Genetics of Human Blood Coagulation

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Modern concepts of human blood coagulation have evolved largely from the study of haemorrhagic disease caused by genetical defects in the clotting mechanism. The first step in identification of most clotting components has been to prepare a fraction from normal blood that will correct the coagulation defect. The 'new' component is then isolated from the fraction and studied until sufficient unique properties are available to differentiate it from all other substances known to participate in clotting. Attempts are made to fit the new information into current theories of coagulation and the component is given a name. In the past this latter and apparently trivial action led to almost paralysing confusion, for by 1959 there were at least 64 synonyms for 10 distinct entities. As suggested by Schmidt in 1861 the non-committal term 'clotting factor' is best retained until a precise chemical notation is available, and through international agreement (Wright, 1959, and subsequently) each recognized component is temporarily assigned a Roman numeral. The international system of nomenclature is given in Table I, together with synonyms still in current use. Coagulation disorders are also qualified by the factor or factors concerned, but the term 'haemophilia' will be retained in historical discussion to cover the clinically identical conditions of factor VIII and factor IX deficiencies.

Previous chaos over terminology was symptomatic of problems that have haunted investigators ever since John Hunter (1794) concluded that the clotting mechanism was as complex as life itself. The fundamental problem is lack of basic information on the structure, metabolism, and physiological function of clotting factors due to difficulties with isolation and chemical analysis. Calcium is an exception, but its ionic concentration in plasma

does not vary sufficiently to affect clotting. Fibrinogen is a fairly well-defined plasma protein, but despite considerable progress over the past two years the remaining factors can only be characterized in gross terms of biochemical and physiological function, though available data suggest that each is a protein. It follows that methods of measuring most factors are indirect and based on reactions in artificial clotting systems. Quantitative assay of clotting factors is difficult and the internal errors of each method are not well defined. Lack of agreement on normal standards invalidates comparison of results obtained in different laboratories and impedes the study of quantitative variation. In addition, such are the technical problems that it is not often practicable to reproduce a given experimental system in order to examine claims based on its use. This inability to test an hypothesis by Newtonian principles has led to many variant interpretations of clotting phenomena, some of which run so contrary to the accumulated mass of experimental observation as to be logically untenable. The persistence of different interpretations and the resulting polemics are additional reasons why blood coagulation is such a difficult and contentious field.

The development of hypotheses on human blood coagulation has been fully reviewed by Biggs and Macfarlane (1962). Briefly, the current theory is based on the classical scheme of Schmidt (1892) and Morawitz (1905), which can be summarized as follows.

There is general agreement on the principles of the thrombin-fibrinogen reaction (Laki and Gladner, 1964). Thrombin acts enzymatically on its substrate, fibrinogen, splitting off polypeptides. Residual molecules (fibrin monomers) polymerize

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TABLE I
NOMENCLATURE OF BLOOD COAGULATION FACTORS AND THEIR GENETICAL DISORDERS

International Classification*	Synonyms Still in Common Usage†	Some Synonyms Still Used for Genetical Disorders‡
I	<i>Fibrinogen</i>	Congenital afibrinogenaemia
II	<i>Prothrombin</i>	Congenital hypoprothrombinaemia
III	<i>Thromboplastin (tissue extract)</i>	—
IV	<i>Calcium</i>	—
V	Pro-accelerin; labile factor accelerator globulin (AcG)	Hypo-accelarinaemia
VI	Not now used	—
VII	Proconvertin; Stable factor; serum prothrombin conversion accelerator (SPCA); autoprothrombin I	Hypoproconvertinaemia
VIII	Antihæmophilic globulin (AHG), antihæmophilic factor (AHF), antihæmophilic factor A (AHA), platelet co-factor I	Haemophilia A, AHG (or AHF) deficiency
IX	Christmas factor plasma thromboplastin component (PTC) antihæmophilic factor B (AHB) platelet co-factor II autoprothrombin II	Christmas disease Haemophilia B PTC deficiency
X §	Stuart factor Stuart-Prower factor	Stuart deficiency
XI	Plasma thromboplastin antecedent (PTA)	PTA deficiency, haemophilia C
XII	Hageman factor	Hageman trait
XIII	Fibrin stabilizing factor (FSF) Lorand—Laki (LL) factor fibrinase	FSF (or fibrinase) deficiency

* Following the recommendations of International Committee on Blood Clotting Nomenclature (Wright, 1959).

† Terms in italics are used in this review.

‡ Accepted usage is currently 'Factor ... deficiency'.

§ Not to be confused with Factor X of Koller (1955), a term used for multiple factor defects resulting from coumarin therapy or vitamin K deficiency.

in the presence of activated factor XIII and calcium ions to form a haemostatically effective fibrin gel. The key to blood coagulation lies in the conversion of prothrombin into the active enzyme, thrombin. Current theory recognizes two independent pathways of prothrombin activation. The first involves plasma clotting factors and is known as the intrinsic or plasma system. This system is initiated by contact of blood with surfaces other than normal vascular endothelium (Nossel, 1964). Surface contact activates factor XII which then activates factor XI, and in like manner factors IX, VIII, X, and V successively participate in the system. Calcium ions and a phospholipid derived from platelets are also required, though their sites of action are not clear. The other prothrombin-activation pathway starts with release of undefined extracts with enzymatic properties from damaged tissue and is termed the extrinsic system. Tissue extracts activate factor X in the presence of factor VII and calcium. All this constitutes the majority view and there are other theories, notably that of Seegers (1964), which are radically different.

For some time hypothetical schemes of blood clotting have been based on the principle of a sequential or chain reaction. It was known that clotting factors occurred physiologically as inactive precursors and that some had proteolytic properties. But it was not possible to envisage a co-ordinated enzymatic system without invoking entirely hypo-

thetical reaction products at various stages. There was, to quote Biggs and Gaston (1960), an atmosphere of fantasy and improbability about the whole subject. The position changed when Macfarlane (1964a, 1965) published a simple and rational hypothesis, well supported by experimental evidence, much of which has been obtained very recently. He proposed a sequential transformation of inactive plasma clotting factors (proenzymes) into the active state (enzymes). As the product of each reaction acted on the next factor in sequence, Macfarlane likened the process to an enzyme cascade or biochemical system of amplification, because at each step a greater weight of protein was involved. Factor X appears to be common to both pathways of prothrombin activation because it acts as a substrate in the intrinsic system (Spaet and Cintron, 1963; Macfarlane, 1965) and the extrinsic system (Hougie, 1959a). A scheme based on the enzyme cascade theory is given in Fig. 1.

Using different experimental data, Ratnoff (Davie and Ratnoff, 1964; Ratnoff, Botti, Crum, and Donaldson, 1965) has arrived at strikingly similar conclusions. Likewise the views of Margolis and Bruce (1964), based on the kinetics of plasma factor reactions, are in general agreement. The latter authors devised a simplified kinetic model to represent the quantitative relation between the concentration of a given clotting factor and its reaction rate (coagulation time) under standard conditions. One conclusion was that the sum of indi-

FIG. 1. Scheme of human blood coagulation based on enzyme cascade theory of Macfarlane (1964a, 1965). ? Va denotes lack of experimental evidence for activation of factor V, though recent work (Davie and Ratnoff, 1964) suggests that activation takes place as depicted. Calcium ions are required for *in vitro* performance of each prothrombin activation system, the thrombin-fibrinogen reaction, and factor XIII activity.

vidual reaction constants could not be exceeded on theoretical or experimental grounds, thus implying that the system was complete and there were no more major clotting factors to be discovered. But an essential assumption was that an ultra-dilute system existed. This is certainly a gross over-simplification because the reaction rate between any two factors must be greatly complicated by the velocity of molecular rearrangements that occur, for instance when active enzymes are rapidly destroyed.

It must be emphasized that blood clotting is not an isolated system but part of an extensive reaction mechanism involving many physiological processes. A close but currently obscure relationship exists between the plasma coagulation factors and the other major components of haemostasis, the platelets, and small blood vessels. This relationship is underlined by rare mutations that cause defects in more than one haemostatic compartment. Conventional classifications of phenotypes into one or other compartment have been made for didactic not biological reasons and are becoming difficult to maintain. For example, it has recently been claimed that such an apparently localized example of gene effect as the non-contractable malformations of small blood vessels in hereditary haemorrhagic telangiectasia (Macfarlane, 1941) is associated with a consistent abnormality of platelet function (Muckle, 1964). The difficult problem of genetically defective haemostasis (involving the blood

clotting system) will be discussed in relation to von Willebrand's disease.

The powerful mechanism for inhibiting activated clotting factors and their reaction products is as yet poorly defined in biochemical terms. Teleologically regarded as a defence against intravascular coagulation, this system rivals the clotting mechanism in complexity. The fibrinolytic system, greatly studied in view of its therapeutic potential, has been recently reviewed (Macfarlane, 1964b). Inhibitors in the earlier stages of clotting have been discussed by Favre-Gilly and Thouverez (1959), Margolius, Jackson, and Ratnoff (1961), and Soulier (1962). As will be shown, it is not certain whether hereditary disorders of naturally occurring inhibitors have been demonstrated. Acquired inhibitors may arise in pregnancy, during the course of malignant or other disease, and occasionally in otherwise healthy persons (Hougie, 1955; Biggs and Macfarlane, 1962). Acquired inhibitors may also develop against the clotting factor that is defective in genetical disorders of blood coagulation. This phenomenon has some features of an immune reaction but its actual mechanism remains unclear (Biggs and Bidwell, 1959; De Vries, Rosenberg, Kochwa, and Boss, 1961; Leitner, Bidwell, and Dike, 1963).

Congenital haemorrhagic states characterized by complete or partial deficiency of a single clotting factor are generally interpreted in terms of current theory as resulting from a genetical disorder of factor synthesis. Knowledge of certain disorders has hardly progressed beyond that point reached by Garrod with the first inborn errors. The customary restraint in dealing with blood coagulation in discussions on biochemical genetics (e.g. Harris, 1959) is well judged. Nevertheless, sufficient information is now available to avoid perpetuating curious beliefs such as listing 'abnormalities in tissue extracts' among genetical defects of the extrinsic clotting system (Clarke, 1964).

The hypothetical status of blood clotting is a serious hindrance to any genetical analysis. Much of the relevant literature is rendered valueless because what is hypothetical has been presented as fact, and there is no record of objective data that would have remained permanently useful. Contemporary ideas with relation to gene action, complex effects of mutation, and the evolution of blood clotting are, with a few exceptions, speculative. But while awaiting more precise chemical data such ideas have heuristic value, and in this regard it is worth while summarizing the considerable contribution to human genetics made by the study of haemophilia during the period when very little was known about blood coagulation.

SOME GENERAL OBSERVATIONS

Contribution of Haemophilia to Human Genetics: Historical

Early examples of hereditary counselling in the form of rabbinical dispensations for circumcision of boys from families where previous male children had died after the operation are recorded in the Talmud (Folios 64, 134a). The description in early texts leaves little doubt that death was due to bleeding, with haemophilia the most likely cause (Rothschild, 1882; Katzenelson, 1884).

The first unequivocal account of haemophilia in the medical literature was published by Consbruch in 1793, though McKusick (1962a) has recently claimed precedence with a convincing report from a Massachusetts newspaper dated 1791. In a report which received widespread attention Otto (1803) emphasized that though females were unaffected they were capable of transmitting haemophilia to their male children. By 1820 sufficient families had been described for Nasse to stress inheritance through normal females (Nasse's law). Henceforth haemophilia was considered the most typical example of sex limitation (Sedgwick, 1861), as the mode of inheritance was called until Morgan (1913) decided that 'sex-linked' was a less misleading description.

During the latter half of the 19th century a haemorrhagic disease appeared among males in Queen Victoria's family. Although no laboratory investigation of affected members has ever been reported, descriptions of bleeding in Prince Leopold (*Brit. med. J.*, 1868, 1884b), the Tsarevich Alix (Curtiss, 1940), and other princes (Benson, 1939) are entirely consistent with haemophilia, and the royal pedigree subsequently became of technical value to Haldane (1938a), a source of conflict over publishing the genetical potential of living persons (Hutt, 1948 versus Iltis, 1948) and a standard illustration of sex-linked recessive inheritance in genetical textbooks. An accurate version is given by McKusick (1964).

One consequence of publicity given to unfortunate princes was that practising physicians became aware of the features of sex-linked inheritance and searched for other hereditary diseases with a similar basis (*Brit. med. J.*, 1884a). Thus the mode of transmission became apparent for one type of ichthyosis (Sedgwick, 1861), pseudo-hypertrophic muscular dystrophy (Gowers, 1909), and was confirmed for partial colour blindness (Horner, 1876). Nevertheless the mechanism of sex-linked inheritance in humans was not entirely clear to the geneticist Bateson (1909), and one reason for this was probably an increasing confusion over the identity of haemophilia. The clarity of early reports had become obscured by uncritical analysis of familial bleeding syndromes. In a comprehensive monograph Grandier (1855) concluded that haemophilia was common in

females, and this led eminent gynaecologists (e.g. Kehler, 1876) to regard profuse uterine haemorrhage without obvious cause as due to haemophilia. Divided opinions on haemophilia in women were closely examined by Bulloch and Fildes (1912) in their monumental review of 949 papers and monographs on haemophilia. Using stringent criteria of diagnosis they could not find unequivocal evidence of abnormal bleeding in females from haemophilic families and wisely concluded that further discussion would be unprofitable until an objective method of detecting impaired haemostasis had been devised. Their views had been previously criticized by Osler (1910) who described a girl with haemophilia-like symptoms of the type found in homozygotes with rare autosomal clotting factor deficiencies. Bulloch and Fildes rejected a family which contained a female subsequently regarded as homozygous for haemophilia. The proposita had bleeding symptoms similar to those experienced by her father (Treves, 1886). Handley and Nussbrecher (1935) enlarged the pedigree and in 1951a Merskey reported investigations on a sister of Treves' proposita and decided that she had haemophilia and being the product of a first cousin marriage was a true homozygote. Finally Gilchrist (1961) described a third sister with bleeding symptoms and laboratory evidence of factor VIII deficiency. However, Valberg (1959) disputed both the laboratory and pedigree evidence and regarded the family as showing the autosomal dominant condition, von Willebrand's disease.

Further controversy surrounded the offspring of haemophiliacs. So few affected males produced children that evidence was sparse, but Lossen (1877) categorically stated that haemophilia was never transmitted through an affected or normal male (Lossen's law). Bulloch and Fildes (1912) supported this view and where haemophilia had apparently been transmitted by the daughter of an affected male they suspected that the haemophilic had married a carrier female and felt that consanguinity through geographical isolation would favour such a mating. Legg (1872) also held this view, noting the apparently high incidence of haemophilia in Germany where cousin marriages were not discouraged. Both Legg and Bulloch and Fildes quoted evidence for their hypothesis from supposedly consanguineous matings in the famous Mampel kindred (Lossen, 1877), but the pedigree was unreliable and contained several bogus haemophiliacs who assumed the diagnosis in order to escape military service in the Napoleonic wars (Klug, 1926).

Bulloch and Fildes confirmed Wachsmuth's (1849) observation that women in haemophilic families were highly fertile, and with the help of Karl Pearson showed how biased ascertainment produced an improbable sex-ratio in reported sibships. They also concluded that a few cases may

'arise *de novo*' but added that a more likely explanation was inability to demonstrate a line of inheritance. Although Osler (1910), Roth (1918), and Boggs (1934) subsequently published convincing evidence to support the 'spontaneous origin' of haemophilia, the concept was grudgingly accepted by the medical profession and in 1960 Quick found it necessary to review the case for fresh mutation.

Following Mendelian predictions, transmission of the gene through daughters of haemophiliacs was demonstrated in pedigrees (Muir, 1906, 1928), proved theoretically (Bauer, 1922), and generally accepted (Schloessmann, 1924). Bauer's opinion that females homozygous for haemophilia would not survive intrauterine life remained unchallenged until breeding experiments with the North Carolina colony of haemophilic dogs (Graham, Buckwalter, Hartley, and Brinkhous, 1949) produced homozygous females with symptoms similar to those in affected hemizygous males.

In 1937 Birch published observations on 98 haemophiliacs and their families in Illinois. She used a prolonged blood coagulation time as laboratory evidence of haemophilia. This test, developed by Almroth Wright (1893), was not in general use at the time of Bulloch and Fildes' survey but subsequently became a diagnostic criterion for haemophilia, thereby delaying recognition of mild forms in which normal results are obtained. Until quite recently the possibility of haemophilia was rejected if the coagulation time was normal (Davidson, Epstein, Miller, and Taylor, 1949). Mild haemophilia was first defined in a clear fashion by Merskey (1951b), though Schloessmann (1930) and Haldane (1935) had previously explained variations in haemorrhagic severity as due to allelic genes.

In 1943 Andreassen published a survey of haemophilia in Denmark which was the first attempt at complete ascertainment in a small relatively immobile geographically defined population. Sköld (1944) followed with a similar survey in Sweden, and a Scandinavian tradition was established with another careful population study of haemophilia from Finland (Ikkala, 1960) and follow-up surveys in Denmark (Sjolin, 1961) and Sweden (Ramgren, 1962a).

Although Davenport (1930) and Hogben (1931) had published some preliminary calculations on segregation, mutation, and linkage, the first rigorous analysis was made when Haldane (1935) used data from Bulloch and Fildes' survey and some additional information collected by Dr. Julia Bell to develop his indirect method of estimating mutation rate at a sex-linked locus. He subsequently applied the method to the data of Birch (Haldane and Philip, 1939) and Andreassen (Haldane, 1947) and also examined linkage between the genes for

colour blindness and haemophilia, first on rather crude data (Bell and Haldane, 1937) and subsequently on Hoogvliet's (1942) excellent material (Haldane and Smith, 1947). From all this work Haldane developed methods of analysis and arguments that remain of permanent value in genetics, though certain specific estimations and conclusions based on what is retrospectively very imperfect data have subsequently become invalid.

During the 1930's experimental work on blood clotting created laboratory techniques which led to the first clear indication of heterogeneity in haemophilia and related states, when Owren (1944) described a female with congenital factor V deficiency. The new era for classical sex-linked haemophilia commenced and in 1952 when Biggs, Douglas, Macfarlane, Dacie, Pitney, Merskey, and O'Brien in England, and Aggeler, White, Glendening, Page, Leake, and Bates in the United States of America independently discovered factor IX deficiency and so demonstrated that the phenotype of 'haemophilia' could be caused by two dissimilar sex-linked genes.

Laboratory Investigations in Blood Coagulation

This is no place for a discussion on the mechanism and relative merits of laboratory systems used in the study of blood clotting. The subject is fully reviewed by Biggs and Macfarlane (1962). Unfortunately some knowledge of established tests is vital in considering matters of genetical importance, and in the past several geneticists have analysed data based on misinterpretation of results or faulty application of a particular test. For instance, Haldane (1947) was misled by Andreassen's (1943) conclusion that the whole blood coagulation time was prolonged in females heterozygous for haemophilia. This is definitely not the case (Nilsson, Blombäck, Ramgren, and von Francken, 1962; Biggs and Macfarlane, 1962), but the assumption influenced Haldane's ideas on mutation rate and did not permit him to reject Sirks' (1937) theory on crossing-over between the X and Y chromosome, despite its theoretical difficulties (Haldane, 1938b). Likewise Sjolin (1961), through using in a population study the sensitive and non-specific thrombin generation test (Macfarlane and Biggs, 1953) in a manner unsuitable for differentiating between defects in the intrinsic pathway of prothrombin activation (Ollendorff, 1960; Nilsson, Blombäck, and Ramgren, 1961), reported among other unique findings the highly improbable occurrence of either factor VIII, factor IX, or factor XII deficiency in different males from the same sibship.

Diagnostic systems are designed for classifying patients with various clotting defects and are generally efficient in differentiating normal from abnormal within the framework of contemporary blood coagulation theory. As theory has changed so has the interpretation of some tests that are retained for their general usefulness. The one-stage prothrombin time test devised by Quick (1935) on the classical Schmidt-Morawitz theory to measure prothrombin was subsequently found to give abnormal results in deficiencies of factors V, VII, or X. Thus reinvestigation of certain patients with inherited haemorrhagic disease and a prolonged prothrombin time has reflected the increase of knowledge. For example, affected members of one family were classified as congenital hypoprothrombinaemia in 1948 (Hagen and Watson), factor VII deficiency in 1953 (Frick and Hagen), and factor X deficiency in 1958 (Gonyea and Krivit). Such reclassification is an obvious hazard in reviewing recorded cases. Coagulation experts may also differ on the validity of previous laboratory investigations. Marder and Shulman (1964) considered there was sufficient evidence to regard 52 reported cases of factor VII deficiency as proven, but of these Owen, Amundsen, Thompson, Spittell, Bowie, Stilwell, Hewlett, Mills, Sauer, and Gage (1964) scored 15 as probably affected, 3 as possibly affected, and one as an unlikely case.

In general, gross disturbances of clotting can be readily classified but there is often difficulty with milder defects, particularly those of factor XI or XII. A critical test is *in vitro* comparison of the patient's plasma with that from a person with a known specific defect, and in this manner factor XI deficiency was confirmed in two patients who had previously been regarded as unclassifiable (Todd and Wright, 1964) or factor XII-deficient (De Vries and Braat-van Straaten, 1964). *In vitro* comparison caused confusion in the past because 'haemophilic plasma' could be deficient in either factor VIII or factor IX and 'hypoprothrombinaemic plasma' might lack any one of four factors. However, Ratnoff and Steinberg (1962) could be confident of using homogeneous data in their genetical analysis of factor XII deficiency, for though their 55 cases were investigated in many different centres throughout the world, with only one exception the diagnosis had been confirmed by comparative tests with plasma from Ratnoff's original patient, Mr. Hageman, or with other factor XII deficient plasma that had been matched with his.

Some tests of clotting function are listed in Table II, together with investigations used in measuring other components of haemostasis. Taking advantage of known properties of each factor basic laboratory procedures are modified in many different ways to give a qualitative estimation suitable for diagnosis. Semi-quantitative estimations are expressed as a percentage of

TABLE II
SOME BASIC LABORATORY TESTS USED IN INVESTIGATION OF HAEMOSTATIC FUNCTION*

Test	Aspect of Haemostatic Mechanism Measured	Conditions in Which Test is Abnormal
Whole blood coagulation time	Over-all coagulation mechanism	Severe coagulation factor deficiencies; factor inhibitors
Thromboplastin generation test	Intrinsic pathway of prothrombin activation	Deficiencies of factors V, VIII, IX, X, XI, and XII qualitative platelet defects; factor inhibitors
One-stage prothrombin time	Extrinsic pathway of prothrombin activation; prothrombin, fibrinogen	Deficiencies of factors V, VII, and X, prothrombin, and fibrinogen; factor inhibitors
Bleeding time†	Platelet function vessel contraction	Quantitative and qualitative platelet defects; capillary defects
Tourniquet test	Platelet function; vessel wall integrity	Qualitative and quantitative platelet defects; capillary defects
Clot observation	(a) Clot retraction (b) Clot lysis (c) Stability in 5M urea	Quantitative and qualitative platelet defects; fibrinolysis; factor XIII deficiency

* Details and further tests in Biggs and Macfarlane (1962). Additional information on investigation of: contact factors, Nossel (1964); platelet function, Johnson (ed.) (1961); fibrinolysis, Macfarlane (ed.) (1964b).

† Techniques discussed by Nilsson, Magnussen, and Borchgrevink (1963).

the 'average normal level' (taken as 100%) with the exception of fibrinogen which can be measured directly. The general principle is to construct an *in vitro* clotting system with the test factor as the only variable. The clotting time of the system is then compared with values on a reference curve obtained by serial dilutions of a standard normal plasma with factor-deficient plasma. Unfortunately there are different interpretations of the 'standard normal plasma' which is usually normal plasma pooled from a varying number of healthy individuals, or in the case of factor VIII incorporates a concentrated source of animal or human factor VIII. Factor content of standard preparations depends on conditions of storage and other variables, and attempts are being made to produce material that will permit comparison of measurements made at different times in different centres (Kekwick and Walton, 1964; Aronson and Marder, 1964). There is little information on the relative precision, accuracy, or specificity of any particular assay system, and it must be emphasized that factor levels recorded on the following pedigrees represent only approximate measurements.

Lack of knowledge on the variation of clotting factor levels in the normal population seriously impedes quantitative analysis of data. With a few

notable exceptions little more than an unqualified 'normal range' is usually given and the frequency distribution limits of levels in those heterozygous for incompletely recessive autosomal or sex-linked genes and normal persons are often obscure. This problem will be further discussed with reference to factor VIII.

There is one technique, the thrombo-elastograph (Hartert, 1952), that differs from usual systems in recording physical changes that take place during clotting. The instrument has been used quite extensively on the Continent, but is affected by ill-defined technical errors and its results can only be interpreted in terms of theory developed from clotting studies in glass tubes. It is therefore generally regarded as having no advantage over conventional methods. Gedda and Poggi (1964) used the thrombo-elastograph in a study of normal twins in order to detect hereditary components of blood clotting, but their results merely underline the undefined variability of sundry physical measurements.

Tests of clotting function are in general technically complex, sensitive to variations in blood collecting and handling of samples, and laborious to perform. Such features invalidate use of the tests in field surveys, though it is possible to employ relatively simple screening tests (e.g. Margolis, 1961; Hardisty, Macpherson, and Ingram, 1962) of the intrinsic prothrombin activating system which is the area where the majority of mild or clinically latent defects are encountered. Screening tests can be performed on capillary (finger-prick) blood, but further elucidation requires full-scale investigation on venous blood in a specialized laboratory. It must be emphasized that the whole blood coagulation time is useless for detecting mild clotting disorders (Diamond and Porter, 1958). Quick (Quick, 1957; Quick and Hussey, 1962a) has constantly reiterated that the one-stage prothrombin time test, which is sensitive to factor defects in the extrinsic prothrombin activating system, gives if performed to his instructions an almost invariably normal result of 12 seconds. Unfortunately other laboratories cannot achieve this constancy (as shown by the normal ranges given in Table IV and the study of Brooks and Copeland (1964), and so it is not possible to use pre-treatment levels in patients attending anticoagulant therapy clinics as a means of detecting the 1-5 second prothrombin time prolongation often found by careful testing of those heterozygous for factor V, VII or X and possibly prothrombin deficiency. Following the reasoning of Graham, Barrow, and Hougie (1957), the combined population frequency of these heterozygotes may be in the order of 0.06-0.08.

Phenotypes in Genetical Disorders of Blood Coagulation: Ascertainment and Prevalence

As a general rule there is a tendency to abnormal bleeding when a plasma clotting factor cannot be detected in the circulation or its concentration is reduced to below approximately one-third of the normal level. Factor XII deficiency is the exception, for despite grossly disordered *in vitro* clotting there is characteristically no haemostatic defect, and affected persons are usually ascertained through the chance finding of delayed blood clotting during unrelated laboratory investigations, blood donation tests, or in the United States of America through the medico-legally inspired but irrelevant use of the whole blood clotting time as a screening test for haemostatic defects before surgery (Diamond and Porter, 1958; Fletcher, 1958).

The pattern of bleeding in inherited disorders of blood coagulation differs from the 'capillary' or 'thrombocytopenic' type (Quick, 1957; Ratnoff, 1960; Biggs and Macfarlane, 1962) common to von Willebrand's disease, and genetical defects of platelets (Quick and Hussey, 1962b; Kanska, Niewiarowski, Ostrowski, Poplawski, and Prokopowicz, 1963; Alagille, Josso, Binet, and Blin, 1964) or capillaries (Blackburn, 1961). Certain groups of clotting disorders have typical features, and though clinical discrimination is too imprecise for distinguishing the underlying defect some characteristics are valuable as a means of case ascertainment from diagnostic records, particularly in the milder states without florid haemorrhagic disease. For instance, umbilical bleeding on separation, not section of the cord (Hurez, Debray, Lebras, and Denimal, 1961) is universal in afibrinogenemia and factor XIII deficiency, but most unusual in haemophilia despite the presence of factor VIII deficiency at birth (R. Biggs, 1965, personal communication). Bleeding of apparently spontaneous origin, intramuscular haematoma, and haemarthroses are invariably encountered in severe haemophilia but uncommon in other states where haemorrhage from epithelial-lined tracts is more typical. Tonsillectomy is a dangerous and often lethal way of detecting mild clotting defects (Krahl, 1955; Biggs and Macfarlane, 1958), but due to the different haemostatic situation appendectomy may cause no abnormal haemorrhage even in severe haemophilia (Kerr, 1964).

Clinical severity in haemophilia is generally related to the clotting factor level, and though measurement of the latter is a preferable way of grading severity most haemophiliacs fall into one of the following clinical categories, each of which is associated with a character-

istic range of factor VIII or factor IX levels (Ikkala, 1960; Kerr, 1963):

Severe haemophilia. Factor level 0–1%, coagulation time usually more than one hour. Abnormal bleeding noted in first year, haemarthroses occur before school age, and haemorrhage may be of apparently spontaneous origin and show a cyclic tendency to recur episodically. Intramuscular haematoma, haematuria, and gastrointestinal bleeding are common. Almost all have permanent physical disability by puberty.

Moderate haemophilia. Factor level 1–3%, coagulation time usually not prolonged over 30 minutes. An intermediate grade with obvious manifestations of lesser intensity than the severely affected.

Mild haemophilia. Factor level 3–30%, coagulation time normal. Abnormal bleeding follows significant trauma, operation, tooth extraction, or is associated with local pathology. Haemarthroses are rare. Affected males lead an entirely normal life participating in body-contact sports, military service, and so on.

It must be emphasized that the above categories apply only to haemophilia and are retained for descriptive usage and in defining clinical grades employed before the advent of modern tests. There is usually close agreement for both symptoms and factor levels among related males with factor VIII or IX deficiency (Digilio, Bacchetta, and Savignoni, 1960; Biggs and Macfarlane, 1962; Joist, 1963; Lewis, Didisheim, Ferguson, and Li, 1963), but on occasion, despite having similar laboratory findings, a patient will differ clinically from his affected relatives in having no apparent haemorrhagic tendency (Quick, 1957; Moor-Jankowski, Truog, and Huser, 1957) or in contrast may have a distinctly increased bleeding tendency (Margolis, 1961). The mechanism of this clinical discordance remains unknown.

Classifying haemorrhagic severity on impressions of a few cases is notoriously unreliable and well demonstrated by the large kindred of McKusick and Rapaport (1962), where, before the days of effective therapy, some haemophiliacs died from exsanguination in infancy and others lived for more than 70 years, the oldest dying when aged 82. The situation is most difficult in mild haemorrhagic states where the question of abnormal bleeding rests on the significance of such variable events as menorrhagia and bleeding after operation, tooth extraction, parturition, or in association with some tissue lesion. A serious haemostatic defect obviously exists, when a woman has von Willebrand's disease associated with many episodes of life-endangering menorrhagia (Jorpes, 1959), or, due to undiagnosed factor XI deficiency, has required massive blood transfusion after several operations (Lisker, Josephson, Werbin, Shapiro, and Rosengvaig, 1957). Yet abnormal bleeding mainly

localized to one particular organ or situation cannot be graded in terms of the generalized haemorrhagic state in haemophilia. Therefore 'severe' or 'marked' is a suitable description for significant bleeding in autosomal disorders, as opposed to 'mild' for vague and ill-defined episodes.

Ascertainment of severe haemophiliacs in certain populations has been reasonably complete, because from an early age the patients inevitably become known to hospitals, special treatment centres, research workers, blood transfusion services, and agencies for the physically disabled (Ikkala, 1960; Ramgren, 1962a; Kerr, 1965). The same reasoning applies to those with more extreme phenotypes due to autosomal genes. In contrast the probability of encountering mildly affected patients remains unknown. A proportion undoubtedly go through life without experiencing any traumatic incident that leads to diagnosis, and others are not detected until middle age (Kerr, 1962). Not unexpectedly there still exists a reluctance to consider haemophilia in an otherwise healthy male, and what is retrospectively an alarming post-operative haemorrhage in such persons is at the time often ascribed to local causes. Under-ascertainment of mild haemophiliacs has certain consequences in population studies, which will be discussed in relation to factor VIII.

The distribution of hereditary clotting defects appears to be world wide in the sense that a similar pattern of disorders is encountered wherever diagnostic facilities are available. Phenotype frequencies in Japan (Abe, 1964) are comparable to those in native and migrant European populations. Excluding von Willebrand's disease whose prevalence is uncertain through differences in diagnostic criteria, factor VIII and factor IX deficiencies account for over 95% of all cases detected in population surveys (Ikkala, 1960; Nilsson *et al.*, 1962; Kerr, 1965). The frequency of factor VIII deficiency relative to factor IX deficiency has been variously estimated from 10:1 (Den Ottolander and Hoorweg, 1955) to 3:1 (Lewis, Ferguson, Fresh, and Zucker, 1957). The ratio in pooled data from 16 collections of patients listed by Jung (1960) was approximately 5:1. A higher proportion of males with factor IX deficiency (25% of the total) was noted in two population studies (Ikkala, 1960; Kerr, 1965). The over-all prevalence of genetical disorders is not clear. The position is unsatisfactory because research centres deal with a selected group of patients and tend to collect certain rare disorders which particularly interest them. Population studies have generally been concerned only with factor VIII and factor IX deficiency. Estimates

on trait frequencies in a population vary from 10^{-4} (Marx, 1959) to more commonly quoted figures within the range $1.5 \times 10^{-4} - 2.5 \times 10^{-4}$ (Den Ottolander and Hoorweg, 1955; Rapaport, Fallon, and Goodman, 1958; Quick, 1957; Fantl, 1961a; Ramgren, 1962b; Biggs and Macfarlane, 1962; Polák, Chrobák, Salavec, Cerňík, and Antalovská, 1964). More extensive data on frequencies in factor VIII and factor IX deficiency will be discussed later. Von Willebrand's disease is remarkably prevalent on some Baltic islands, affecting 1 in 10 of the population (Eriksson, 1961) as compared to the incidence of diagnosed cases in Australia, 1 in 300,000 (Fantl, 1961a). Homozygotes for incompletely recessive genes with marked bleeding symptoms are extremely rare (for each condition q^2 is unlikely to be greater than 10^{-6}). It is impossible even to hazard a guess at the frequency of factor XII and factor XI deficiencies with typically absent or very mild haemorrhagic symptoms because ascertainment is so largely dependent on chance.

MAJOR CLINICAL TYPES

(1) Fibrinogen

Current knowledge on the chemistry of fibrinogen and its conversion to fibrin has been thoroughly reviewed by Laki and Gladner (1964). Molecules of fibrinogen are ellipsoid, measure some $500 \times 40 \text{ \AA}$, and have a molecular weight of about 330,000. Data are available on amino acid composition of fibrinogen from man, ox, pig, sheep, dog, horse (Blombäck and Yamashina, 1958), and lamprey eel (Doolittle, Oncley, and Surgenor, 1962). During clotting, thrombin enzymatically alters the molecular structure of fibrinogen by releasing fibrinopeptides, and the parent molecules (fibrin monomers) then polymerize to form the fibrin network. The bovine fibrinogen molecule contains about 2700 amino acids incorporated into six peptide chains (Blombäck and Yamashina, 1958), and there is evidence that the specificity of thrombin action is dependent on the amino acid sequence (Clegg and Bailey, 1962). The evolutionary significance of comparative studies in fibrinogen and the fibrinogen-fibrin transition will be discussed later.

It is agreed that fibrinogen is synthesized by the liver and the plasma level is reduced in extensive disease, extirpation, or necrosis of that organ (Favre-Gilly, 1947). Little is known about metabolism, but by comparison with other plasma proteins not concerned with coagulation the rapid physiological turnover of fibrinogen suggests a continuous *in vivo* consumption (Astrup and Van Creveld, 1961), and Miller, Titthasiri, and Hanavan

(1961) noted that the rate of fibrinogen formation was dependent on the blood level, which implied a feedback system of regulation.

Acquired fibrinogen deficiency due to decreased production, rapid utilization through widespread clotting, or destruction by circulating fibrinolysins is far more common than a congenital deficiency (Biggs and Macfarlane, 1962). No more than two unrelated cases with the latter condition have ever been reported from any large blood coagulation research centre. Two hereditary varieties have been recognized, each distinguished by a descriptive term based on the degree of fibrinogen deficiency. Congenital afibrinogenemia, first described by Rabe and Salomon (1920), is characterized by a haemorrhagic state, infinitely prolonged whole blood coagulation time, and absence of plasma fibrinogen, as measured by techniques based on the clotting properties of thrombin, salt-precipitation of plasma fibrinogen, and electrophoretic analysis. The other variety, congenital hypofibrinogenemia, is less well defined as diagnosis is dependent on 'significantly' reduced fibrinogen levels, and haemorrhagic symptoms are generally vague. Needless to say, lack of knowledge on the distribution of fibrinogen values in normal populations and the internal variation of methods of estimation has hindered classification of reported cases and analysis of family studies. The normal range of variation is generally taken as 250-400 mg./100 ml. (Biggs and Macfarlane, 1962; Laki and Gladner, 1964; and the majority of authors listed in Table III). However, a few surveys of levels in several hundred healthy subjects suggest a far wider range of variation (e.g. Hill and Trevor-row, 1942; Revol, 1962), and the carefully controlled comparison of several methods by Gross, Schwick, Lang, Nies, Rahn, Becker, and Hengstmann (1963) suggest a source of individual and technical variation for each estimation.

Previous reviewers of congenital afibrinogenemia have noted a sex distribution of cases at variance with an autosomal hypothesis (Frick and McQuarrie, 1954; Graham, 1957), have differed on the significance of findings in heterozygotes (e.g. Graham, 1957; Harris, 1959), and have considered heterogeneous case material as a single entity (Bommer, Künzer, and Schröer 1963b). In addition, individual and familial details of the same patients have been scattered through several different reports of clinical studies. For these reasons, all cases with sufficient data to support the diagnosis of congenital afibrinogenemia are recorded in Table III.

Congenital Afibrinogenemia. No fibrinogen was detected in the blood of 61 patients (Table III)

TABLE III
REPORTED CASES OF CONGENITAL AFIBRINOGENAEMIA

Case No.	Source(s)	Sex Sibs	Age at Last Investigation (yr.)	Age at Death	Parental Consanguinity†	Findings in Relatives‡
1	Rabe and Solomon (1920) Breckhoff (1924)	M	13	22	Yes	Parents normal CT
2	Opitz and Frei (1921)	F	8½ mth.	8½ mth.	Yes	—
3	Macfarlane (1938) Biggs and Gaston (1960)	M	8	28	Yes	Sister + H, father 75, mother, brother normal F
4	Paillard, Jeune, and Revol (1938) Revol and Favre-Gilly (1947)	F	26		No	
5	Schönholzer (1939)	M	12	13	Yes	Sister + H, sister 130, parents, 8
6	Fritzsche (1939)	F	2½	2½	No	other relatives normal F
7	Glanzmann, Steiner, and Keller (1940) Brönnimann (1954)	M	15		No	
8	Van Creveld (1941)	M	6 wk.	7 wk.	?	Mother, brother normal F
9	Castex, Pavlovsky, and Bonduel (1943) Pavlovsky and Berg (1962)	M	24		Yes	Parents, 3 brothers, normal F
10	Henderson, Donaldson, and Scarborough (1945)	M	11		Yes	Sister + H, parents, 2 sibs normal F
11	Biddau and Ammaniti (1946)	F	6	9	Yes	Brother + H, parents, 4 brothers, normal F
12	Pinniger and Prunty (1946) Pinniger and Franks (1951) Hardisty and Pinniger (1956) Ingram, Pinniger, and Vallet (1960)	F	30		Yes	Parents, 2 sibs normal F
13	Corbett (1947)	M	8 days		No	—
14	Revol and Favre-Gilly (1947)	M	4		No	—
15	Diamond, Wolff, and Borges (1948)	M	14		No	Parents normal F
16	Gitlin and Borges (1953)	M *	5		No	
17	Alexander <i>et al.</i> (1954) Rausen, Cruchaud, McMillan, and Gitlin (1961)	F	1½		Yes	Parents normal F
18	Buček (1951)	F	12		?	2 brothers + H, parents, sister normal CT
19	Prentice (1951)	M	19	19	No	Parents, 2 sibs normal F, maternal aunt 120
20	De Silva and Thanabalasundaram (1951)	M	11 mth.	1	No	
21	Lawson (1953)	F	20		No	Brother + H, mother 150
22	Van Nuffel and Verstraete (1953) Vandenbroucke, Verstraete, and Verwilghen (1954) Sokal (1962)	M	11		No	Parents normal F
23	Albeggiani and La Grutta (1954)	M	3		Yes	1 brother + H
24	Frick and McQuarrie (1954)	M	7		Yes	Mother F 163, father 196, brother 190, sister 178, sister and 6 other relatives normal F (> 250)
25	Lewis and Ferguson (1954)	M	3½		No	Mother normal F
26	Mahoudeau, Soulier, Dubrisay, Larrieu, and Pringuet (1954) Brault and Prat (1955) Soulier, Larrieu, Dubrisay, and Mahoudeau (1955)	F	20		Yes	1 sister + H
27	Pritchard and Vann (1954)	M	19 days		No	—
28	Caussade, Neimann, Pierson, and Manciaux (1954)	F*	8½		Yes	1 sister + H, father F 184, paternal grandmother 126, mother 257
29		F*	2			
30		F*	1 wk.			
31	Brault and Prat (1955) Soulier <i>et al.</i> (1955)	M	5½		Yes	
32	Camelin, Revol, Favre-Gilly, Vailhe, Ardry, and Meiral (1955)	F*	12		No	Parents, 3 sibs normal F
33	Maupin, Vigne, Perrot, Leroux, Raby, Storck, and Lacassie (1962a)	M*	5½			
34	Maupin, Moulllec, and Kherumian (1962b)	M*				
35	Laurinsich, Gatto, Gueli, and Imperato (1954)	M	20 mth.		No	
36	Sebok and Szabo (1955)	M	13		No	Mother normal CT
37	Hule and Preis (1956)	F	13 mth.		?	Mother F 78, maternal aunt 88, maternal grandmother 234, father, 2 sibs normal F (> 300)

Cont'd

TABLE III—continued

Case No.	Source(s)	Sex Sibs	Age at Last Investigation (yr.)	Age at Death	Parental Consanguinity†	Findings in Relatives‡
38	Wilson and Gutteridge (1956)	F	11		?	—
39	Fernando and Dharmasena (1957)	M	22		Yes	Parents, 4 sibs normal F
40	** Grossmann and Carter (1957)	?	Newborn	3 hr.	?	Parents, sister normal F
41	Morita and Kagami (1957)	F	7		No	Mother normal F
42	Cuttillo and Schettini (1958)	M	3 dy.		Yes	Parents normal F
43	Lato, Severi, and Antonelli (1958)	M	18 days	3 wk.	No	—
44	Orlando and Manara (1958)	M	9		No	Parents normal F
45	Van Creveld and Liem (1958) Astrup and Van Creveld (1961)	F	1		No	—
46	Kranz and Ruff (1959)	M	13		Yes	—
47	Garbin and Garofalo (1960)	M	5 dy.		Yes	Parents normal F
48	Rodriguez and Bidegain (1960)	F	3½		?	—
49	De Vries <i>et al.</i> (1961)	F	16	16	Yes	Sister + H, brother + H, father 5 sibs normal F
50	Gugler and Lüscher (1961)	M	2 mth.	2 mth	No	—
51	Jain and Parande (1962)	F	8		No	Sister + H, mother 33, father 65
52	Niewiarowski <i>et al.</i> (1962)	M	3		No	Brother + H, parents, 3 sibs normal
53	Kozłowska <i>et al.</i> (1964)	F	13		Yes	2 brothers + H, parents normal
54	Guimbretière and Harousseau (1962)	M*	4	4	No	Parents 3 sibs normal
55		M*	7	7		
56	Gross <i>et al.</i> (1963)	F	19		No	†† Mother 75, father 100, sister 83, aunt 73, paternal grandmother 325, sister 348
57	Oseid and Svendsen (1963)	M*	14		No	Parents, 3 sibs, 11 other relatives normal F
58		F*	6			
59	Werder (1963)	M	4		No	Mother 170, sister 170, 4 maternal aunts 163, 130, 163, 163, father 222
60		M	2½		No	mother 155
61	Bommer <i>et al.</i> (1963a)	F	15		No	—

* Sibs.

† ? = Not mentioned.

‡ CT = Coagulation time; + H = Died from haemorrhage; F = Plasma fibrinogen level (mg./100 ml.). Values are given in italics stated to be significantly reduced and there is evidence of partial deficiency.

§ Dizygous twins.

** Doubtful case.

†† Levels using a salt-precipitation method, similar results found using two other techniques.

by conventional methods, though the use of sensitive immunological techniques revealed a trace of fibrinogen on several occasions (Gitlin and Borges, 1953; Niewiarowski, Kozłowska, Gulmantiowicz, Pelczarska-Kasperka, 1962; Werder, 1963; Gross *et al.*, 1963; Bommer, Künzer, and Schröer, 1963a) but not on others (Hardisty and Penniger, 1956; Werder, 1963; Oseid and Svendsen, 1963). Overt liver disease was invariably excluded as a cause of fibrinogen deficiency, but objective evidence of an otherwise intact haemostatic mechanism and, in particular, a normal fibrinolytic system, was consistently available only for cases reported since 1957. Excluding five young sibs of patients, all cases included in Table III presented with haemorrhagic symptoms. Authors were in general agreement on the remarkable mildness of bleeding symptoms by comparison to other genetical disorders of clotting. The most frequent manifestations were

bleeding from the umbilicus (almost invariable in newborn infants), nose, gingivae, small wounds, and after teeth extraction. Haemarthroses were uncommon, occurring in six patients (Alexander, Goldstein, Rich, Le Bulloch, Diamond, and Borges, 1954; Lewis and Ferguson, 1954; Kranz and Ruff, 1959; De Vries *et al.*, 1961). Menstruation was remarkably normal (Lawson, 1953; Hardisty and Pinniger, 1956; De Vries *et al.*, 1961; Gross *et al.*, 1963), and menorrhagia is only rarely encountered (Werder, 1963). Nevertheless, the serious nature of the haemostatic defect is underlined by 14 deaths from haemorrhage and the fact that 16 undiagnosed but presumably affected younger sibs died as infants, mostly from umbilical bleeding. To date no patient has had offspring.

Family studies support homozygosity for an autosomal gene. Including sibs who died in infancy from haemorrhage, 17 of 55 sibships con-

tained more than one affected person. There was parental consanguinity in 21 (43%) of 49 matings. No instance of vertical transmission occurred. Evidence of gene effect in presumptive heterozygotes has been sought ever since Macfarlane (1938) detected a low level of plasma fibrinogen in the father of his patient. Frick and McQuarrie (1954) interpreted slightly reduced levels in four relatives as significant, but as Graham (1957) pointed out it was impossible to accept these findings in the absence of data on the normal distribution of values. Subsequent studies (Table III) with more adequate definition of normal range and internal errors indicated that heterozygotes could be detected in some families (Jain and Parande, 1962; Gross *et al.*, 1963; Werder, 1963) but not in others (De Vries *et al.*, 1961; Niewiarowski *et al.*, 1962; Oseid and Svendsen, 1963; Kozłowska *et al.*, 1964).

Graham (1957) noted that patients recorded by mid 1955 had a significantly abnormal sex ratio (21 male: 8 female $\chi^2 = 6.03$) for an autosomal hypothesis. This distribution was difficult to explain as any bias in ascertainment would tend towards exclusion of males through being diagnosed as haemophilic, on the classical grounds of haemorrhagic disease in a male associated with a prolonged coagulation time. However, the previous sex ratio was probably due to chance, for though its effect is still present with 36 males: 24 females, χ^2 is now 2.40. More conclusive evidence against abnormal segregation comes from the sex distribution of cases reported since 1955 (13:11) ($\chi^2 = 0.166$) and of sibs of propositi who died in infancy from haemorrhage and whose sex could hardly account for any bias (9:7) ($\chi^2 = 0.250$).

It is generally held that the genetical defect in congenital afibrinogenemia results in reduced synthesis of the protein because isotopically labelled fibrinogen has a similar half-life of 3–4 days in normal (Volwiler, Goldsworthy, MacMartin, Wood, MacKay, and Fremont-Smith, 1955) and affected (Gitlin and Borges, 1953; Lewis and Ferguson, 1954; Werder, 1963) individuals. Traces of apparently normal fibrinogen detected immunologically in plasma and by Sokal (1962) on platelets from a patient with congenital afibrinogenemia support the concept of diminished synthesis. Acquired fibrinogen inhibitors have developed in the circulation of two patients (Brönnimann, 1954; De Vries *et al.*, 1961), but there is no evidence to suggest genetically-controlled excessive fibrinogen destruction. An altered molecule cannot be entirely excluded, though it is unlikely that another species of fibrinogen comprising over 5% of the total plasma protein would remain undetected. However, there is a strong possibility that one instance of genetically altered fibrinogen

has been found. Ménaché and Boivin (1964) reported a man who had no bleeding symptoms and no fibrinogen detectable by usual methods, but preliminary studies strongly suggested the presence of abnormal fibrinogen with distinct physico-chemical properties. The patient's son had an apparently identical defect. It is also known from experiments on fibrinogen from six different species that thrombin hydrolyses bonds between arginine and glycine residues (Blombäck and Yamashina, 1958). Laki and Gladner (1964) point out that if, through an error of synthesis, lysine adjacent to glycine were replaced by arginine, the critical bond would then be split by thrombin in trace amounts and as a result fibrinogen would become unclottable. These authors go on to suggest that the defect in afibrinogenemia may be no more than a replacement of lysine by arginine in one of the six peptide chains of fibrinogen.

Congenital Hypofibrinogenemia. In 1935 Risak found that apparently low levels of fibrinogen were associated with mild haemorrhagic symptoms in several members of two different families and first suggested the possibility of an inherited trait. Several case reports followed where congenital hypofibrinogenemia was considered responsible for abnormal bleeding. This diagnosis is difficult to maintain on retrospective examination of some widely quoted cases. For instance, Nissen's (1938) patient had fibrinogen levels of over 200 mg./100 ml. on several occasions; members of Heinild's (1944) family had a complex haemostatic defect with thrombocytopenia more prominent than fibrinogen deficiency, and the infant described by Allibone and Baar (1943) suffered from extensive hepatic disease. Bommer *et al.* (1963b) included Nissen's and Heinild's cases together with seven similar patients in a review of congenital afibrinogenemia regarding hypofibrinogenemia as a genetical variant of the more extreme trait. In contrast, Revol (1962) analysed 26 reported cases with fibrinogen levels between 10 and 50 mg./100 ml. and where a primary cause including fibrinolysis had been eliminated. He concluded that the aggregation of subnormal levels within sibships or in two generations rendered a genetical basis probable. In agreement with Imperato and Dettori (1958), Revol considered there were no grounds for relating the two varieties of fibrinogen deficiency or regarding those with the partial defect as heterozygous for congenital afibrinogenemia. Available evidence suggested at least two alleles at different loci.

Three reasonably well-documented cases with fibrinogen levels of less than 70 mg./100 ml. resulted from a consanguineous mating (Lida and Banfi, 1953; Penati and Gaidano, 1953; Gerkowicz and Krawczynski, 1954).

The condition has been noted in a pair of monozygotic twins (Kugelmass, 1953). Hasselback, Marion, and Thomas (1963) recently described a family where 5 members of 2 generations had mildly abnormal bleeding and fibrinogen levels between 58 and 158 mg/100 ml. The authors postulated autosomal dominant inheritance, but as their report, like so many others, did not contain information on the normal limits of the method used to estimate fibrinogen it is clearly desirable to avoid drawing any genetical conclusions on congenital hypofibrinogenaemia at present.

(2) Prothrombin

The properties of prothrombin (factor II) have been extensively discussed by Seegers (1962) whose many studies indicate that the protein is the sole precursor of thrombin. The Seegers group maintain that factors VII and IX are also derived from prothrombin, which as explained by Biggs and Macfarlane (1962) is contrary to the majority view.

Purified prothrombin migrates electrophoretically as an α_2 globulin, and has an elongated stable molecule and a molecular weight estimated at 68,000 (Lamy and Waugh, 1953). Acquired hypoprothrombinaemic states are relatively common but inherited disorders are extremely rare. It must be recalled that cases diagnosed as congenital or familial hypoprothrombinaemia on the basis of an abnormal one-stage prothrombin time cannot be accepted unless deficiencies of factors V, VII, and X and excess anti-thrombin activity have been excluded. This applies to cases reported before 1959 excepting those described by Quick, Pisciotta, and Hussey (1955), which were subsequently reinvestigated (Quick and Hussey, 1962a).

Details recorded for 9 cases studied by modern techniques are given in Table IV. Bleeding symptoms dating from infancy were noted in 8 instances, being marked in 6 (Case 2 and 3 of Quick and Hussey, 1962a; Pool, Desai, and Kropatkin, 1962; Bastos, Reno, and Correa, 1964), and less severe in 2 others (Borchgrevink, Egeberg, Pool, Skulason, Stormorken, and Waaler, 1959; Josso, Prou-Wartelle, and Soulier, 1962), which latter had each lost a presumably affected sib from the effects of haemorrhage. The prothrombin time was prolonged to varying degrees and all cases with abnormal bleeding had greatly reduced plasma prothrombin levels as measured by the two-stage method (Ware and Seegers, 1949). There is insufficient evidence to draw conclusions on the genetical mechanism in these cases though homozygosity for an autosomal mutation is supported by the findings in relatives (Table IV). Two unpublished kindreds from Oxford are shown in Fig. 2. Members in two generations had prolonged prothrombin times and reduced prothrombin levels associated with very mild or absent bleeding symptoms. Increased anti-thrombin activity of unknown significance was noted in kindred B. There was no parental consanguinity.

Family 1 of Quick and Hussey (1962a) differed in that a slightly prolonged prothrombin time associated with normal prothrombin levels and no bleeding tendency was detected in 10 members of 3 generations. Quick and Hussey explained these findings on the hypothesis (Quick and Stefanini, 1948) that only a small proportion of prothrombin is 'active' (i.e. measurable by the one-stage prothrombin time) and the 'inactive' remainder becomes converted to the active state in the two-stage

TABLE IV
CONGENITAL HYPOPROTHROMBINAEMIA

Source	Case No.	Sex and Age	Bleeding Symptoms	Prothrombin Time (PT) (sec.)*	Prothrombin Assay (%)	Parental Consanguinity	Abnormal Findings in Relatives
Quick and Hussey (1962a)†	1	M	Nil	16(12)	120	Nil	1 sister, 2 brothers, father, 5 other relatives similarly affected
	2	M	Marked	19-29(12)	11	Nil	1 brother similarly affected, nil in 5 sibs PT slightly prolonged in 2 sisters and ? father
	3	F 12	Marked	73(12)	2	Nil	
Borchgrevink <i>et al.</i> (1959)		F 38	Mild	14.4(12-13)	9	Yes	Not sought; 1 brother died from haemorrhage
Pool <i>et al.</i> (1962)		M 7	Marked	25(13-15)	2-4	Nil	Nil in parents and sibs
Josso <i>et al.</i> (1962)		M 23	Mild	16-18.5(13-15)	10-13	Yes	Nil in 4 sibs; 1 brother died from haemorrhage
Bastos <i>et al.</i> (1964)‡	1	F 11	Marked	28(11-13)	1	} Yes	Patients are sibs; 1 other child died from umbilical bleeding; parents and other relatives not studied
	2	M 8	Marked	25(11-13)	1		
	3	M 11/12	Marked	34(11-13)	1		

* Normal mean or range is given in parenthesis.

† Additional details in Quick (1947) and Quick *et al.* (1955).

‡ Laboratory findings are not complete for this family.

FIG. 2. Congenital hypoprothrombinaemia. Unpublished families by kind permission of Dr. R. Biggs (Family A) and Dr. F. C. Bolton (Family B). Tonsillectomy in the propositus of Family A was described by Livingstone and Johnstone (1963).

(assay) procedure which latter therefore measures the 'total' prothrombin. The family in question could thus have a genetical defect in the mechanism that controls the level of 'active' prothrombin fraction which would account for the abnormal time in the presence of a normal assay result.

It may be concluded that haemorrhagic disease is occasionally due to a genetical disturbance of prothrombin metabolism, but clarification awaits further knowledge of the pathways involved.

(3) Factor V

The existence of factor V in normal plasma was postulated by Quick (1943) and its presence confirmed by Owren (1944) who described a woman with haemorrhagic symptoms dating from childhood and deficiency of the factor. Although the study of factor V has been hampered by its lability and other problems (Fantl,

1957), some progress has recently been made with material isolated and purified by methods discussed by Blombäck and Blombäck (1963). The factor has general properties of a protein and Papahadjopoulos, Hougie, and Hanahan (1964a) working with bovine factor V estimated a molecular weight of roughly 400,000.

Acquired defects are associated with the defibrination syndrome, carcinomatosis, and certain infections probably through factor V acting as a substrate for a stimulated fibrinolytic system (Biggs and Macfarlane, 1962). Congenital factor V deficiency is very rare. Bachmann (1958) and Hörder and Hiemeyer (1963) have reviewed 33 recorded cases for which there is reasonable evidence to support the diagnosis. Haemorrhagic symptoms vary in severity but generally consist of ecchymoses or bleeding from mucous membranes, and patients may experience few serious episodes (Friedman, Quick, Higgins, Hussey, and Hickey, 1961; Borchgrevink and Owren, 1961). Haemarthroses have not been reported and menorrhagia does not necessarily prove troublesome, though Brink and Kingsley (1952) described a presumably affected girl who bled to death at the menarche. Epidermolysis bullosa was present in one patient (Alexander and Goldstein, 1952), multiple congenital anomalies in another (Hörder and Sokal, 1956), and syndactyly was segregating independently in the family of De Vries, Matoth, and Shamir (1951).

There is little evidence on which to draw genetical conclusions but the family data given in Table V support autosomal inheritance. An additional

TABLE V
FAMILY STUDIES OF FACTOR V DEFICIENCY

Source(s)	Sex Sibs*	Age	Factor V % PT time (sec.)	Consanguinity	Findings in Relatives††
Brink and Kingsley (1952)	F*	25	5	Yes	Parents 40-60, 2 brothers, 2 sisters 24-60, 2 brothers 'normal' 7 other relatives reduced levels 80, 12 had 'normal' levels
	F*	32	5		
	M*	13	5		
	†M*	8	5	Yes	Mother 30, father 29, brother 42, sister 103
	F*	9	5		
Lewis and Ferguson (1955)	F	3	0	No	Mother 38, father 33, brother 85, sister 34
Owen and Cooper (1955)	M	47	6	No	2 children, 32 and 48, 10 other relatives 'normal'
Hörder and Sokal (1956)	F	27	3	?	4 brothers 43, 34, 120, 46, 2 sisters 39, 23, daughter 80
Friedman <i>et al.</i> (1961)	M*	13	§PT = 51	No	Father PT 13-14, mother PT = 13-14, sister PT = 12
	‡F*	5	PT = 41		
Retief and Coetzee (1964)	F*	5	PT = 56	No	Parents normal PT
	F*	3	**PT = 120		
	F*	5/12	PT = 120		
	F*	5/12	PT = 120		

* Sibs: PT time is prothrombin time.
† Although highly probable, direct relationship to other sibship could not be proved.
‡ Monozygous twins. Normal PT = 12 sec. §, 10.5 sec. **
†† Factor V value is in italics if stated to be evidence of partial deficiency, similarly prothrombin time (PT).

FIG. 3. Factor V deficiency. By kind permission of Dr. R. Biggs. (Details given by Rush and Ellis in 1965.)

family is shown in Fig. 3. Autosomal dominant inheritance was proposed by Owen and Cooper (1955) who considered that reduced factor V levels in a man and his two children indicated a single gene with varying expression. Although semi-quantitative assay of factor V is technically difficult, the available evidence (Table V) favours homozygosity for an incompletely recessive gene. Information on consanguinity has been inconsistently reported but parents were related on three occasions (Brink and Kingsley, 1952; Kingsley, 1954; Seibert, Margolius, and Ratnoff, 1958). There has been no unequivocal instance of abnormal bleeding in a person with an intermediate level of factor V.

(4) Factor VII

A few years after the existence of factor VII was predicted to explain the potentiating effect of serum on prothrombin conversion by tissue extracts (Mann, Hurn, and Magath, 1947), the first patient with an inherited deficiency was described by Alexander, Goldstein, Landwehr, and Cook (1951). The factor is not consumed during clotting, is relatively stable, and has electrophoretic characteristics of a β globulin (Owen and McKenzie, 1954). Additional properties are summarized by Alexander (1961).

It is agreed that factor VII originates from the liver because reduced blood levels are found after experimental hepatectomy (Mann, Shonyo, and Mann, 1951), liver disease, or the administration of hepato-toxic drugs (Seegers, 1951), including salicylates and propylthiouracil (Craddock, Shotton, Crockett, and Leavell, 1951).

In addition, the factor appears to be synthesized by rat liver slices (Pool and Robinson, 1959) and suspension cultures of hepatic cells (Prydz, 1964). Significantly elevated factor VII levels have been noted during pregnancy (Owen *et al.*, 1964), clinical (Poller, 1959) or experimental (Fisher, Kupfer, and Kagan, 1962) thromboembolic states, and after administration of 4-amino-antipyrine to guinea-pigs (Weiner and Dayton, 1960). The part played by factor VII in the extrinsic pathway of prothrombin activation remains obscure and current views are discussed by Macfarlane (1965).

Factor VII deficiency has been thoroughly reviewed by Hall, Rapaport, Ames, and deGroot (1964), Marder and Shulman (1964), and Owen *et al.* (1964). Each group differed somewhat on criteria for assessing the reliability of diagnosis in previously reported cases. Owen *et al.* used the most stringent classification defining 'proven' patients as those whose plasma had been matched with that from other patients known to have factor VII or factor X deficiency. On this basis there were 24 unequivocal instances, 15 in whom the diagnosis was probably correct, though factor X deficiency had not been definitely excluded, 52 with lesser chances of being factor VII deficient, and finally 8 disproved cases where factor X was found to be the defective factor on reinvestigation.

Most patients with factor VII deficiency have been of European origin, but it has occurred in a negro (Barnett, 1957) and in American Indians (Miller, 1959; Marder and Shulman, 1964). Bleeding symptoms are notoriously variable and fall into three main categories. One type becomes evident during infancy and is characterized by umbilical bleeding melaena, haematemesis, bruising epistaxis, and gingival haemorrhage. Affected children can be severely disabled by recurrent episodes (Zollinger, 1963; Marder and Shulman, 1964). Deaths have occurred during infancy from umbilical or intracranial haemorrhage and the latter manifestation has been described in three sisters with factor VII deficiency (Van Creveld, Veder, and Blans, 1956). The second variety has a more insidious clinical onset at a later age with such symptoms as epistaxis, ecchymosis, and gingival bleeding associated with tooth eruption or extraction. Haemarthroses occurred in 55% of male patients reviewed by Owen *et al.* (1964) and may represent the only manifestation of disordered haemostasis (Tveten, 1958). Menorrhagia is frequent in adult females (Hicks, 1955; Voss and Waaler, 1959; Kupfer, Hanna, and Kinne, 1960). Although tooth extraction is consistently followed by prolonged bleeding (Hall *et al.*, 1964), some major

operations have been performed without complication (Marder and Shulman, 1964). The final clinical variety comprises those patients who have experienced no abnormal bleeding despite having a factor VII level of less than 1% (Godal, Madsen, and Nissen-Meyer, 1962; Hall *et al.*, 1964).

Family studies initially indicated that factor VII deficiency resulted from homozygosity for an incompletely recessive autosomal gene (Chevallier,

expected on an autosomal hypothesis. Similar conclusions were made by Owen *et al.* (1964) in an equally extensive study and by Zollinger (1963) who investigated an inbred Swiss kindred. A pedigree which contains an unusual type of mating (Marder and Shulman, 1964) is given in Fig. 4. There is general agreement that the partial factor VII deficiency of heterozygotes is not associated with an abnormal bleeding tendency (Hall *et al.*, 1964; Owen *et al.*, 1964).

(5) Factor VIII

In 1937 Patek and Taylor demonstrated that a plasma globulin was lacking in haemophilia and thereby diverted attention from the hypothesis that platelets were defective (Fonio, 1936). Characterization of the factor in general physico-chemical terms was delayed for many years through inadequacies of clotting theory and consequent difficulty in constructing suitable laboratory tests. Some progress has been made more recently despite marked instability of factor VIII *in vitro* and technical problems in separating the factor from fibrinogen, its co-precipitant in plasma fraction I of Cohn. The current position on factor VIII isolation and purification has been clearly summarized by Surgenor (1964). Studies on partly purified preparations indicate that factor VIII has general properties of a protein (Biggs and Macfarlane, 1962; Blombäck, 1964).

Little is known about the origin and metabolism of factor VIII. It appears that the liver is not involved in synthesis as severe hepatic damage and coumarin overdosage in animals have no effect on plasma levels (Graham, Collins, Godwin, and Brinkhous, 1951). Pool and Spaet (1954) found that drug-induced bone-marrow depression in rats was associated with factor VIII deficiency, but a causal relation remains unsubstantiated. Cross-circulation experiments between haemophilic and normal or splenectomized dogs (Weaver, Price, and Langdell, 1963) suggested that the spleen had a homeostatic function because in contrast to normal dogs the splenectomized animals were unable to maintain factor VIII levels during cross-infusion with haemophilic blood and subsequently showed greatly impaired regeneration of factor VIII.

The manner in which factor VIII is utilized during clotting and undergoes metabolic degradation in the circulation is also obscure. Nevertheless the weight of experimental evidence favours the hypothesis that the plasma level of factor VIII reflects equilibrium between synthesis and destruction. A minority consider that factor VIII deficiency is due to a circulating inhibitor (Mammen, 1963) or that partial inhibitor activity can be consistently detected in plasma from patients (Nour-Eldin and Wilkinson, 1958). However, the evidence on

FIG. 4. Factor VII deficiency. From Marder and Shulman (1964), by kind permission of Dr. N. R. Shulman. Albinism was also segregating in one sibship, but direct evidence of consanguinity of parents (Navajo Indians) was not obtained. The mother of the proposita had factor VII estimations on three occasions (at times when she was not pregnant), but normal values were always recorded.

Bernard, Fiehrer, Bilski-Pasquier, Samama, and Cerf, 1955; Zollinger and Hitzig 1958; Caen, Yanotti, Varangot, and Bernard, 1959; Voss and Waaler, 1959; Kupfer *et al.*, 1960; Cleton and Loeliger, 1960). Alexander (1961) expressed doubts on the specificity of certain assay procedures used to detect partial factor VII deficiency in heterozygotes, but more recent studies have greatly strengthened the earlier views on inheritance. In a well-controlled investigation of 81 relatives of 3 affected sibs, Hall *et al.* (1964) demonstrated clear segregation of factor levels in accordance with predicted genotypes and included evidence that 8 children with one factor VII deficient (homozygous) parent had a partial factor deficiency with levels more than two standard deviations from the normal mean. Ratios for gene segregation and sex were those

FIG. 5. Autosomal dominant factor VIII deficiency. Family from Rotterdam to be published by Hensen *et al.* (1965) and reproduced by kind permission of Dr. E. A. Loeliger.

which these claims are based can be alternatively interpreted to fit with conventional theory (Hougie, 1959b; Biggs and Macfarlane, 1962).

Excluding the development of a factor VIII inhibitor through unknown cause or in association with generalized disease (Margolius *et al.*, 1961;

Horowitz and Fujimoto, 1962), no acquired form of factor VIII deficiency is known. The most prevalent type of sex-linked haemophilia (henceforth termed factor VIII deficiency) will be discussed here. Autosomal traits in which factor VIII is defective are von Willebrand's disease, combined factor VIII and factor V deficiency, and a few other less well-defined conditions with multiple haemostatic defects. These will be considered later. There remains Loeliger's unique kindred (Hensen, Mattern, and Loeliger, 1965) which is the first clear demonstration of apparently isolated factor VIII deficiency resulting from autosomal mutation (Fig. 5). Normal bleeding times and lack of factor VIII elevation after infusion of factor VIII-deficiency (haemophilic) plasma are strong evidence against von Willebrand's disease, and indicate that this inherited haemorrhagic disorder represents the third distinct variety of autosomal factor VIII deficiency.

Genetical Component of Factor VIII Variation in Normal Persons. It is generally agreed that factor VIII levels in healthy individuals vary from about 50 to 200% of an average normal standard designated as 100% (Brinkhous, Langdell, Penick, Graham, and Wagner, 1954; Biggs, 1958; Cooperberg and Teitelbaum, 1960; Pitney, Kirk, Arnold, and Stenhouse, 1962; Hawkey, Anstall, and Grove-Rasmussen, 1962; Perkins, Rolfs, and Acra, 1962; Preston and Barr, 1964, and others). The frequency distribution approximates to, or

FIG. 6. Distribution of factor VIII levels (%) in 217 normal individuals with superimposed normal curve (from Kerr *et al.*, 1965). Mean, $95 \pm 25\%$, range 32-200%.

TABLE VI
SOME NON-GENETICAL CAUSES OF FACTOR VIII VARIATION

Reference	Source of Variation	Effect on Plasma Factor VIII
Cooperberg and Teitlebaum (1960)	Increasing age	Elevation*
Pitney and Elliot (1960)	Male sex	Elevation
Rizza (1961)	Hypergammaglobulinaemia	Elevation
Ingram (1961)	Muscular exertion	Elevation
Bergentz and Nilsson (1961)	Adrenaline	Elevation
Egeberg (1962a)	Fracture (dogs)	Reduction
Egeberg (1963)	Coronary artery disease	Elevation
	Experimental fever induction; hypothyroidism	Elevation
Davidson and Tomlin (1963)	Post-operation	Reduction
Strauss and Diamond (1963)	Pregnancy	Elevation
Preston (1964)	Pregnancy	Elevation
Egeberg and Owren (1963)	Oral contraceptives	Elevation
Preston and Barr (1964)	Blood group A	Elevation†

* Pitney *et al.* (1962), Preston and Barr (1964), found a significant sex but not age effect.

Kerr *et al.* (1965) found neither. Nilsson *et al.* (1962) found significantly higher levels in post-menopausal women.

†Relative to blood group O.

fits, a normal curve (Fig. 6). Attempts to establish a genetical component in variation are compromised by undefined limits of non-genetical variability. A remarkable number of environmental effects influence factor VIII levels (Table VI). Many can be eliminated during planned investigation, but others, notably 'anxiety' at venepuncture and errors inherent in assay techniques, remain an unknown source of variability.

With one widely used technique, the random error increased proportionately with the plasma factor VIII concentration (Biggs and Macfarlane, 1962). Instability of standard factor VIII preparations is a potential source of systematic error in quantitative analysis and much more information on the reliability of standards is required. It will be noted from Table VI that conclusions differ on the effect of age and sex on factor levels. The significantly higher mean value found in association with blood group A (Preston and Barr, 1964; Kerr, Preston, Barr, and Biggs, 1965) requires further examination and could for instance merely reflect less stability of factor VIII in a group O blood sample.

The theory that X-linked alleles were responsible for varying phenotypes in haemophilia (Schloessmann, 1930; Haldane, 1935) was extended by Brinkhous *et al.* (1954) to cover factor VIII level distribution in normal persons through assuming that haemophilia appeared as a threshold effect. But difficulties with a hypothetical X-linked allelic series became apparent with the demonstration of similar distributions in normal males and females (Fischer, Landbeck, and Lenz, 1958) and the discovery of autosomal mutations that influenced the factor level. Graham (1959 and subsequently) developed theories based on the control of factor

VIII synthesis at an X-linked and at least one autosomal locus (as discussed in section on von Willebrand's disease). Pitney and Arnold (1959) suggested that normal levels were controlled by autosomal genes. The question has been further examined in two studies of factor VIII levels in members of normal families (Pitney *et al.*, 1962; Kerr *et al.*, 1965). Allowing as far as possible for known sources of non-genetic variability, both studies agreed in that, first, variation in factor VIII levels between parents from different families did not differ significantly from variation between husband and wife, secondly, variation was significantly less among sibs than between children from different families and, thirdly, the observations would best be explained by a series of genes at autosomal loci. Kerr *et al.* compared intrafamily correlation coefficients with those expected on an additive autosomal gene hypothesis (Maynard-Smith, Penrose, and Smith, 1961) and obtained sufficiently encouraging results to proceed with a twin study (in progress). There is an obvious limit to interpretation of data founded on such an imprecise biological measurement, but the evidence at present suggests a complex genetical control of factor VIII involving several autosomal loci.

Factor VIII Deficiency. Bulloch and Fildes (1912) regarded haemophilia as a disease of native or migrant Western Europeans, but factor VIII deficiency has been subsequently detected among many other ethnic groups. These include South African Bantu (Feldman and Lewis, 1952), tribal North American Indians (Painter and Ellett, 1960), Arabs (El-Mehairy and El-Masri, 1960), an

indigenous Filipino (Castillo, Jongco, and Pascual, 1960), and a Melanesian (Champness, 1962). Bass and Yaghamai (1962) noted that 32 cases had been reported among American Negroes, and Abe (1964) reported that 340 patients with factor VIII deficiency attended one haemophilia clinic in Japan.

Many statements on the prevalence of factor VIII deficiency have been based on the number of patients attending large blood coagulation centres. Estimations from population surveys utilizing multiple sources of ascertainment are far less prone to bias. Ikkala (1960) in Finland, Ramgren (1962a) in Sweden, and Kerr (1965) in New South Wales reached similar conclusions in estimating the trait frequency in males at birth as within the range $0.98-1.7 \times 10^{-4}$, which is interestingly within the limits of $0.4-1.7 \times 10^{-4}$ predicted long ago by Haldane (1935). The proportions of severe and mildly affected patients were roughly the same in each population survey, and it was recognized that the actual prevalence of mildly affected persons remains entirely unknown. Certainly only a proportion of cases experience sufficient operative trauma or some other injury to cause abnormal bleeding, and even then many have to await further episodes before a clotting defect is suspected and appropriate diagnostic tests performed (Kerr, 1962). In contrast, one aspect of the difficult problem in calculating trait frequency at birth has changed recently with radically improved diagnosis and therapy over the past decade and consequently less chance of infants dying undetected. The effect of improved therapy is becoming evident in that progressively more severe haemophiliacs are now entering older age-groups (Kerr, 1962).

The evidence for X-linkage is incontrovertible (Haldane, 1947; Lewis *et al.*, 1963) not only from formal analysis of human data but also from breeding experiments with the North Carolina haemophilic dogs. For instance, 10 matings between haemophilic dogs and homozygous affected females yielded 47 offspring of either sex, all with factor VIII deficiency (Graham, 1962).

Analysis of a single family where males had mild factor VIII deficiency and females had reduced factor levels (Graham, McLendon, and Brinkhous, 1953) led to the suggestion of autosomal inheritance, and, by contrast, females heterozygous for severe haemophilia were thought to show no evidence of gene effect (Graham, 1959; Joist, 1963). However, with the development of more reliable methods of factor VIII assay it subsequently became apparent that reduced levels were present in the blood of females from families with mild or severe factor VIII deficiency (Didisheim, Ferguson, and

Lewis, 1958; Pitney and Arnold, 1959; Nilsson *et al.*, 1962; Lewis *et al.*, 1963; Kerr *et al.*, 1965). The evidence indicates a recessive X-linked gene with inconstantly detectable effect in heterozygotes, and there has never been a definite instance of male-to-male transmission in families where mild factor VIII deficiency is segregating. As Haldane suggested in 1935, the most likely explanation for phenotype variation is an allelic series at an X-linked locus. The earlier hypothesis of genetical dichotomy misled Sutton (1960) and Woolf (1962, 1963) into proposing different mechanisms for each variety, i.e. production of an altered molecule with some factor VIII activity in the mild form, and suppression of synthesis or the presence of a functionally inert molecule in the severe form.

Bullock and Fildes (1912) observed abnormal segregation for sex and haemophilia in their collected pedigrees, but making appropriate corrections Hogben (1931) and Haldane (1935) showed close agreement with theoretically predicted ratios for each character. In the segregation analysis of families containing 202 patients with factor VIII, Lewis *et al.* (1963) found a significant excess of haemophiliacs among males born to sisters of haemophiliacs but not among the sons of daughters of affected males. Morton (1965) has challenged the statistical basis of this observation. Be this as it may, Lewis *et al.* eliminated differences in family size as a possible cause but were otherwise unable to explain the distorted segregation. The latter was not observed by Haldane on analysis of Birch's (Haldane and Philip, 1939) or Andreassen's (Haldane, 1947) data, nor by Ikkala (1960).

Following Haldane's (1932) classical arguments on equilibrium of the 'haemophilia gene' in large human populations, attempts have been made to estimate the rate of spontaneous mutation which must account for a proportion of the 25-40% of sporadic cases with either factor VIII or factor IX deficiency in recorded series (listed by Ramgren, Nilsson, and Blombäck, 1962). Two frequently quoted observations that are claimed as direct evidence of fresh mutation are the family described by Davenport (1930) in which haemophilia and colour blindness were segregating in a manner that allowed no other interpretation (Haldane, 1935), and the occurrence of haemophilia in one of monozygotic twins (Quick and Conway, 1949); though zygosity in the latter was subsequently admitted to be in doubt (Quick, 1960).

Haldane's (1935) first estimation of mutation rate (μ) in 'haemophilia' was about 2×10^{-5} per X chromosome per generation. Andreassen (1943) using Haldane's indirect method calculated μ —

1.9×10^{-5} , but Haldane (1947) showed that Andreassen had probably given too high an estimate of effective fertility through biased selection of fertile haemophilic ancestors and the use of younger and reproductively immature brothers as controls. Haldane gave a revised $\mu = 3.2 \times 10^{-5}$. Vogel (1955) calculated $\mu = 2.2 \times 10^{-5}$ from data on haemophilia in Switzerland collected by Fonio (1954), and Ikkala (1960) estimated the over-all rate for factor VIII and factor IX deficiency in Finland as 3.4×10^{-5} . For factor VIII deficiency μ was 3.2×10^{-5} , and Ikkala used a figure for effective fertility based on severe haemophilia assuming that the rate of mutation to mild alleles was negligible. Bitter (1963) calculated a somewhat higher rate, $\mu = 4.1 \times 10^{-5}$, from data on factor VIII deficiency collected in Hamburg.

Through accepting Andreassen's claim that mothers of sporadic haemophiliacs had a prolonged coagulation time and assuming that the observed excess of familial haemophilia denoted more frequent mutation on sperm-borne X chromosomes, Haldane (1947) estimated that the mutation rate in males was about ten times greater than in females. Vogel (1955) drew similar conclusions, but Kosower, Christiansen, and Morton (1962) after exhaustive criticism of Haldane's data and methods of analysis were unable to find any evidence for a sex difference in mutation rate. Bitter (1963) concluded from comparison of data on mothers with a single child and those with other affected male relatives that mutation was much greater in sperm (i.e. grand-paternal), but this was based on laboratory identification of heterozygosity which, as will be shown, is still a contentious matter.

Bell and Haldane (1937) concluded from very sparse data that genes for haemophilia and colour blindness were closely linked. Haldane and Smith (1947) subsequently examined more informative pedigrees including eight recorded by Hoogvliet (1942) and gave a maximum likelihood estimate of about 10% recombination within limits of about 5 and 20%. In a recent study where both the type of haemophilia and colour blindness were specified, Whittaker, Copeland, and Graham (1962) detected linkage with 6% recombination between the loci for deutan colour blindness and factor VIII deficiency. Initial studies with the Xg blood groups (O'Brien, Harris, Race, Sanger, Tippett, Hamper, and Gavin, 1962) suggested no linkage with the factor VIII deficiency locus. Davies, Gavin, Goldsmith, Graham, Hamper, Hardisty, Harris, Holman, Ingram, Jones, McAfee, McKusick, O'Brien, Race, Sanger, and Tippett (1963) subsequently analysed data from 35 suitable families with factor VIII deficiency

by the lod score method and calculated a recombination fraction of 0.40 with wide confidence limits. Harrison (1964) estimated 50% recombination between the loci from studying another 10 families, and so the evidence suggests very loose or absent linkage with the unfortunate consequence that Xg blood grouping will provide no information for counselling purposes. However, as Haldane and Smith (1947) illustrated on a pedigree, linkage with a colour blindness locus can under certain conditions prove valuable in estimating the probability of heterozygosity in a female relative of a haemophiliac. At present, linkage studies indicate the following order of loci on the provisional X chromosome map (distances in centimorgans):

Xg ²⁷ G6PD ⁵ Deutan ¹² Factor VIII

Certain congenital abnormalities have been found in association with factor VIII deficiency such as a gross cardiovascular malformation (Bogedain, Carpathios, Kalemkeris, and McMahon, 1962), anomalous karyotype (Elves and Israëls, 1962), and Marfan's syndrome (Erdohazi, Cowie, and Lo, 1964). No unusual association was suggested from the distribution of ABO groups (Chaudhuri, 1960; Ikkala, 1960) and haptoglobin types (Kamel, Davies, and Cumming, 1963) in small series of patients.

Factor VIII Deficiency in Females. There are at least 60 well-documented reports of females with haemorrhagic symptoms and an isolated deficiency of factor VIII. The majority are from haemophilic families and are related to affected males as mother (Fantl and Margolis, 1955; Serafini, Felici, and Bacchetta, 1958), daughter (Douglas and Cook, 1957), or sister (De la Chapelle, Ikkala, and Nevanlinna, 1961), and sometimes less closely, but always with a genetical probability of being heterozygous (McGovern and Steinberg, 1958; Mellman, Wolman, Wurzel, Moorhead, and Qualls, 1961; Bond, Levin, Celandier, and Guest, 1962). Two examples are shown in Fig. 7. Such women have factor VIII levels below 20% and usually mild bleeding symptoms, though the girl studied by De la Chapelle *et al.* (1961) was severely affected. These cases are usually regarded as heterozygotes who show unusually severe expression of the incompletely recessive gene, but some authors have speculated on the possibility of homozygosity through disturbances of cell division or coincidental mutation on a sperm-borne X chromosome.

Homozygous females with haemorrhagic disease and factor VIII deficiency have resulted from mat-

FIG. 7. Females with partial factor VIII deficiency and haemorrhagic symptoms. Details of proposita in family A (by kind permission of Dr. R. Biggs) were noted in Taylor and Biggs (1957). The proposita in family B (unpublished, by kind permission of Dr. J. Margolis) has symptoms rather more marked than those in her father and other male relatives.

FIG. 8. Females homozygous for factor VIII deficiency. Pedigree of Pola and Svojitka (1958) reproduced by kind permission of the Editor, *Folia Haematologica*, Leipzig.

ings between heterozygotes and affected hemizygous males who were cousins (Merskey, 1951a; Gilchrist, 1961; Wilkinson, Israëls, Nour-Eldin, and Turner, 1957; Pola and Svojitka, 1958; Morita, Kagami, Ebata, and Yoshimura 1964) or not related (Israëls, Lempert, and Gilbertson, 1951). On a family containing a double first-cousin mating (Pola and Svojitka, 1958) is shown in Fig. 8. In every instance homozygous females had bleeding symptoms and *in vitro* findings similar to those in affected male relatives, which is evidence against X-linked gene dosage effect. There was remarkably little trouble with menstruation in 8 documented cases and all except Pola and Svojitka's proposita had produced offspring, usually but not always associated with serious post-partum haemorrhage.

A unique and convincing explanation of severe factor VIII deficiency in an apparent female was given by Nilsson, Bergman, Reitalu, and Waldenström (1959) on detecting no sex chromatin bodies in the child's cells. The patient was subsequently found to have testicular feminization and male karyotype, thus accounting for clinical and laboratory features identical to affected male relatives (Fig. 9). Further studies (I. M. Nilsson, 1965, personal communication) revealed that another

FIG. 9. Factor VIII deficiency in a phenotypic female with testicular feminization. From Nilsson *et al.* (1959) by kind permission of Dr. I. M. Nilsson who supplied additional details recorded on pedigree and in the text.

sib with female phenotype and a normal factor VIII level also had testicular feminization and a male karyotype, while a third sib, with physical and chromosomal features of a female, had a reduced factor VIII level implying heterozygosity. Dr. Nilsson suggests that these findings indicate autosomal rather than X-linked transmission of testicular feminization.

The final category of females with clinical evidence of factor VIII deficiency concerns those in whose families exhaustive investigations had failed to reveal affected male relatives (Choremis, Zervos, Tseverenis, Apostolopoulou, and Mandakaki, 1956; Case 1 of Quick and Hussey, 1958; Stefanovic, Rolovic, and Zujevic, 1959; Braun and Stollar, 1960; Lusher, Staub, and Belote, 1964). Clinical and laboratory features were similar to those in women with genetical evidence of heterozygosity, but a female karyotype was sought and confirmed in only two cases (Braun and Stollar, 1960; Lusher *et al.*, 1964). It was generally held that these females were recipients of a fresh mutation.

There have been many conflicting reports on gene effect in heterozygous females. Studies prior to the development of factor VIII assays were fully reviewed by Margolius and Ratnoff (1956) who themselves detected a plasma abnormality in only one of 27 proven or possible carriers. Subsequent

studies based on various methods of factor VIII assay (detailed by Miller and Siggerud, 1964) have given equally variant results (Table VII). Many investigations were undertaken in the hope that a suitably sensitive technique would clearly distinguish normal from heterozygous females. Some results are difficult to interpret through lack of data on the normal distribution of levels and undefined errors associated with a single estimation.

Nilsson *et al.* (1962) who recorded the highest frequency of reduced levels (40 of 41 genetically proven or probable heterozygotes of fertile age) based their conclusions on a single factor VIII estimation in over half the cases. The single 'normal' exception in their series was a female whose plasma level varied from 44 to 100% on different occasions. Two others included as having significantly low levels had values that ranged respectively from 69 to 28% and 61 to 45% (normal range 60-160%, mean $100 \pm 117.5\%$). Under the conditions of their well-controlled study of 35 proven or probable carriers, Rapaport, Patch, and Moore (1960) concluded that 75% of heterozygotes and 66% of normal females could be detected in a mixed population. They predicted the likelihood of heterozygosity for a woman with an observed level through calculating the cumulative frequency of control and carrier female values in relation to a given factor VIII level, and plotting the ratio of normal to total population against observed values. Miller and Siggerud (1964) used a

TABLE VII

STUDIES OF FACTOR VIII LEVELS IN FEMALE RELATIVES OF FACTOR VIII DEFICIENT MALES

Source	Total Females Examined	Genotype Specified				Genotype Unspecified	
		Heterozygous*		Possibly Heterozygous†		(Proven and Possible Heterozygotes)	
		Normal Factor VIII	Reduced Factor VIII	Normal Factor VIII	Reduced Factor VIII	Normal Factor VIII	Reduced Factor VIII
Gardikas, Katsiroumbas, and Kottas (1957)	10	10					
Biggs and Macfarlane (1958)	16					8	8
Didisheim <i>et al.</i> (1958)	63					44	19
Pitney and Arnold (1959)	16	10	6				
Alagille and Prou-Wartelle (1960)	17	9	8				
Bentley and Krivit (1960)	26					3	23
Rapaport <i>et al.</i> (1960)	35					18	17
Ikkala (1960)	17	6	2	6	3		
Deutsch and Kock (1962)	10	4	6				
Nilsson <i>et al.</i> (1962)	79	2	31	30	26		
Githens and Wilcox (1962)	31					20	11
Bradlow (1962)	10					5	5
Lewis <i>et al.</i> (1963)‡	76	42	34				
Bergna and Pavlovsky (1964)	22	9	3	8	2		
Miller and Siggerud (1964)	30					2	28

* Daughters of affected males, or females, with at least 2 haemophilic relatives in appropriate lineage.
† Mothers of a sporadic affected male or females with $\frac{1}{2}$ or less chance of being heterozygous.
‡ From data on families where 2 or more female carriers were studied.

similar analysis and obtained a somewhat higher degree of discrimination. Some authors recorded the frequency of mild bleeding symptoms as noted by 50% (Didisheim *et al.*, 1958; Deutsch and Kock, 1962) to 77% (Bentley and Krivit, 1960) of the women. However, little weight can be placed on these observations owing to the high prevalence of similar subjective beliefs among normal individuals (Biggs and Macfarlane, 1962; Owen *et al.*, 1964). Doubtless some females with a factor VIII level reduced below one-third of normal did experience the effects of impaired haemostasis, and for this reason it is wise to assay factor VIII in all potential female carriers before a surgical operation.

FIG. 10. Distribution of factor VIII levels by genotype in 144 members of 25 families with factor VIII deficiency (from Kerr *et al.*, 1965).

It is generally concluded from these studies that factor levels in relatives of affected males segregate according to genotype but with considerable overlap between values in heterozygous females and normal persons (Fig. 10). The variation in values among definite heterozygotes is well demonstrated by the daughters of two factor VIII deficient males in Fig. 11. Levels in heterozygous females are normally distributed with a mean value approximately half that of normal females (Rapaport *et al.*, 1960; Miller and Siggerud, 1964; Kerr *et al.*, 1965) and a large variance (Table VIII). This distribution was also found in a group of heterozygous female dogs (Parks, Brinkhous, Harris, and Penick, 1964) and has been generally interpreted in terms of the inactive X chromosome theory of gene dosage compensation (Lyon, 1961), assuming a small number of factor VIII precursor cells at the time of inactivation. In a speculative

TABLE VIII

COMPARATIVE DISTRIBUTION OF FACTOR VIII LEVELS IN FEMALES FROM FAMILIES WITH FACTOR VIII DEFICIENCY. (From Kerr *et al.*, 1965)

	No.	Factor VIII Levels (%)		
		Mean	Range	Coeff. Variation
Normal females	99	93	32-200	29.21
Heterozygous females*	27	50	26-126	45.12

* Daughters of hemizygous affected males, or females with at least 2 factor VIII deficient relatives in appropriate lineage.

FIG. 11. Variation of factor VIII levels in heterozygous daughters of males with mild factor VIII deficiency.

[sic] analysis, Miller and Siggerud (1964) showed that levels in 30 female carriers fitted the distribution expected if random inactivation of the X chromosomes occurred at a time when five precursor cells were in existence. It is difficult to propose an alternative explanation for observations in heterozygotes at present, but, bearing in mind differences over similar interpretations of varying enzyme levels in females heterozygous at the X-linked locus for glucose-6-phosphate dehydrogenase deficiency (Beutler and Baluda, 1964; Brewer and Tarlov, 1964), any final conclusions should be withheld until it is possible to seek direct evidence of a mosaic cell population with regard to factor VIII.

There are some isolated reports claiming success in carrier detection through the use of methods based on physical measurements of blood coagulation or different interpretation of clotting theory. These include thromboelastography (De Nicola, 1957), plasma electrophoresis (Stefanini and Moschides, 1957), and measuring the osmotic resistance of platelets (Bruster, 1962).

It may be concluded that it is possible to detect a proportion of heterozygotes with the confidence required for genetic counselling. But several factor VIII assays must be performed on each suspected carrier with a carefully standardized technique and the result related to an adequately defined distribution of normal values. Counselling without evidence of heterozygosity can be exceedingly difficult, particularly with female relatives of sporadic cases, and Binet, Sawers, and Watson (1958) have given a mathematical treatment of this problem.

As yet there is no evidence that families are deliberately limited in size after the birth of an affected boy (Biggs and Macfarlane, 1958; Katz, 1959). Physicians commonly record that it is their responsibility to explain genetical risks but not to influence any decisions on procreation which should be left to the parties concerned (Pavlovsky, 1957; Oppé, 1960). By contrast, Scandinavian physicians supported by the appropriate legislation strongly advocate abortion and sterilization of female carriers to the extent of misquoting Pope Pius XII (1958) on the moral aspects of preventing conception (Jorpes and Ramgren, 1962). Their efforts have had slight success in Sweden where, during 1943-1960, sterilization was performed on 3 (1.2%) males with factor VIII deficiency and 18 (15%) known female carriers (Jorpes and Ramgren, 1962). However, Borgström in 1958 noted that only one carrier in Finland had been sterilized. From Denmark, Riis and Fuchs (1960) described determination of foetal sex by examining cells in amniotic fluid obtained during the second trimester of pregnancy from two females who each had a quarter chance of conceiving a male infant with factor VIII deficiency. The policy was to terminate pregnancy if no sex chromatin bodies were detected, but female sex was predicted in each case and confirmed at term. There are obvious problems associated with this procedure, not the least being cytodiagnostic, and it is unlikely to become an accepted method of dealing with the distressing situation that confronts possibly heterozygous women who desire children.

(6) Factor IX

Factor IX is relatively stable and has properties of a protein which include electrophoretic migration with serum β -globulin. The factor is not readily

detectable in plasma (Schiffman, Rapaport, and Patch, 1963), and its presence in normal serum (presumably in the activated state) led to the discovery of factor IX deficiency in 1952. Despite clinical and genetical similarity to factor VIII deficiency, the *in vitro* differences suggested non-allelism of respective X-linked genes (Biggs *et al.*, 1952; Aggeler *et al.*, 1952). Apart from an occasional disclaimer (Wiener, 1953), this view is generally accepted and supported by linkage studies and a doubly hemizygous male from a family where both traits were segregating (Robertson and Trueman, 1964).

It appears to some (McKusick, 1962b) that factor IX deficiency is on the average a milder haemorrhagic disorder than factor VIII deficiency. Distribution of severity among small groups of patients (Rosenthal and Sanders, 1954; Biggs and Macfarlane, 1958; Ikkala, 1960) favoured this conclusion but no such distinction was found in larger series (Wilkinson, Nour-Eldin, Israëls, and Barrett, 1961; Ramgren, 1962b; Lewis *et al.*, 1963). Very severe forms of factor IX deficiency have been described (Carter, Hougie, and Menk, 1960; Kerr, 1962), and the clinical variability (Ramot, Angelopoulos, and Singer, 1955a; Favre-Gilly, Burgensis, Thouverez, and Belleville, 1962) suggests there is an allelic series as postulated for factor VIII deficiency.

Genetical studies of factor IX deficiency have followed the pattern for factor VIII deficiency. In a magnificent study of the famous Tenna family of bleeders, Moor-Jankowski *et al.* (1957) demonstrated that males with moderately severe haemorrhagic disease had factor IX levels ranging from 2.5 to 6%. The family live in a remote Swiss valley where the population has features of an anthropological isolate (Ikin, Mourant, Kopeć, Moor-Jankowski, and Huser, 1957) little changed in structure since the fourteenth century (Joos, 1946). Transmission of the gene was reliably recorded through 11 generations, and there were 13 affected males living in 1956. Selection against the latter was formerly more extreme. The mean age at death of those born between 1676 and 1800 was 27 years in contrast to 43 years for those born since 1880. One reason for persistence of the gene was suggested by Rosin, Moor-Jankowski, and Schneeberger (1958) who showed that heterozygous sisters of affected males had significantly larger families than homozygous normal sisters. In another study, Simpson and Biggs (1962) also found that heterozygous sisters appeared to contribute more than the expected number of mutant genes to the next generation and Lewis *et al.* (1963) noted a similar tendency in their families.

Females with haemorrhagic symptoms and low factor IX levels have been reported from families

with affected males (Hardisty, 1957) or as a sporadic case (Nilehn and Nilsson, 1962).

The assay of factor IX has proved technically difficult and has hampered studies on heterozygous females. In 13 investigations before 1960 (listed by Didisheim and Vandervoort, 1962) 33% of 66 proven or probable carriers gave abnormal results for a variety of tests. More recently Nilsson *et al.* (1962) and Didisheim and Vandervoort (1962) investigated a total of 37 carriers and claimed low levels in over 75%. By contrast Simpson and Biggs (1962) found that 26% of 53 genetically proven heterozygotes had levels below the limit of the normal range and only 13% had levels less than twice the standard deviation of the mean for normal females. Frota-Pessoa, Gomes, and Calicchio (1963) interpreted distribution of values in heterozygous females as indirect evidence in favour of the inactive-X theory of dosage compensation.

Quick (1960) suggested that the relative trait frequencies of factor VIII and factor IX deficiencies would be reflected in the corresponding mutation rates at each locus. The rate at the factor IX locus would thus be about one-fifth that for factor VIII deficiency. From data recorded in 7 surveys, the proportion of families with an apparently sporadic male is not greatly different for each mutation, being 32.5% for factor VIII deficiency and 25% for factor IX deficiency (from De Ottolander and Hoorweg, 1955; Rapaport *et al.*, 1958; Biggs and Macfarlane, 1958; Deutsch, 1957; Ikkala, 1960; Ramgren, 1962b; Lewis *et al.*, 1963). Despite difficulties with gene frequency, effective fertility, and other problems that would appear to invalidate estimates of mutation rate (Simpson and Biggs, 1962), both Ikkala (1960) and Bitter (1963) have calculated similar rates, respectively 0.2 and 0.3×10^{-5} .

The locus for factor IX deficiency has not been linked with any other X-linked gene and at present cannot be placed on the provisional X-chromosome map. Lack of linkage with the factor VIII locus was demonstrated by Whittaker *et al.* (1962) on factor VIII or factor IX deficient families in which either deuteranopia or protanopia was also segregating. No measurable linkage between the Xg and factor IX loci has been detected (Davies *et al.*, 1963; Harrison, 1964).

(7) Factor X

Factor X was discovered through reinvestigation (Hougie, Barrow, and Graham, 1957) of a man (Mr. Stuart) previously thought to have factor VIII deficiency (Lewis, Fresh, and Ferguson, 1953),

and by studying (Telfer, Denson, and Wright, 1956) a hitherto unique clotting defect in a woman (Miss Prower). The factor was subsequently characterized as a protein with electrophoretic properties of an α globulin (Denson, 1958) and an approximate molecular weight of 87,000 (Esnouf and Williams, 1962).

Factor X acts as a substrate for several physiological and non-physiological agents prior to participation in prothrombin conversion. Advantage is taken of its activation by Russell's viper venom to construct diagnostic systems and to investigate the role of factor X in blood coagulation (Macfarlane, 1965). Raised factor levels are found in pregnancy (Brody and Finch, 1960). Acquired factor X deficiency has occurred transiently through unknown cause (Graham, Barrow, and Wynne, 1959), or in association with liver disease (Biggs and Macfarlane, 1962) and amyloidosis (Korsan-Bengtson, Hjort, and Ygge, 1962; Howell, 1963; Ménaché and Boivin, 1964).

Few cases of factor X deficiency have been recorded. In the last extensive review, Bachmann (1958) listed details of 19 patients from 12 families in whom the diagnosis had been unequivocally confirmed. Clinical features are similar to those for factor V deficiency, though haemarthroses have occasionally been noted (Hougie *et al.*, 1957; Chevallier, Bernard, Bilski-Pasquier, De Grouchy, and Samama, 1959). Practically all information of genetical relevance has come from the careful study of three families. In the initial account of the Stuart kindred where the parents of the propositus were related to each other as aunt and nephew, Graham *et al.* (1957) noted segregation of factor X levels into three classes. Assuming autosomal inheritance of an incompletely recessive gene, those with intermediate levels corresponded to heterozygotes. Some of the latter were thought to show mildly abnormal bleeding symptoms, an observation on which there has been no subsequent confirmation. Roos and Huizinga (1959) drew similar genetical conclusions from the detailed analysis of a family (Roos, van Arkel, Verloop, and Jordan, 1959) where a large sibship resulted from a consanguineous mating. Of 12 children, 6 had bleeding symptoms and factor X levels of about 5%, 4 had intermediate levels and no symptoms, and 2 were entirely normal. It was shown that this distribution was not improbable for genotypes assumed on an autosomal hypothesis. In a third study of an inbred kindred (partly shown in Fig. 12), Bachmann (1958) provided further segregation and laboratory data in favour of autosomal inheritance with partial expression in the heterozygote. Chevallier *et al.*

FIG. 12. Factor X deficiency. From Bachmann (1958), by kind permission of Dr. F. Bachmann. Only part of the family containing the proposita is shown. The full pedigree of 508 persons, of whom 61 had coagulation studies is in *Arch. Klaus-Stift. Vererb-Forsch.*, vol. 33.

(1959) described the occurrence of factor X deficiency in two first cousins whose mothers were sisters, but whose fathers were unrelated. On rather incomplete data the authors regarded heterozygosity as most probable because bleeding had occurred only after severe trauma and the laboratory evidence favoured that genotype. One other variant observation was made by Rabiner and Kretschmer (1961) who interpreted results from the electrophoresis of serum eluates to indicate that factor X resided in both α and β globulin fractions of serum. Evidence of a defect in both fractions was found in a patient with factor X deficiency and because an isolated defect of one or another fraction was found in relatives, it was postulated that factor X deficiency resulted from a pleiotropic gene or independent segregation of two genes. An alternative explanation may be suspected in view of the recent demonstration of a change in molecular properties of factor X on activation (Papahadjopoulos, Yin, and Hanahan, 1964b).

(8) Factor XI

Properties that distinguish factor XI from factor XII in the contact phase of clotting have been reviewed by Nossel (1964). Haemorrhagic symptoms in the inherited deficiency state are mild and generally follow operations or severe injury (Rapa-

FIG. 13. Factor XI deficiency. From Rapaport *et al.* (1961) by kind permission of Dr. S. I. Rapaport. Partial deficiency was assumed at factor XI levels below 65%.

port, Proctor, Patch, and Yettra, 1961). Some entirely symptomless cases have been detected during unrelated laboratory investigation (Egeberg, 1962b; Todd and Wright, 1964). Both sexes are affected, and though females occasionally suffer from menorrhagia only one instance of post-partum bleeding has been reported (Ramot, Angelopoulos, and Singer, 1955b).

Diagnosis may prove difficult (De Vries and Braat van Straaten, 1964) and some studies with qualitative methods have been interpreted to indicate that factor XI deficiency is caused by an autosomal dominant gene with varying expression (Rosenthal, Dreskin, and Rosenthal, 1955; Cavins and Wall, 1960; Cuttillo, Gargano, Regoli, and Anichini, 1962). But using a quantitative assay Rapaport *et al.* (1961) found that segregation of levels in the families of 8 probands fitted distributions expected for an incompletely recessive autosomal gene. Presumptive heterozygotes had no bleeding symptoms. Excluding the family of Campbell, Mednicoff, and Dameshek (1957) which had some unusual features, there is no real evidence to dispute this mode of inheritance (now accepted by Rosenthal, 1964). Two of Rapaport's pedigrees are given in Fig. 13.

Almost all patients whose nationality has been recorded were of Jewish origin (Rosenthal *et al.* 1955; Rapaport *et al.*, 1961; Biggs and Macfarlane, 1962). This may account for the uneven distribution of factor XI deficiency among different research centres. From the laboratory in New York where factor XI was discovered in 1953, Rosenthal (1964) has collected 72 cases from among 46 Jewish families. The situation may be analogous to that

for Tay-Sachs disease which is practically confined to the Jewish community of the same city (Volk, Aronson, and Saifer, 1964).

(9) Factor XII

Factor XII was discovered in 1955 by Ratnoff and Colopy through investigation of a man with grossly defective blood clotting but no haemorrhagic symptoms. The factor has properties of a basic protein (Margolis, 1963) and is activated in mammalian plasma through adsorption to glass and other insoluble substances (Hardisty and Margolis, 1959) or by certain organic compounds that are characterized by O-dihydroxyl groups (Ratnoff and Crum, 1964). The activated factor reacts enzymatically with factor XI to initiate clotting and is also concerned with the release of plasma kinins, a capillary permeability factor (Margolis, 1963), and fibrinolysin (Holemans and Roberts, 1964).

There are typically no haemorrhagic symptoms in factor XII deficiency, though minor bleeding has been noted in a few instances (Soulier and Larrieu, 1958; Didisheim, 1962; R. Biggs, 1965, personal communication). Haemarthroses and deep tissue bleeding have never been reported and with one dubious exception (Haanen, Hommes, Benraad, and Morselt, 1960) operations have not been followed by abnormal haemorrhage (Fantl, Morris, and Sawers, 1961; Didisheim, 1962; Abildgaard, Cornet, Alcalde, Schulman, and Fort, 1963).

Factor XII deficiency has been noted among Caucasians of many different nationalities (Ratnoff and Steinberg, 1962) and in one American Negro family (McCain, Chernoff, and Graham, 1959).

Careful family studies initially suggested homozygosity for an autosomal gene (Margolius and Ratnoff, 1956; McCain *et al.* 1959), and this has been impressively supported by an analysis of data on 55 cases in 37 families (Ratnoff and Steinberg, 1962). The sex distribution of cases was approximately equal, the trait was present in 28% of examined sibs, and no parent or offspring of propoiti was affected. Consanguinity of parents was noted in three families (Margolius and Ratnoff, 1956; McCain *et al.*, 1959) and was probably present in one other (Loeliger and Hensen, 1960). Using non-specific tests, no clotting defect could be noted in 33 presumptive heterozygotes, but with a crude semi-quantitative technique a decreased level of factor XII (to the order of 25–60%) was found in about one-third of heterozygotes.

(10) Factor XIII

Factor XIII, the fibrin stabilizing factor, was isolated from normal plasma in 1948 by Laki and Lorand. It is a thermolabile protein that reacts with fibrinopeptides during fibrin formation and converts a loosely cross-linked urea-soluble fibrin gel into a stable urea-insoluble clot. Calcium ions and free sulphhydryl groups are required for the reaction. Other properties are discussed by Lorand and Jacobsen (1958) and in a series of papers by Loewy, Dunathan, Gallant, and Gardner (1961). It is not yet agreed whether the factor acts stoichiometrically or as an enzyme. Factor XIII was subsequently found on platelets (Lüscher, 1957), and

an apparently identical substance has been isolated from several body tissues (Tyler and Lack, 1964). An acquired deficiency was detected in persons with acute leukaemia (Nussbaum and Morse, 1964).

An inherited defect of factor XIII was discovered by Duckert, Jung, and Shmerling (1960) whose propoiti characteristically commenced bleeding some 24–36 hours after severe trauma and continued to bleed episodically from a poorly healing wound for several weeks. In contrast to normal fibrin the patient's blood clot dissolved in 5M urea and 1% monochloroacetic acid. Subsequent studies revealed that the clot was abnormally susceptible to fibrinolysis (Bickford and Sokolow, 1961), could not support effective growth of fibroblast cultures (Beck, Duckert, and Ernst, 1961), and had an unstable structure (Duckert, Jung, and Shmerling, 1961).

The propoiti of Duckert *et al.* (1960) and two similarly affected sibs were offspring of a consanguineous mating in a highly inbred kindred (Fig. 14). Four children among three other sibships had died from bleeding, and the only affected survivor, a female cousin of the propoiti, had a similar clot defect. Autosomal recessive inheritance was initially proposed (Shmerling, Jung, and Duckert, 1961), and with the subsequent development of a more sensitive technique (Sigg and Duckert, 1963) it was possible to detect evidence of partial factor XIII deficiency among presumptive heterozygotes (Duckert, 1965) as shown in Fig. 14.

FIG. 14. Factor XIII deficiency. Part of the revised pedigree (Duckert, 1965) originally reported by Shmerling *et al.* (1961), redrawn by kind permission of Dr. F. Duckert. Two additional sibships each containing presumptive homozygotes are shown in the original.

TABLE IX
CASE REPORTS OF FACTOR XIII DEFICIENCY

Source	Sex and Age	Umbilical Haemorrhage	Clinical Features		Parental Consanguinity	Abnormal Findings in Other Relatives
			Delayed Onset Bleeding	Intracranial Haemorrhage		
Duckert <i>et al.</i> (1960)	M 7 M 1½	+	+	+	Yes	Patients are brothers; other relatives affected as in Fig. 14
Ikkala and Nevanlinna (1962)	M 2	+	—	—	No	No clot defect in parents; affected younger brother mentioned by Ikkala <i>et al.</i> (1964)
Mandelli (1963)	F 2	+	+	—	Yes	No clot defect in parents
Masure (1963)	M 6	+	+	—	No	No clot defect in parents or brother; 1 sister died from umbilical bleeding
Josso, Prou-Wartelle, Alagille, and Soulier (1964)	M 1 M 4	+	+	+	No Yes	No clot defect in mother; no clot defect in parents, brother, or sister
Ikkala, Myllylä, and Nevanlinna (1964)	F	+	—	—	Yes	Not investigated

Six case reports have been subsequently published (Table IX). All patients experienced umbilical bleeding during the neonatal period and post-traumatic haemorrhage of delayed onset was common. Intracranial haemorrhage in two young patients emphasizes the serious haemostatic defect. Parental consanguinity in 3 of 6 subsequent families supports Duckert's evidence for an autosomal recessive gene, though using diagnostic tests no partial gene effect was found in available parents or sibs.

(11) Platelet Clotting Factors

It has been known for many years that platelets exert a profound influence on blood clotting and haemostasis in general. Recent studies have shown that during their 7–9 days life-span these cells contain or carry on their surface many substances which include several clotting factors, adenosine triphosphate (ATP), 5-hydroxytryptamine, and other amines, and enzymes for a glycolytic cycle. In addition there are several specific platelet clotting factors designated by Arabic numerals (Biggs and Macfarlane, 1962). The most important of these, platelet factor 3, is a phosphatide (Troup, Reed, Marinetti, and Swisher, 1961) required for the efficient performance of the intrinsic pathway of prothrombin activation. This factor appears defective in certain genetical disorders of platelet function. The position is none too clear because platelet biochemistry is still in its infancy as are studies on the two vital phenomena of platelet aggregation (metamorphosis) and adhesion to foreign surfaces (Sharp, 1964).

Classification of these disorders is difficult. Braunsteiner (1955) defined two groups each characterized by bleeding symptoms, prolonged bleeding time, and normal numbers of platelets. One group, the 'thrombopathies', was characterized by abnormal coagulation tests, normal clot retraction, and platelet aggregation. The other, 'thrombasthenia' had in contrast normal clotting tests and abnormal clot retraction and platelet aggregation. Both groups are often loosely described as Glanzmann's disease, but in recent years the eponym has generally been retained for the thrombasthenia category (Larrieu, Caen, Lelong, and Bernard, 1961; Pittman and Gaham, 1964; Friedman, Bowie, Thompson, Brown, and Owen, 1964). However, variant findings have necessitated several sub-categories (Ulutin, 1961), and in a clarifying review Hardisty, Dormandy, and Hutton (1964) gave good reasons why thrombasthenia should be redefined as a congenital bleeding disorder characterized by a normal platelet count, a long bleeding time, and failure of platelet aggregation on addition of adenosine diphosphate (ADP). Abnormal clotting tests inconstantly found in thrombasthenia are ascribed to defective release rather than deficiency of platelet clotting factors (Johnson, Monto, and Caldwell, 1958; Ulutin, 1961; Hardisty *et al.*, 1964). The latter authors suggested that clotting anomalies were related to non-reaction of platelets with ADP and that all haemostatic abnormalities might be attributable to a single defect of the cell membrane. Parental consanguinity in over 10% of earlier case reports (Larrieu *et al.*, 1961) and in 3 of 6 more recent ones (Pittman and Gaham, 1964; Friedman *et al.*, 1964; Hardisty *et al.*, 1964) favours autosomal recessive inheritance, but there has been no record of any heterozygote effect, which is hardly surprising at a time when tests of platelet function are still being developed.

The thrombopathy group has been recently reviewed by Kanska *et al.* (1963) and Alagille *et al.* (1964). Both sexes were affected by moderately severe bleeding mainly from mucous tracts and into the skin. Platelets are characteristically large and show defects in tests of the intrinsic clotting system generally ascribed to a deficiency of platelet factor 3. Using the electron microscope, Braunsteiner (1961) found normal platelet pseudopod formation in contrast to abnormal spreading in thrombasthenia. Gross (1961) demonstrated abnormal platelet glycolytic enzymes in thrombasthenia but not in thrombopathy. In the latter state homozygosity for an autosomal recessive gene is suspected, because in recent reports 5 of 7 sets of parents with affected children were related (Bernard, Caen, and Maroteau, 1957; Kanska *et al.*, 1963; Alagille *et al.*, 1964). Kanska *et al.* found partial defects of platelets in presumptive heterozygotes.

It is possible that genetical defects of plasma clotting factors influence platelet function. Bowie, Thompson, and Owen (1964a) have in fact suggested this to explain an apparently unique congenital platelet disorder, but most ideas on such a relationship have been centred on von Willebrand's disease.

(12) Combined Genetical Defects of Clotting Factors and Other Components of Haemostasis

Congenital and inherited haemorrhagic states, affecting either sex and characterized by an abnormally prolonged bleeding time, were regarded as genetical disorders of capillaries or platelets until 1953, when Alexander and Goldstein reported two such patients who also had significantly reduced levels of factor VIII. It was subsequently realized that another characteristic of this group was marked phenotypic variability which, together with exaggerated reliance on imprecise laboratory techniques, led to such confusion over presumptive pathogenesis and classification, that by 1959 von Willebrand's (1931) unfortunate term 'pseudohaemophilia' had been applied to at least eight distinct varieties of haemorrhagic disease (Gross, 1959). The first attempt at an objective classification based on laboratory findings, which did not worsen the situation by proposing new terminology, was made by Valberg and Brown (1958). They assumed that the prolonged bleeding time was due to abnormal capillaries and recognized three categories: defects confined to capillaries (subsequently reviewed by Blackburn, 1961), capillary defects associated with abnormal coagulation, and capillary defects asso-

ciated with abnormal platelets. Unfortunately the cause of a prolonged bleeding time remains unknown and can only occasionally be ascribed with any confidence to abnormal vessels. At present, each variety is best described in unequivocal clinical, laboratory, and genetical terms, which is admittedly cumbersome but avoids the vague and hypothetical terminology that prevents further analysis of many previous studies. Von Willebrand's disease is the only disorder in this group that can be regarded as reasonably homogeneous in that there is general agreement on clinical, diagnostic, and genetical characteristics. Consequently, current knowledge on inherited haemostatic disorders will be discussed in relation to von Willebrand's disease.

Von Willebrand's Disease. In 1926 the Finnish physician von Willebrand studied a girl with severe haemorrhagic symptoms who lived on the Åland archipelago in the North Baltic sea. Investigation of the girl's family revealed that 23 of 66 available members suffered from abnormal bleeding of varying severity. Federley, geneticist at the University of Helsinki, concluded that in contrast to classical haemophilia an autosomal dominant gene was responsible, though in a later report von Willebrand (1931) published speculation on the possibility of X-linked dominant inheritance, which subsequently became untenable on finding instances of male-to-male transmission (von Willebrand and Jürgens, 1933). The main laboratory findings were a consistently prolonged bleeding time in the presence of a normal coagulation time. Through observing some minor structural changes as the sole abnormality in platelets, von Willebrand postulated a functional platelet disorder and suggested the term 'pseudohaemophilia' following the nomenclature of Glanzmann (1918) and Frank (1925). These authors had previously described congenital platelet defects which von Willebrand subsequently recognized as quite distinct from the Åland diathesis.

In 1930 Jürgens investigated von Willebrand's patients with an apparatus designed on contemporary coagulation theory to measure platelet function (Morawitz and Jürgens, 1930) and interpreted the results as confirming a functional platelet disorder (von Willebrand and Jürgens, 1933). 'Von Willebrand's pseudohaemophilia' was accordingly renamed 'die Konstitutionelle Thrombopathie (von Willebrand-Jürgens)' and classified among the inherited platelet defects (Jürgens, 1941). Despite clear definition, both the original and revised term were subsequently used to cover a variety of hereditary bleeding disorders with obviously different pathogenesis (Gross and Mammen, 1958). More or less by

convention it became permissible to diagnose von Willebrand's disease when a congenital haemorrhagic state was associated with any combination of the following characteristics: prolonged bleeding time, normal or slightly prolonged coagulation time, normal or slightly reduced platelet count, normal or abnormal platelet morphology, normal clot retraction tests, indicative of faulty prothrombin consumption, and autosomal dominant inheritance. The latter feature was confirmed by Favre-Gilly, Guy, Beaudoin, and Roger (1954) in 80% of reported families where the diagnosis remained within limits imposed by von Willebrand and Jürgens, though there were a few instances of multiple cases in sibships with neither parent nor other relative apparently affected.

Alexander and Goldstein (1953) who first recorded the association of low (5–10%) levels of factor VIII with congenital haemorrhagic disease, prolonged bleeding time, and normal platelets postulated a dual haemostatic defect separately involving capillaries and factor VIII. Their laboratory investigations were soon confirmed (Larrieu and Soulier, 1953; Van Creveld, Jordan, Punt, and Veder, 1955; Darte, 1955). Initial family studies (Schulman, Smith, Erlandson, and Fort, 1955; Singer and Ramot, 1956) suggested that a dominant gene was concerned with regulating factor VIII, and by demonstration of a father-son transmission (Matter, Newcomb, Melly, and Finch, 1956) sex-linkage could be rejected. Schulman *et al.* (1955) detected unduly tortuous nail-bed and conjunctival vessels in their parents and proposed that the apparent plasma-capillary defect be known as 'vascular haemophilia'. Singer and Ramot (1956) favoured 'pseudohaemophilia B' and others (Matter *et al.*, 1956; Achenbach and Klesper, 1957) regarded 'angiohaemophilia' as most suitable. Quattrin (1954) accounted for all aetiological possibilities with 'plasma-telangi-thrombopathia'.

Meanwhile, Nilsson (1956) had studied a Swedish girl with severe bleeding symptoms, prolonged bleeding time, and low factor VIII (5%). When factor VIII concentrated from human plasma (fraction 1–0, Blombäck and Blombäck, 1956) was administered therapeutically not only did bleeding cease and the factor VIII level rise, but the bleeding time was shortened to near normal limits. This finding was confirmed on patients from other Swedish families where the trait was inherited as an autosomal dominant (Nilsson, Blombäck, Jorpes, Blombäck, and Johnson, 1957b).

The Swedish workers then went to the Åland islands and examined patients some of whom had been originally studied by von Willebrand. They concluded (Nilsson, Blombäck, and von Francken, 1957a) that the islanders had a disorder identical

to that on the Swedish mainland despite lack of a connecting geneological link between the ethnically related populations. The results of this investigation justify retaining an eponymic substitute for the full descriptive term 'inherited autosomal diathesis with factor VIII deficiency and prolonged bleeding time' (Nilsson *et al.*, 1957a; Spurling and Sacks, 1959; Cornu, Larrieu, Caen, and Bernard, 1961).

Due to confused views on von Willebrand's disease there is little indication of its prevalence in populations outside Scandinavia. The most recent estimate from Sweden (Nilsson and Blombäck, 1963) implied a trait frequency of about 1 in 80,000 which is in agreement with the view of Achenbach (1963) in Western Germany and unpublished data (R. Biggs, 1965 personal communication) from Oxford that von Willebrand's disease is encountered in clinical practice about as frequently as factor IX deficiency. The disease is prevalent among the inbred communities of the Åland archipelago (total population about 30,000) with over 10% of all persons affected on certain islands (Eriksson, 1961).

Typical forms of bleeding in heterozygotes are epistaxis, superficial bruising, gingival haemorrhage, and following minor trauma (Biggs and Macfarlane, 1962; Blombäck, Jorpes, and Nilsson, 1963a). Prolonged haemorrhage is usual after operations and exodontia, and sometimes (Silwer and Nilsson, 1964), though not necessarily (Deutsch, Mlczochna, and Ulm, 1959), follows parturition. Haemarthroses and intramuscular haemorrhages are rare (Nevanlinna, Ikkala, and Vuopio, 1962; Blombäck *et al.*, 1963a) and the pattern of bleeding is thus quite different from that in haemophilia. Bleeding symptoms become less frequent with advancing years and, excluding menorrhagia, cause little trouble by middle age (Achenbach, 1963; Silwer and Nilsson, 1964). The assignment of clinical severity is hazardous and usually gauged by rather unreliable events such as menstrual loss or post-operative bleeding.

Heterozygotes ascertained through haemorrhagic symptoms generally have a factor VIII level below 20% and a very prolonged bleeding time (Nilsson *et al.*, 1963). Matter (1963) found a close correlation between the degree of factor VIII reduction and prolonged bleeding time. However, with heterozygotes ascertained through a propositus, Silwer and Nilsson (1964) concluded that there was no correlation between clinical severity and the degree of laboratory abnormality. Some with haemorrhagic symptoms have a defect confined to only one haemostatic parameter (Pitney and Arnold, 1960; Blombäck *et al.*, 1963a). Eriksson (1961) and Eriksson, Hiepler,

Jürgens, Lehmann, and Schulz (1961) used an abnormality in capillary fragility as a measure of gene effect in possible heterozygotes with no *in vitro* defect.

Presumptive homozygotes have been described (von Willebrand, 1931; Eriksson, 1961) with symptoms and haematological findings that were not very different from those in heterozygotes, but Graham, Barrow, and Roberts (1965) reported more extreme findings in two homozygotes. Geiger and Rath (1963) described monozygotic male twins probably hemizygous for factor VIII deficiency and heterozygous for von Willebrand's disease, who came from a family where both traits were segregating and who had haemorrhagic disease similar to their haemophilic male relatives. Quick and Adlam (1963) drew similar conclusions from another family where evidence for dual segregation was less convincing.

There is general agreement that the laboratory variation observed among related heterozygotes is not a technical artefact, and as widely differing results can be obtained from the same patient over a period of time (Blombäck *et al.*, 1963a) some non-apparent modifying agents probably influence the mutant gene effect. Such variation hampers genetical analysis, and this is well discussed by Raccuglia and Neel (1960) and illustrated by extensive studies of an Åland (Eriksson, 1961) and Swedish kindred (Silwer and Nilsson, 1964). Different

diagnostic criteria were used in each study, and some investigators consider that evidence of abnormal capillary fragility, as obtained by Eriksson in cases where the bleeding time and factor VIII level were normal, is an unreliable measure of haemostasis (Nevanlinna *et al.*, 1962; Geiger and Rath, 1963) and unrelated to a prolonged bleeding time (Matter, 1963). In addition the Duke method of estimating bleeding time, used by Eriksson, gave in the hands of Silwer and Nilsson abnormal results in only 4 of 46 cases in contrast to the Ivy technique which yielded a significantly prolonged bleeding time in all their 46 cases. It must be noted that the bleeding time test gives most variable results: for instance, Roskam (1954) using the Duke method on the ear lobes of the same patient obtained a normal result (3 minutes) from one ear and an abnormal result (48 minutes) from the other. These and other technical problems led to equivocal findings, but in neither kindred was there an instance of transmission through an apparently normal person (as suspected in one member of Raccuglia and Neel's 1960 family).

Intrafamilial variation in laboratory findings is illustrated by part of Silwer and Nilsson's pedigree (Fig. 15). Genetical analysis of Scandinavian families gave results consistent with autosomal dominant inheritance of a single gene with varying penetrance and a wide range of expression. This is in agreement with conclusions from other family studies (Valberg and Brown, 1958; Raccuglia and Neel, 1960; Nevanlinna *et al.*, 1962; Blombäck *et al.*,

Fig. 15. Von Willebrand's disease. Part of Swedish kindred of Silwer and Nilsson (1964) by kind permission of Dr. I. M. Nilsson.

FIG. 16. Von Willebrand's disease. Part of Åland kindred of Eriksson (1961), by kind permission of Dr. A. W. Eriksson.

1963a). Part of Eriksson's consanguineous kindred is given in Fig. 16 including the propoita who, despite being classified as a severe bleeder (and possibly homozygous), bore three children. Eriksson detected no obvious effect of selection, and though in the past bleeding was commonly fatal in childhood (von Willebrand, 1931; Lehmann, 1959) there is now reliable and successful therapy (Jorpes, 1963).

There have been a few reports of syndromes clinically and genetically analogous to von Willebrand's disease but where a prolonged bleeding time is associated with a deficiency of factor IX (Achenbach and Klesper, 1957; Gross and Mammen, 1958; Blombäck *et al.*, 1963a), factor XI (White, Yunis, Colliander, and Krivit, 1963) or both factor VIII and factor XI (Perry, Oppell, and Baker, 1960). These disorders possibly represent unique mutations, and their study along lines currently used in von Willebrand's disease is awaited.

Current Views on Pathogenesis of Von Willebrand's Disease and Studies on the Genetic Control of Factor VIII. It is not known whether the prolonged bleeding time in von Willebrand's disease is due to defective capillaries, abnormal platelets, or a plasma factor deficiency. Morphological changes in capillaries have been reported (Macfarlane, 1941; Schulman *et al.*, 1955; Marx, 1959) but most investigators listed by Blombäck *et al.* (1963a) have detected no vessel abnormalities. Jamra, Lichtenstein, Vieira, and Ribeiro Leite (1952) and Buchanan and Leavell (1956) found great variation in normal capillary

structure and concluded that it was impossible to distinguish so-called pathological changes.

Observations on raising the factor VIII and shortening the bleeding time in von Willebrand's disease by infusions of factor VIII-deficient (haemophilic) plasma (Nilsson, Blombäck, and Blombäck, 1960; Cornu, Larrieu, Caen, and Bernard, 1963; Biggs and Matthews, 1963) led Swedish workers (Blombäck *et al.*, 1963a) to postulate that the primary genetical defect was in a plasma anti-bleeding factor (or vascular factor) that influenced the bleeding time and the production or activation of factor VIII. The vascular factor has not been characterized in detail but appears to be a protein concentrated in the fibrinogen fraction of plasma (Biggs and Matthews, 1963).

The von Willebrand-Jürgens school of Åland investigators have always held that some platelet abnormality is basically responsible for defective haemostasis and reduced factor VIII levels (Jürgens, Lehmann, Wagelius, Eriksson, and Hiepler, 1957), though some of their findings such as abnormal platelet function in the thromboplastin generation test (Eriksson, 1961) and diminished clot retraction (Lehmann, Jürgens, and Eriksson, 1964) await confirmation. Structural changes in platelets have been detected by electron microscopy (Raccuglia and Neel, 1960; Eriksson *et al.*, 1961; Marx and Jean, 1964). Reduction in platelet adhesiveness was noted both *in vivo* (Borchgrevink, 1961) and on glass surfaces (Zucker, 1963; Lehmann *et al.*, 1964). Although platelet aggregation is normal with standard concentrations of ADP, unexplained abnormalities have been observed at very low concentrations (Vainer and

Caen, 1963; Odegaard, Skalhegg, and Hellem, 1964). Caen (1963) also claimed that platelet ATP levels were significantly raised in von Willebrand's disease, which suggested that diminished ADP release during haemostasis would prevent effective platelet aggregation and reduce platelet adhesion to the vessel wall. Some workers (Zucker and Borelli, 1956; Ingram, 1956) have reported that platelet 5-hydroxytryptamine was defective, but Nilsson *et al.* (1957a) were unable to confirm this.

On the assumption that platelets are abnormal in von Willebrand's disease, Borchgrevink (1961) postulated that the capillary type of bleeding might be due to failure of a normal reaction between damaged endothelium and platelets. The condition may not be homogeneous with regard to platelets. Blackburn, Macfie, Monaghan, and Page (1961) described a family with typical features of von Willebrand's disease with the exception that some affected members had thrombocytopenia.

Theories on the genetical control of factor VIII have been designed by J. B. Graham to accommodate findings in von Willebrand's disease and factor VIII deficiency. Graham (1959) initially proposed double heterozygosity for two dominant genes to explain the dual laboratory defect in von Willebrand's disease, but this view became untenable when family studies revealed close association of defects rather than the independent segregation demanded by a two-gene hypothesis. Close-linkage could also be excluded on grounds of the improbability in encountering two uncommon genes, almost invariably in the coupling phase. Graham (1963) now considers a pleiotropic gene most likely, and, from experimental observations on factor VIII deficiency and von Willebrand's disease interpreted in terms of current views on synthesis of bacterial and mammalian proteins, he has described hypothetical models of factor VIII synthesis (McLester and Graham, 1963, 1964; Graham *et al.*, 1965). Each model was designed to account for control of factor VIII production at two loci; dominance of the von Willebrand allele and recessivity of the factor VIII deficiency gene; and the increase of plasma factor VIII after infusion of factor VIII deficient (haemophilic) plasma into a person with von Willebrand's disease but not conversely. The models, though speculative and certainly over simplified, have heuristic value in that some assumptions may be tested. For instance in a 'regulatory' model, factor VIII deficiency (haemophilia) could result from mutation of an X-linked structural gene, and von Willebrand's disease from a dominant mutation of a regulator gene at the autosomal locus for 'vascular

factor' (McLester and Graham, 1964). If the model is valid it is essential that post-infusional factor VIII 'synthesized' in von Willebrand's disease be structurally identical with normal plasma factor VIII, and this has to some extent been confirmed (Barrow, Roberts, Pons, and Graham, 1964). However, further infusion experiments have favoured a hypothesis that factor VIII is a polymer formed by at least two polypeptide chains each coded by a structural gene at either an X-linked or autosomal locus (Graham *et al.*, 1965).

(13) Combined Clotting Factor Deficiency

Congenital haemorrhagic disorders characterized by deficiencies of two or possibly three clotting factors comprise a difficult and disputed group. It is not easy to distinguish an acquired inhibitor associated with factor VIII or factor IX deficiency from a dual clotting factor deficiency (Margolius *et al.*, 1961; Nilsson *et al.*, 1962), and in the absence of such information it is impossible to accept some instances of alleged factor VIII-factor IX deficiency (Hill and Speer, 1955) or factor IX-factor VII deficiency (Bell and Alton, 1955). In a review of eight case reports, Graham (1957) noted that all those affected were males allegedly hemizygous for both X-linked mutants, or for either factor VIII or factor IX deficiency combined with an autosomal mutant. Graham pointed out that under Hardy-Weinberg conditions double hemizygosity for factor VIII and factor IX deficiency would be expected with negligible frequency (estimated at $0.26 - 3.1 \times 10^{-9}$), but hemizygosity for factor VIII deficiency combined with heterozygosity for factor V deficiency (to explain findings of Oeri, Matter, Isenschmid, Hauser, and Koller, 1954 and Iverson and Bastrup-Madsen, 1956) might occur with less improbability (Graham's figure was $0.35 - 2.5 \times 10^{-7}$). Subsequent reports have in no way clarified the position, but some alternative conclusions have been drawn from recent studies where quantitative factor assays were performed.

Combined factor VIII and factor V deficiency is supported by relatively convincing laboratory data (Seibert *et al.*, 1958; Jones, Rizza, Hardisty, Dormandy, and MacPherson, 1962): 7 patients (4 males and 3 females) from 5 families with bleeding symptoms varying from marked (4) to mild (3) have been described. Three of five matings were consanguineous (Oeri *et al.*, 1954; Iversen and Bastrup-Madsen, 1956; Jones *et al.*, 1962) and a fourth possibly so (Seibert *et al.*, 1958). Excluding a sister of Iversen and Bastrup Madsen's propositus

who had isolated factor V deficiency, no clotting defect was detected in a total of 7 parents and three sibs. Factor V and factor VIII have some similar properties (Bowie, Thompson, and Owen, 1964b; Macfarlane, 1965), and it has been suggested that the combined defect is due to a genetically induced block in synthesis of some common precursor protein (Oeri *et al.*, 1954; Seibert *et al.*, 1958; Jones *et al.*, 1962; Rapaport, Schiffman, Patch, and Ames, 1963).

There is only one instance where evidence for combined factor VIII and factor IX deficiency is supported by convincing laboratory and genetical data. Robertson and Trueman (1964) reported a family where both traits were segregating and there was pedigree evidence for double heterozygosity in a female whose son had a dual factor deficiency. He had mild symptoms similar to those in male relatives with either factor VIII or factor IX deficiency, implying no excessive haemostatic consequence from double hemizygoty.

A unique combination of factor VII and factor X deficiency has recently been studied in 242 members of a large family by Kroll, Alexander, Cochios, and Pechet (1964). Sixty members of either sex had decreased levels of factor X; and of these 28 were also deficient in factor VII. None had an isolated defect of factor VII. Bleeding symptoms were mild, and 12 persons also exhibited carotid-body tumours, which appeared to be segregating independently in one part of the family. The authors considered several genetical hypotheses such as a single autosomal gene at the factor X locus which also modified factor VII synthesis, or segregation for two genes at the respective loci. The latter possibility could not be rejected on the available data.

Claims for combined factor VIII and factor VII deficiency are not supported by convincing quantitative evidence of factor VII reduction, as in a family where factor VIII deficiency was segregating (Constandoulakis, 1958) or where a factor VII level of 55% was found in a male with factor VIII deficiency (Gaston, Mach, and Beck, 1961). The same holds for alleged factor VII deficiency in males with factor IX deficiency (Bell and Alton, 1955; Nour-Eldin and Wilkinson, 1959; Aresu and Spinnozola, 1960), particularly as Verstraete, Vermeylen, and Vandenbroucke (1962) found reduced levels of factor VII in random males with sex-linked factor IX deficiency.

In two recent studies of sibs with marked haemorrhagic disease and a complex clotting defect, it was concluded that factor XI was deficient in combination with factors VIII and IX (Angelopoulos, Kourepi Vicatou, and Mourdjinis, 1964) or factor VIII (Schulz, Nowotny, Schmutzler, and Duckert, 1964). Due to the difficulties in measuring factor XI (Nossel, 1964) some reservations must remain on interpretations of *in vitro* findings in these families. Nevertheless a small resi-

dium of persons with a congenital disorder of clotting, the nature of which remains obscure with present techniques (Biggs and Macfarlane, 1958), probably contains some with multiple factor deficiencies of genetic origin.

(14) Clotting Factor Inhibitors

Claims of congenital defects in the inhibitor system associated with haemorrhagic disease have not usually reached the limits of validity demanded by some authors (Margolius *et al.*, 1961; Soulier, 1962). However, Quick (1957) diagnosed congenital hyperheparinaemia in a woman who first bled abnormally when aged 3 and a similar disorder was described by Heni and Krauss (1956) in a father and daughter. In both studies the *in vitro* clotting defect was restored by protamine sulphate and toluidine blue, but this reaction is not specific for heparin (Soulier, 1962). However, Quick also achieved *in vivo* correction of the defect in his patient.

Brown, Diamant, Galbraith, and Wilson (1963) reported a family with mild haemorrhagic disease in two generations. Members of two sibships were investigated with non-specific tests of clotting function. The results were interpreted as evidence of an inherited antithrombin. Specific factor assays were not made, nor some other procedures considered necessary for definitive diagnosis (Margolius *et al.*, 1961; Soulier, 1962). Earlier reports of congenital disorders in the inhibitor system (Bell, 1951; Speer, Hill, Maloney, and Roberts, 1955) can be alternatively explained in retrospect as due to an acquired factor VIII inhibitor in factor VIII deficiency. Egeberg (1965) has recently given a preliminary account of a family where several members in two generations experienced recurrent thrombo-embolic episodes from an early age. This was associated with diminished activity of blood antithrombin. Further studies are required for confirmation that this family illustrates a genetical disturbance of clotting, which leads to obstructive vascular disease.

(15) Inherited Resistance to Coumarin Anticoagulant Drugs

Coumarin and structurally related indanedione drugs have a complex effect on clotting, which causes reduction in plasma levels of prothrombin and factors VII, IX, and X. The drugs are regarded physiologically as anti-metabolites that compete with vitamin K in an enzyme system responsible for synthesis of the above factors in hepatic cells.

Remarkable tolerance to toxic amounts of anticoagulant poison (warfarin) was noted in certain members of a rat (*Rattus norvegicus*) colony in West Scotland (Boyle, 1960). The colony was eradicated but in a recurrent

infestation three years later further warfarin-tolerant animals were detected (Cuthbert, 1963). There were no further data to support the impression that tolerance was inherited.

An important contribution to human pharmacogenetics was made by O'Reilly, Aggeler, Hoag, Leong, and Kropatkin (1964) who described a family (Fig. 17), where some members were extraordinarily resistant to all but massive doses of warfarin sodium, other coumarin derivatives, and phenindione. The 72-year-old propositus required

FIG. 17. Inherited coumarin resistance. From O'Reilly *et al.* (1964) by kind permission of Dr. R. A. O'Reilly who points out that an X-linked gene cannot be excluded on present data.

145 mg. warfarin per day to achieve depression of clotting factors within the therapeutic range. This dose was some 45 standard deviations above the average normal requirement (7 ± 3 mg.). Similar resistance was found in an identical twin and five other relatives in three generations. This suggested control by a dominant gene. As to the possible mechanism of coumarin resistance, O'Reilly *et al.* clearly excluded decreased absorption and increased destruction of the drug and also overproduction by the system responsible for synthesizing vitamin K-dependent clotting factors. Excess vitamin K storage appeared unlikely and the most probable explanation was that affected persons had an abnormal enzyme or receptor site with either a decreased affinity for warfarin or an increased affinity for vitamin K. In each case vitamin K-warfarin competition would be distorted. Subsequent studies have demonstrated increased sensitivity to different vitamin K analogues (R. A. O'Reilly, 1965, personal communication). Dr. O'Reilly also pointed out the possible relevance of Olson's work on vitamin K-induced prothrombin

production in chicks. Olson (1964) interpreted his findings in terms of Jacob-Monod theory to show that vitamin K normally acts with the product of a regulator gene to de-repress an operon containing structural genes for each vitamin K-dependent clotting factor. Mutation at the regulator gene locus would thus account for greatly increased response to vitamin K— a hypothetical situation that would explain the findings in the human mutation.

COMPARATIVE AND THEORETICAL ASPECTS

Evolution of Blood Clotting. Mutation in other Species

Heilbrun (1961) and Gregoire and Tagnon (1962) reviewed studies on coagulation of coelomic fluid, haemolymph, and blood of invertebrates, and concluded that the multi-stage process of mammalian blood clotting had evolved from a simple mechanism based on cellular functions. When coelomic fluid escapes, the only type of circulating cell, the amoebocytes, are incorporated into a mesh, but whether this represents syncytia or transformation into a gelis controversial (Gregoire and Tagnon, 1962).

Specialized cells in the haemolymph of insects appear to undergo alteration on contact with foreign surfaces (Gregoire and Florkin, 1950). This is followed by various degrees of plasma gelification (Wigglesworth, 1959). The arthropods, particularly Crustacea, have been most extensively studied and variations in clotting throughout the group range from complete absence of any coagulum to the formation of a firm clot. It has long been known (Hardy, 1892) that amoebocytes in crustacean haemolymph are specifically involved in coagulation. Indirect evidence that plasma proteins also take part has been obtained by gravimetric (Morrison and Morrison, 1952) and chemical (Booolootin and Giese, 1959) analysis of the coagulum. Duchateau and Florkin (1954) claimed extraction of fibrinogen from crustacean haemolymph which differed from the mammalian variety in solubility and electrophoretic mobility. However, Levin and Bang (1964), working with *Limulus*, the horse-shoe crab, found that cell-free haemolymph was incoagulable. Moreover, amoebocytes underwent alterations during clotting remarkably like those of mammalian blood platelets, and the authors suggested that as clots formed only in their presence the amoebocytes might be the sole source of coagulable protein.

The assumption that invertebrate clotting systems are largely dependent on cellular reactions is an attractive hypothesis from which to launch teleological arguments on the ultimate complexity of cell-free clotting systems in mammals. But the apparent

simplicity of clotting in lower organisms is suspect, because contemporary biochemical methods have yet to be employed. There is an obvious analogy with concepts of human blood coagulation during the relatively uncomplicated era of classical theory. In addition, experiments performed with artificial clotting systems are of doubtful validity when species-specificity of natural reactions are not represented. It is surely unjustified to draw conclusions, as did Heilbrun (1961), on fundamental similarities between *in vitro* clotting of mammalian plasma and gelification of extruded protozoan cytoplasm merely because both systems require calcium ions and tissue enzymes, are accelerated by thrombin, and inhibited by heparin. Contemporary studies on vertebrates are to date fragmentary, beset with problems of species-specificity and far too incomplete to establish more than a speculative outline of the phylogenetic relationships of certain species in general terms of the clotting mechanism. An exception is the relatively advanced chemical analysis of fibrinogen and fibrinopeptides from different vertebrates (Laki and Gladner, 1964; Doolittle and Blombäck, 1964), which together with studies on the thrombin-fibrinogen reaction have already yielded information on homologies of peptide chain structure from animals with an established evolutionary relationship.

Doolittle and Surgenor (1962) studied coagulation in fish including the most primitive group, the cyclestomes. They found the process similar to that in mammals and notably, two pathways of prothrombin activation. The main difference was in the intrinsic system where thrombocytes assumed the central role, and the function of plasma clotting factors remained obscure. Doolittle (1963) further examined coagulation in the dog fish which appeared to have a haemorrhagic state by some mammalian standards. This proved to be the result of extreme reactions under certain experimental conditions, and Doolittle again found a general similarity with the mechanism in higher orders. An ancillary process was described in fresh-water fish by Wolf (1959). On contact with water, red cells underwent hydrophilic swelling to form a solid mass.

Various plasma factors resembling those of mammals were found in different amphibia (Hawkey, 1960; Hackett and LePage, 1961; Fantl, 1961b), but opinions differed on their identity. Thrombocytes appeared to be the major element in coagulation. The clotting mechanism of toads who live in a hot (Hackett and LePage, 1961) or cold (Anstall and Huntsman, 1960) environment appeared remarkably adapted to the relevant temperature range. Other instances of adaptation involve hibernating or aestivating animals who

develop such degrees of low heart rate, increased blood viscosity, and circulatory stasis that intravascular thrombosis would seem inevitable without some compensatory mechanism. The solution appears to be increased anticoagulant activity in the plasma of hibernating bats (Smith, Lewis, and Svihla, 1954) and hedgehogs (Björck, Johansson, and Nilsson, 1962) and also in cold-induced torpor of turtles (Jacques, 1963). Hjort and Eliassen (1963) tentatively identified the anticoagulant in hibernating hedgehogs as heparin and noted that other complex changes in clotting also took place.

Contact factors appear to be deficient in the blood of certain birds (Soulier, Wartelle, and Ménaché, 1959; Didisheim, Hattori, and Lewis, 1959) and the snake (Fantl, 1961b). The intrinsic plasma system is relatively inactive in these species. Studies in mammals have so far been limited to common domestic and laboratory animals, and despite problems of species specificity, which confounds quantitation of all factors and qualitative identification of some (Didisheim *et al.*, 1959; Quick, Collins, Taketa, and Hussey, 1961; Caillard, Devant, and Klepping, 1962), there is general agreement that the over-all mechanism is similar to that of humans (Biggs and Macfarlane, 1962).

Genetical disorders of clotting in domestic animals have proved valuable in research as shown by previous references to the North Carolina colony of haemophilic dogs. The latter are Irish setters (Field, Rickard, and Hutt, 1946) and have factor VIII deficiency with clinical, genetical, and laboratory characteristics that appear identical to those of the human variety (Brinkhous, 1964). Sex-linked factor VIII deficiency has also been noted in beagles (Brock, Buckner, Hampton, Bird, and Wulz, 1963), greyhounds (Sharp and Dike, 1964), mongrel dogs (Didisheim and Bunting, 1964), and a Shetland sheepdog (Wurzel and Lawrence, 1961). Nossel, Archer, and MacFarlane (1962) described equine factor VIII deficiency. Studies of inherited haemorrhagic disease among domestic swine (Cornell and Muhrer, 1964; Muhrer, Lechler, Cornell, and Kirkland, 1965) indicated an autosomal recessive trait characterized by factor VIII deficiency and a prolonged bleeding time with results of plasma infusion experiments similar but not quite analogous to results obtained on humans with von Willebrand's disease. There are also reports of canine factor IX deficiency (Mustard, Rowsell, Robinson, Hoeksema, and Downie, 1960) and canine factor VII deficiency (Mustard, Secord, Hoeksema, Downie, and Rowsell, 1962). Both conditions had characteristics similar to those in the corresponding human varieties. A complex hereditary haemorrhagic disorder involving prothrombin and extrinsic system factors was studied among inbred mice by Meier, Allen, and Hoag (1962).

The identification of amino-terminal residues in fibrinogen from different vertebrates (Blombäck and Yamashina, 1958; Doolittle *et al.* 1962), together with studies of amino acid sequence of fibrinopeptides proteolytically removed from mammalian fibrinogen molecules by thrombin (Doolittle and Blombäck, 1964) have provided information that has considerable evolutionary implications. Differences in the rate of thrombin-fibrinogen reaction depend on the species from which the proteins originate. Bovine thrombin clots bovine fibrinogen more quickly than fibrinogen from other species (Laki and Gladner, 1964). Lamprey thrombin clots human fibrinogen very slowly, but human thrombin clots lamprey fibrinogen nearly as rapidly as does lamprey thrombin (Doolittle *et al.*, 1962). Laki and Gladner (1964) argue that changes in the primary structure of both thrombin and fibrinogen must have occurred to account for the latter observation. They point out that the principle of splitting acidic peptides from fibrinogen to ensure clot formation was operating at the lamprey (lowest vertebrate) stage of evolution.

Thrombin is not only a protein-specific enzyme but also bond-specific in that only arginine-glycine bonds are hydrolysed during the clotting of all vertebrate fibrinogens so far examined. As mentioned above, Laki and Gladner (1964) speculated on the possibility that congenital afibrinogenemia might result from a single amino acid substitution in the critical arginine-glycine sequence with production of a non-functioning protein. It is also possible that neighbouring sequences influence the rate of thrombin action as reflected in species-specific reactions.

Doolittle and Blombäck (1964) described varying amino acid sequences in fibrinopeptide chains in seven mammals. They discussed how sequence variation could be used to classify phylogenetic relationships, and showed how selection for certain amino acids during evolution would account for the observed alignment of sequences in different animals. A variant human fibrinopeptide chain A has been described (Blombäck, Blombäck, Doolittle, Hessel, and Edman, 1963b) which differs from the normal peptide A in that the N-terminal alanine is absent. No functional effect was apparent in the parent molecule. As the amino acid differences between species must have originated within a single species, such changes may not be too infrequent and some could conceivably disturb the fibrinogen-fibrin transition with haemorrhagic consequences.

Until the molecular mechanisms of enzyme action in the prothrombin activating pathways are understood it will not be possible to examine certain

problems of blood coagulation where the solution rests on tracing the structural history of a single protein molecule through evolutionary time. The problems include genetical defects involving combinations of clotting factors or complex haemostatic disorders. The elucidation of relationships between the coagulation mechanism and other physiological systems at a molecular level will undoubtedly prove a formidable task, as shown by the participation of factor XII in several different reactions (Fig. 18). Initial studies of functional (Marx and Appel, 1962) and biochemical (Mitchell and Sharp, 1964) properties of platelets in different animals indicate the presence of considerable interspecies variation in these cells.

FIG. 18. Initiation of clotting and release of peptides by activated factor XII. From Margolis (1963) by kind permission of Dr. I. Margolis.

Recent theories on human blood clotting have important teleological consequences which will remain of heuristic value until analysis of elementary units and component systems yields explanations in terms of molecular evolution. Macfarlane (1964a) visualized the sequential pro-enzyme-enzyme transformation (cascade) clotting mechanism as a biochemical device to ensure that the minute amount of factor XII activated by surface contact would finally cause the explosive conversion of a relatively large amount of fibrinogen into fibrin.

Subsequently Macfarlane (1965) devised experiments to support the hypothesis that additional stages in the mammalian system would lead to greater haemostatic efficiency by comparison with simple mechanisms in lower animals. He showed that if trypsin activated factor X which in turn activated prothrombin, ten times more thrombin was produced than when trypsin reacted with prothrombin directly. Macfarlane suggested that a trypsin-like enzyme derived from damaged tissue might act on different substrates, including prothrombin, in primitive clotting systems. If mutation resulted in a substrate that yielded a prothrombin activator, the modified system would be more efficient and have a

TABLE X
SUMMARY OF GENETICAL FEATURES IN HEREDITARY DISORDERS OF BLOOD COAGULATION

Clotting Factor	Hereditary Defect		Inheritance*	Typical Haemorrhagic Variation of Phenotype†	Major Phenocopies‡	Gene Effect in Heterozygotes §	Provisional Gene Frequency (q) and Mutation Rate Estimations (μ per X chromosome per generation)	Other
	Isolated	Combined						
Fibrinogen	Afibrinogen-aemia		(Incomplete) autosomal recessive	Severe-moderate	Hepatic disease; malabsorption; defibrination syndromes	Partial effect in some; no effect in others	(q = 10 ⁻³)	Ill-defined trait
	Hypo-fibrinogen-aemia		(Autosomal)	Mild	As above			
Prothrombin	Hypoprothrombin-aemia		(Autosomal recessive)	Severe-mild	Hepatic disease; malabsorption; coumarin, indanedione anticoagulant drugs	—	—	—
Factor V	Factor V deficiency		Incomplete autosomal recessive	Moderate-mild	Hepatic disease; defibrination syndromes	Partial effect	q = 5-10 × 10 ⁻⁴	
		V + VIII deficiency	(Autosomal recessive)	Moderate-mild	—	Nil	—	
Factor VII	Factor VII deficiency	VII + X	Incomplete autosomal recessive (Autosomal dominant)	Severe-nil Mild	As for prothrombin —	Partial effect —	q = 10 ⁻³ - 10 ⁻⁴ —	Unique mutation
Factor VIII	Factor VIII deficiency		Incomplete sex-linked recessive	Severe-mild	—	Full (haem), partial or no effect	q = 1-1.7 × 10 ^{-4**} μ = 3-4 × 10 ⁻⁶	Linked with deuteranopia, not linked with Xg locus High prevalence on Åland archipelago Unique mutation
		Von Willebrand's disease	Autosomal dominant	Severe-nil		Marked variation (haem-nil)		
	Autosomal dominant factor VIII deficiency (Loeilger)		Autosomal dominant	Moderate	—	Full (haem)	—	
Factor IX	Factor IX deficiency		Incomplete sex-linked recessive	Severe-mild	As for prothrombin	Full (haem), partial or no effect	q = 4-5 × 10 ^{-5**} μ = 2-3 × 10 ⁻⁶	Not linked with partial colour blindness, factor VIII, or Xg loci
Factor X	Factor X deficiency		Incomplete autosomal recessive	Severe-mild	As for prothrombin	Partial effect (? haem)	q = 5-10 × 10 ⁻⁴	
Factor XI	Factor XI		Incomplete autosomal recessive	Mild	—	Partial effect	—	Most cases reported in Jews
Factor XII	Factor XII deficiency		Incomplete autosomal recessive	Nil	—	(Partial effect)	—	
Factor XIII	Factor XIII deficiency		(Incomplete) autosomal recessive	Moderate-mild		(Partial effect)	—	

* Provisional assumptions are in parentheses.

† Categories of severity defined in text.

‡ Excludes idiopathic factor inhibitors.

§ Haem = haemorrhagic symptoms

** Frequencies calculated at birth (see text).

selective advantage. In view of the similar properties in two groups of clotting factors, Macfarlane postulated that factors IX, X, VII, and prothrombin may have evolved from a basic prothrombin molecule, and factors V, VIII, fibrinogen in like manner from a prototype fibrinogen molecule.

Prospects for the Future

Further progress with blood clotting obviously depends on advances at the molecular level. An important first step has been the replacement of vague and confusing theories by schemes based on a series of enzymatic reactions. The latter provide a rational basis for enzyme or physical chemists and others whose techniques will ultimately yield fundamental information on properties and reactions.

The limits of present methods have not been fully explored, particularly in the present context of defining gene effect and the quantitative distribution of clotting factors in the normal population. The study of quantitative variation would be greatly assisted by agreement on standard laboratory systems for comparative purposes. Utilizing current methods, the re-examination of clotting in invertebrates and certain animals would provide valuable data on reactions at fluid-solid interphases and the increasing dependence on plasma proteins in mammalian blood coagulation. The comparative study of cellular participation in different species may also throw light on the role of platelets in mammalian haemostasis, though any assumptions in terms of chemical evolution that are made at a non-molecular level must naturally remain tentative.

The aims of blood coagulation research have always been directed towards solution of practical problems associated with bleeding and intravascular clotting. With a few exceptions the study of hereditary mechanisms has been of secondary interest as shown by the inconsistently reported data on specific mutations. The state of present knowledge is summarized in Table X. Nevertheless, some genetical hypotheses deserve equal rank alongside theories with a teleological basis. For instance, if the assumptions of the inactive-X chromosome theory apply to the factor VIII deficiency locus, then a proportion of heterozygous females will have plasma levels within the range for normal homozygous females. Therefore claims that complete separation of genotypes can be made may be misjudged, particularly if little attention has been paid to the errors associated with factor assay. Attempts to devise more 'sensitive' (and hence more complicated and artificial) *in vitro*

systems to distinguish normal from abnormal would likewise be misguided on the inactive-X theory. In addition, the multi-factorial character of clotting factor distribution in normal persons emphasises the extent of data required if assumptions are to be made on all but gross examples of gene effect.

A population study of hereditary clotting defects that conforms to conditions suitable for formal analysis of relevant genetical parameters has yet to be undertaken. Recent Scandinavian surveys are greatly superior to the older and still extensively quoted studies, but retrospective investigations are prone to many well-known biases and difficulties and cannot account for mild phenotypes which are generally detected only in the laboratory. Relatively simple methods for screening coagulation defects are now available, though their performance on a large population would still present formidable practical problems. The search for variant fibrinogen in essentially normal subjects has been started, and there are obvious possibilities with coumarin resistance, but present techniques are quite unsuited for investigating the existence of potential polymorphisms among other clotting factors.

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