is important to remove all the fibrin as it is formed by winding it onto the pipette.

At 1 minute, 0·1 ml is removed from the initial mixture and added to the first tube of fibrinogen. A stop-watch is triggered and the clotting time recorded. The operation is repeated at 1 minute intervals until the clotting time exceeds 180 seconds.

The technique is repeated using the patient's plasma.

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Using a thrombin–fibrinogen curve, the clotting time of the fibrinogen at each subsampling is interpreted in thrombin units. A curve of thrombin formation against time is drawn for both patient and normal plasma. The area enclosed by the curve is computed by any one of the following methods:

1) Using a planimeter;
2) Weighing cut-out areas;
3) Counting the squares enclosed.

The area obtained for the abnormal is expressed as a percentage of the normal.

An alternative, simpler method is to total the thrombin units at each subsampling time for the patient's plasma sample, and divide this figure by the total of the thrombin units for the normal sample, expressing the result as a percentage. If this is done sampling must of course be carried out at standard times which are the same for normal and the patient's plasma.

$$\frac{\text{Patient's plasma (thrombin units)}}{\text{Normal plasma (thrombin units)}} \times 100$$

$$\text{e.g.} \quad \frac{37 \cdot 4}{41 \cdot 2} \times 100 = 90 \text{ per cent}$$

Thrombin–fibrinogen dilution curve. Thrombin dilutions are prepared to contain 20, 15, 10, 5, 3, 2, 1 and 0·5 units of thrombin per ml. A series of tubes containing 0·4 ml of fibrinogen solution are placed in a water bath at 37°C and to these 0·1 ml of each of the thrombin solutions is added; the clotting time is recorded in each case. The results of one experiment are shown in Figure 4. From this figure it will be seen that if the clotting times are plotted against the reciprocal of the concentration of thrombin the curve can be expressed as a straight line passing almost through the origin. For the thrombin–fibrinogen reaction it is possible to relate the clotting time to the concentration of thrombin as follows:

$$\text{C.T.} = \frac{K}{T}$$

where C.T. = clotting time, T = the relative concentration of thrombin, and K = a constant that varies with the concentration of thrombin.

From this curve, clotting times of 0·4 ml amounts of fibrinogen can be read in terms of thrombin units. These thrombin units have no absolute significance but are useful for comparing relative amounts of thrombin in the two-stage prothrombin test.

D. METHODS BASED ON THE KAOLIN–CEPHALIN CLOTTING TIME (INTRINSIC PROTHROMBIN ACTIVATION)

1. *Kaolin-Cephalin Clotting Time*

This test is a refinement of the recalcification time and partial thromboplastin time methods (the recalcification time with the addition of phospholipid). The former test suffers from two disadvantages. First, platelets are variable; second

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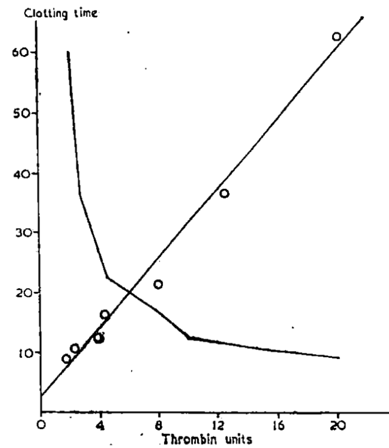

Figure 4. Thrombin—fibrinogen dilution graph: the results of plotting reciprocal of concenration against clotting time.

contact activation in the plasma sample will depend on the nature of the surface of the clotting tube, the length of time the plasma sample is exposed to the surface, and whether the blood sample has been collected into a glass tube or a plastic tube.

The first disadvantage of the recalcification time test is eliminated in the partial thromboplastin time test by recalcifying plasma in the presence of a high and constant excess of platelet substitute. Both disadvantages are overcome in the kaolin–cephalin clotting time (KCCT) by recalcifying plasma in the presence of platelet substitute after the plasma has been exposed for a standard preliminary period of 2 minutes to a suspension of kaolin. The KCCT is an overall test of clotting function and reduced levels of any of the clotting factors I, II, V, VIII, IX, X, XI and XII will be reflected in a prolonged clotting time. It should be realised however that although the test may be profitably included in a battery of preliminary tests it should under no circumstances be used as a single screening test to replace all others. The very nature of this test and its scope in encompassing the entire clotting process from contact activation to fibrin formation renders it vulnerable to more variation than specific tests. For example, elevated levels of one or more individual factors or the presence of active intermediate products of coagulation that would tend to reduce the clotting time may mask a prolongation of the clotting time due to deficiency of another factor. On the other hand, minor deficiencies in several factors may each contribute toward prolonging the clotting time. Attention to technical detail is important. The tubes should be tilted in a standard manner in successive tests otherwise wide variations in clotting times result. Kaolin sediments rapidly from suspension and this should be prevented by expelling a small stream of air bubbles through any sediment that forms by means of the teat and pipette used for transfer when removing a sample.

Whatever its shortcomings this is a valuable ancillary test. It may, for example, be the only test to reveal abnormality in the presence of certain inhibitors associated with the dysproteinaemias and collagen diseases.

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Reagents (*a*) Citrated plasma from the patient and a normal control collected into plastic tubes.

(*b*) Kaolin 5 mg/ml in saline.

(*c*) Cephalin–1/100 Bell and Alton platelet substitute.

Technique 0·1 ml of plasma and 0·1 ml of Bell and Alton platelet substitute are pipetted into a clotting tube and left for 1 to 2 minutes in the water bath to reach 37°C. 0·1 ml of kaolin suspension is then added and after 2 minutes of intermittent gentle shaking 0·1 ml of M/40 $CaCl_2$ is added and a stop-watch started. The tube is gently tilted at 5 to 10 second intervals and the clotting time recorded. The normal range varies from 45 to 65 seconds, depending largely upon the preparation of platelet substitute in use.

2. *Assay methods based on the KCCT*

The KCCT technique may be modified by the use of different substrate plasmas to differentiate among various other deficiencies. Because of its technical simplicity the modification of the technique as an assay of Factors VIII and IX will be considered. In such modifications the unknown sample is tested at high dilution and the substrate plasma supplies all factors other than the one under assay in high and constant concentration. The technique still suffers from many of the inherent technical disadvantages associated with the KCCT and in addition the substrate plasma sometimes progressively activates towards the end of a series of tests resulting in shorter clotting times.

Considerable quantities of factor VIII-or factor IX-deficient substrate plasmas (which must be completely deficient in the appropriate factor) are required. Samples containing as little as 0·5 to 1 per cent of the deficient factor are unsuitable for use as substrate plasma.

The advantages of the one-stage method are that it is quick and superficially technically simpler than the two-stage method, and that no elaborate reagents have to be prepared. Once the standard dilution curve has been constructed on a given day it is not unreasonable to expect to perform 50 assays in a normal day's work. The result for a given sample can be obtained within 5 to 10 minutes of receiving the plasma. The method may be modified to assay factor IX by substituting factor IX-deficient plasma for that deficient in factor VIII.

We do not find these one-stage methods very reliable for the control of treatment in haemophilic and Christmas disease patients, preferring the two-stage methods based on the thromboplastin generation test. However the one-stage methods are undoubtedly more reliable than the unmodified KCCT and should be used if diagnosis is difficult or emergency surgery is necessary. The one-stage method for the assay of factor VIII will be described in detail and may serve as a model for other one-stage assay systems.

3. *One-stage Assay of Factor VIII based on the Kaolin-Cephalin Clotting Time*

Reagents (*a*) *Citrate/buffer diluent for samples:* one volume of 3·8% sodium citrate is added to five volumes of imidazole buffer pH 7·3 (0·68 g glyoxaline and 1·17 g NaCl are dissolved in 196 ml H_2O to which 3·7 ml M HCl is added).

(*b*) *Platelet substitute* (chloroform extract of brain, Bell and Alton 1954): 1 in 100 dilution in saline is usually optimal.

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(c) *Kaolin:* a suspension of kaolin at 5 mg/ml of saline is satisfactory. Higher concentrations have been used, but these usually result in poor clot formation.

(d) *Substrate plasma:* haemophilic plasma completely deficient in factor VIII is required. This can be stored deep frozen at −20°C in small aliquot amounts in plastic tubes.

(e) *0·05 M $CaCl_2$.*

(f) *Pool of fresh normal plasma.*

(g) *Test sample at 1 in 10 dilution.*

Preparation of Dilutions for Calibration Curve Dilutions of normal plasma at 1 in 10, 1 in 20, 1 in 100, 1 in 200, 1 in 1 000 are prepared in citrate buffer diluent and arbitrary values of 100 per cent, 50 per cent, 10 per cent and 1 per cent, respectively, assigned to these dilutions.

Technique 0·1 ml of substrate plasma is pipetted into each of three tubes followed by 0·1 ml of buffer. 0·1 ml of platelet substitute and 0·1 ml kaolin are then added to each tube and the tubes incubated for 2 minutes with tilting at 10 to 20 second intervals. Finally 0·1 ml of 0·05 M $CaCl_2$ is added, a stop-watch is started and the clotting time recorded. The mean of the clotting times is obtained: this represents the 'blank' value on the substrate plasma. The procedure is repeated, substituting each of the dilutions of normal plasma and a 1 in 10 dilution of test plasma in turn for the buffer. The clotting times of the normal plasma dilutions are plotted against the percentage concentration of plasma on double logarithmic graph paper whence a straight line should be obtained for values between 100 and 5 per cent. A typical calibration curve is shown in Figure 5. The clotting time of the 1 in 10 dilution of the unknown sample is converted to a percentage of factor VIII by interpolation from the curve. If the clotting time of the unknown sample is shorter than that of the 1 in 10 normal sample, the unknown sample is tested again at a 1 in 20 dilution, and the result obtained from the curve doubled.

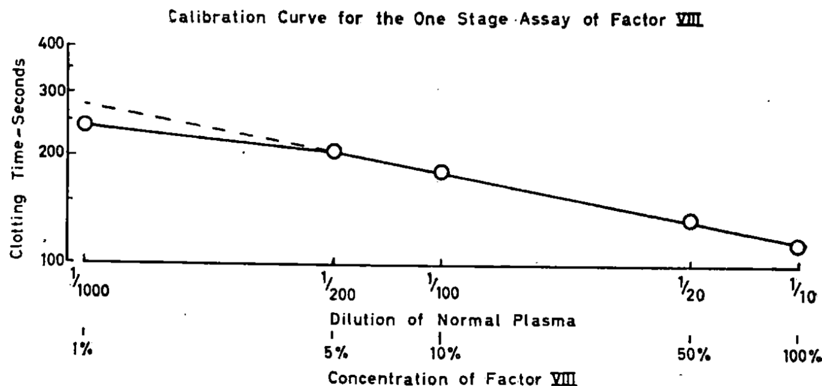

Figure 5. One-stage factor VIII dilution graph.

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During clotting, the tubes should be tilted in turn at about 10 second intervals. If the tubes are left undisturbed during clotting, the clotting times tend to be shorter although somewhat erratic. It is important however that the technique of tilting should be identical from one test to another. If some tubes are shaken more vigorously than others, considerable variation between replicates is obtained and this is one of the main sources of variability and inaccurate results.

It should be noted that if the substrate plasma contains 1 per cent of factor VIII activity the normal calibration curve will cease to be linear for low factor VIII concentrations. The 1 in 100 dilution of normal plasma added to such substrate plasma contains 1 per cent of the normal factor VIII and the amount contributed by the substrate is large in comparison. The use of these substrates will introduce inaccuracy in assessing factor VIII concentrations below 10 per cent.

4. *Test for Inhibitor to Factor VIII based on the KCCT*

The test depends on the fact that inhibitors to factor VIII destroy its activity progressively. When a plasma containing inhibitor is mixed with normal plasma, the KCCT will be long after 1 hour of incubation because the inhibitor has destroyed the factor VIII.

Technique Into four glass tubes are pipetted 0·1 ml of normal plasma and 0·1 ml of test plasma to give mixtures in equal parts of normal patient's plasma. Two of these tubes are tested for clotting at once by the addition of 0·2 ml of cephalin and 0·2 ml of kaolin.

After two minutes incubation at 37°C, 0·2 ml of M/40 $CaCl_2$ is added and the clotting time is recorded. The other two tubes are incubated at 37°C for 1 hour and the KCCT, carried out as for the first two tubes. Control tests are made using 0·2 ml of the same normal plasma tested at once and after 1 hour's incubation at 37°C and 0·2 ml of the patient's plasma tested at once and after 1 hour's incubation at 37°C. In addition samples of the normal plasma and patient's plasma should be mixed as before *after* they have incubated for 1 hour at 37°C and tested. In the presence of a factor VIII inhibitor the mixture of normal and patient's plasma that has been incubated for 1 hour should give a substantially longer clotting time than that of the mixture tested at once or of the mixture tested after the samples have been incubated separately. Obviously the mixtures should also give a longer clotting time than the normal sample.

This is, however, only a rough qualitative test for inhibitor. A better method using the two-stage assay of factor VIII is described by Biggs and Macfarlane (1966).

E. MISCELLANEOUS TESTS

1. *The Fibrinogen Titre* (Sharp *et al*, 1958)

0·5 ml of 0·85 per cent saline is pipetted into each of 14 tubes which are placed in 2 rows of 7. To the first tube in one row is added 0·5 ml of normal citrated plasma. Doubling dilutions of this are made by transferring 0·5 ml from the first tube to the second and then transferring 0·5 ml from this tube to the third and so on, discarding 0·5 ml from the last tube. Similar dilutions are made from the patient's plasma in the second row of tubes. 0·1 ml of bovine or human thrombin of 10 to 30 units/ml is added to each tube and the contents mixed. The tubes are incubated at 37°C for 15 minutes without being disturbed and are then inspected for the presence of clots.

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The method is useful for the diagnosis of the defibrination syndrome. The normal sample will show clots in all dilutions up to 1 in 128. The plasma of a clinically severely affected patient will produce no clots in any dilution whilst the plasma from a patient mildly affected may have clots in the 1 in 4 or 1 in 8 dilutions.

2. *The Assessment of Functional Activity of Platelets. Use of the KCCT with High and Low Spun Plasma* (Hardisty, Dormandy & Hutton, 1964).

When low spun (platelet-rich) plasma is incubated with kaolin, the active thromboplastin principle is liberated from the platelets and absorbed at the surface of the kaolin. The clotting time of such plasma on recalcification is very short, but still depends on the presence of normal amounts of the intrinsic clotting factors in the plasma. When a mixture of equal proportions of normal platelet-rich plasma and normal platelet-poor plasma are incubated with an equal volume of a suspension of kaolin at 5 mg/ml and the mixture recalcified with a volume of 0·035 M calcium chloride, the clotting time lies in the narrow range of 27 to 31 seconds. Platelet function may therefore be assessed by performing such a kaolin clotting time test on mixtures of normal high spun plasma and patients' low spun plasma, and on patients' high spun plasma and normal low spun plasma. The use of these opposite mixtures eliminates any possible effect on the clotting time of differences in the concentration of plasma coagulation factors between patient and control, and ensures that the only variable is the source of platelets.

Technique Equal volumes (0·1 ml) of platelet-rich plasma and platelet-poor plasma are incubated with 0·2 ml of kaolin suspension for 20 minutes at 37°C. 0·2 ml of 0·035 M calcium chloride solution is then added and the clotting time recorded.

The normal range is 27 to 31 seconds for platelet counts of the mixed plasma of between 100 000 and 300 000 per cu. mm. The variations in platelet numbers normally encountered thus have very little effect on the normal range. Hardisty *et al* (1964) obtained clotting times ranging from 41 seconds to 75 seconds in three cases of thrombasthenia.

3. *ADP Aggregation of Platelets*

Some patients who have a clinical history of excessive bleeding are found to have platelets that fail to agglutinate with adenosine diphosphate (ADP). This property is easy to test and patients with this defect may need platelet transfusion. It is suggested by Hardisty, Dormandy & Hutton (1964) that the term 'thrombasthenia' should be used to describe the clinical syndrome associated with this defect.

Normal platelets, when exposed to surface contact such as glass or kaolin, liberate ADP. It is supposed that the liberated ADP causes the platelets to aggregate and adhere to the contact surface. Thrombasthenic platelets fail to liberate ADP and it is thought that this feature, as well as lack of aggregation of the platelets by added ADP, is due to an abnormality of the platelet membrane. In the few patients examined failure of the platelets to aggregate in the presence of added ADP appears usually to be associated with abnormal thromboplastic activity of platelets. The ADP aggregation test is a useful and simple preliminary test.

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Reagents (*a*) Adenosine diphosphate (British Drug Houses Ltd, Poole, Dorset). 20 μg/ml. This should be freshly prepared.

(*b*) Platelet-rich plasma.

Technique 0·2 ml of platelet-rich plasma is placed in a clotting tube at 37°C and 0·1 ml of the adenosine diphosphate solution added.

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Appendix 2: Haemophilia Centres in Great Britain and Northern Ireland

ADDRESS	TELEPHONE NO.
Aberdeen Dr A. A. Dawson Department of Medicine Royal Infirmary Foresterhill Aberdeen AB9 2ZB	0224 23423
Alton Dr P. G. Arblaster Treloar Haemophilia Centre Lord Mayor Treloar Hospital Alton	Alton 2811
Belfast Professor M. G. Nelson Department of Clinical Pathology Royal Victoria Hospital Belfast BT12 6BA	0232 30503
Birmingham Dr M. J. Meynell Haematology Department Queen Elizabeth Hospital Edgbaston Birmingham B15 2TH	021–472 1311
Dr J. Stuart Department of Haematology The Children's Hospital Ladywood Middleway Ladywood Birmingham B16 8ET	021–454 5851
Bournemouth Dr D. Stern Royal Victoria Hospital Shelley Road Boscombe Bournemouth BH1 4JG	0202 35201 x GRO-C (At night and at weekends please ask for the number of the doctor on call for haemophiliac control)
Bradford Dr R. Turner Royal Infirmary Bradford BD9 6RJ	0274 42200
Bristol Dr A. B. Raper Department of Medicine The Royal Infirmary Bristol BS2 8HW	0272 22041

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ADDRESS	TELEPHONE NO.
Cambridge Professor F. G. J. Hayhoe Haematology Clinic Department of Medicine University of Cambridge Hills Road Cambridge CB2 1QT	0223 45171 (At night and at weekends please telephone Addenbrookes Hospital – Cambridge 55671 – and ask for Professor Hayhoe's senior house officer)
Cardiff Dr A. L. Bloom Department of Haematology University Hospital of Wales Cardiff C4F 1XW	0222 755944 (After 5 pm and at weekends please ask for the house physician to the Haematology Department)
Carlisle Dr A. Inglis Department of Pathology Cumberland Infirmary Carlisle CA2 7HY	0228 23444
Derby Dr C. R. R. Wylie Royal Infirmary Derby DE1 2QY	0332 47141
Dundee Dr G. R. Tudhope Therapeutics Unit Maryfield Hospital Dundee DD4 7TL	0382 40011
Edinburgh Professor R. H. Girdwood University Department of Therapeutics The Royal Infirmary Edinburgh EH3 9YW	031–229 2477 x GRO-C
Dr S. H. Davies Department of Haematology The Royal Infirmary Edinburgh EH3 9YW	031–229 2477 x GRO-C
Exeter Dr J. O. P. Edgcumbe Department of Pathology Royal Devon and Exeter Hospital Exeter EX1 1PQ	0392 72261, 59261

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ADDRESS	TELEPHONE NO.
Glasgow Dr C. R. M. Prentice Department of Medicine Royal Infirmary Glasgow OSS4	041-552 3535 (If not available ask for the receiving physician)
Hull Dr J. J. Taylor Department of Pathology Kingston General Hospital Beverley Road Hull HU3 1UR	0482 28631
Inverness Dr I. A. Cook Regional Blood Transfusion Centre Raigmore Hospital Perth Road Inverness IV2 3UJ	0463 34151
Leeds Dr L. M. Swinburne St James' Hospital Leeds L59 7TF	0532 33144
Liverpool Dr T. Black Liverpool Royal Infirmary Pembroke Place Liverpool L35 PU	051-709 5511
London Professor P. L. Mollison Haematology Department St Mary's Hospital Praed Street Paddington London W21 PG	01-262 1280 x GRO-C
Professor R. M. Hardisty Haematology Department The Hospital for Sick Children Great Ormond Street London WC1N 3HJ	01-405 9200 x GRO-C (At night and at weekends please ask hospital telephone operator for the resident assistant physician)
Dr P. Barkhan Department of Haematology Guy's Hospital London SE1 9RT	01-407 7600

HSOC0000616_0042

ADDRESS	TELEPHONE NO.
Dr R. S. Mibashan Haematology Department Royal Postgraduate Medical School Hammersmith Hospital Ducane Road London W12 6HS	01–743 2030 x GRO-C (At night and at weekends please ask operator for the haematology registrar on duty)
Dr G. C. Jenkins Haematology Department The London Hospital Whitechapel Road London E1 1BB	01–247 5454
Professor W. M. Davidson Haematology Department King's College Hospital Denmark Hill London SE5 8AF	01–274 6222
Dr C. A. Holman Haematology Department Lewisham Hospital High Street Lewisham London SE13 6LH	01–690 4311
Professor J. W. Stewart Bland Sutton Institute of Pathology The Middlesex Hospital Mortimer Street London W1PDB	01–636 8333 (At night and at weekends please ask hospital telephone operator for the duty pathologist)
Dr Katharine M. Dormandy The Haemophilia Centre The Royal Free Hospital (NW Branch) Lawn Road London NW3 2XJ	01–794 4561 (After 5.30 pm and at week-ends please ask for the resident pathologist on call for the Haemophilia Centre)
Professor T. A. J. Prankerd Haematology Department University College Hospital Gower Street London WC1 6JJ	01–387 5050

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ADDRESS	TELEPHONE NO.
Dr G. I. C. Ingram Department of Haematology St Thomas' Hospital London SE1 7EH	01–928 9292 x GRO-C (At night and at weekends please ask hospital telephone operator for the doctor on duty for haemophilia)
Dr J. G. Humble Haematology Department Westminster Hospital Dean Ryle Street London SW1 P24P	01–828 9811
Professor P. T. Flute Haematology Department St George's Hospital Blackshaw Road London SW17 0QT	01–672 1255
Manchester Dr I. W. Delamore Department of Clinical Haematology The Royal Infirmary Manchester M13 9WL	061–273 3300
Dr D. I. K. Evans Department of Pathology Royal Manchester Children's Hospital Pendlebury Manchester M27 1HA	061–794 5321
Margate Dr H. Sterndale Isle of Thanet District Hospital (Margate Wing) St Peter's Road Margate CT9 4AN	0843 20222
Newcastle Dr T. H. Boon Royal Victoria Infirmary Newcastle upon Tyne NE1 41P	0632 25131
Oxford Dr Rosemary Biggs Dr C. Rizza Oxford Haemophilia Centre Churchill Hospital Oxford OX3 7LJ	0865 64841 x GRO-C (After 5 pm and at weekends please ask the hospital telephone operator for the doctor on call for the Haemophilia Centre)

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ADDRESS	TELEPHONE NO.
Portsmouth Dr J. R. O'Brien Central Laboratory St Mary's General Hospital (East Wing) Milton Road Portsmouth PO3 6AG	0745 22331
Sheffield Professor E. K. Blackburn The United Sheffield Hospitals Department of Haematology The Royal Infirmary Sheffield S6 3DA	0742 20977 (At night and at weekends please ask for the doctor on call for the Haemophilia Centre)
Southampton Dr J. Leslie Royal South Hants Hospital Fanshawe Street Southampton S09 4RE	0703 26211

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