DISORDERS OF HAEMOSTATIC FUNCTION

by

A. S. DOUGLAS

CONTENTS

Part IV. The Diagnosis of Haemorrhagic Disease

Part V. Platelet-Vascular Disorders

Part VI. Disorders of Coagulation

PREFACE

The work described in this thesis was carried out over a seven year period from 1949 to 1956. It was started in 1949 in the University Department of Medicine, Royal Infirmary, Glasgow, and continued there until 1951, during which time I was registrar to Professor L.J. Davis. From 1951 to 1953 the studies were continued during the tenure of a Medical Research Council Research Fellowship, in the Department of Clinical Pathology, Radcliffe Infirmary, Oxford, under Dr. R. G. Macfarlane, and later in the Department of Haematology, Postgraduate Medical School, London, under Dr. J. V. Dacie. From 1953 to 1956 the research was again carried out in the University Department of Medicine, Royal Infirmary, Glasgow, at which time I was a lecturer in medicine. My interest in these problems was encouraged and directed by Professor L. J. Davis and Dr. Alexander Brown in Glasgow, by Dr. R. G. Macfarlane and Dr. Rosemary Biggs in Oxford, and by Dr. J. V. Dacie in London. It is a particular pleasure to acknowledge my gratitude to all of these.

In addition to the facilities used by virtue of the appointments mentioned above, during 1953 to 1956 the work was, in part, financed by grants to the department, from

the Advisory Committee on Medical Research for Scotland and from the Medical Research Council.

The work done during these years can be considered to fall into two main categories. The first has been concerned with the physiology of blood coagulation, in particular the details of the interactions involved in thromboplastin formation and prothrombin conversion. The second has been concerned with the application of these advances, to problems of blood coagulation in clinical medicine. This thesis deals with these latter aspects.

Some of the work described in this thesis has been published, but a large part is as yet unpublished. In certain of the published papers I am the sole author while in others I am co-author. Where the work was carried out jointly I have indicated in the text the aspects for which I am responsible.

The following is an outline of the plan of the thesis. The first three chapters (1, 2, 3) -the history of blood coagulation, the thrombin-fibrinogen reaction and the dissolution of the classical theory, are introductory. The succeeding three chapters (4, 5, 6) deal with blood thromboplastin. Chapter (4) deals very briefly with the advances in our understanding of blood thromboplastin.

This describes, in outline only, the work published jointly with Dr. Rosemary Biggs and Dr. R. G. Macfarlane (J. Physiol.1953, 119,89; J. Physiol. 1953, 122,538; J. Physiol. 1953, 122, 554). The fifth chapter deals with my share in the discovery of Christmas disease. This was published jointly with Dr. Rosemary Biggs,Dr. R.G.Macfarlane, Dr. J. V. Dacie, Dr. W.R. Pitney, Dr. C. Merskey, and Dr. J.R. O'Brien. (Brit. med. J. 1952, 2, 1378). Chapter (6) describes an atypical constitutional coagulation defect. The next two chapters describe investigations into the mode of action of anticoagulant drugs - heparin and the coumarin drugs. This work has, in part, been published. (Brit. med. Bull. 1955, 11, 39; Clin.Sci.1955, 14, 601; J. clin. Invest. 1956, 35, 533). Chapter (9) describes the diagnostic approach to haemorrhagic disorders. This also has been partly published. (Glasg. med. J. 1955, 36, 238 Included in this chapter is a description of the thromboplastin generation test published jointly with Dr. Rosemary Biggs. (J. clin. Path.1953, 6, 23). The next two chapters deal with platelet disorders and vascular defects. (unpublished work). In chapter (12) is given a general account of disorders of blood coagulation, which arise as a result of deficiencies of coagulation factors. Some of the rarer

conditions are described in detail in this chapter, the others in the succeeding chapters. The case of prothrombin deficiency mentioned in this chapter has been reported jointly with Dr. Rosemary Biggs (J. clin. Path. 1953, 6, 15). The other material is unpublished. Chapter (13) describes investigations on "hypoprothrombinaemias" except for the coumarin defect, described earlier. The part of this work on newborn infants has been published (Arch. Dis. Childh. 1955,30,509), the remainder is unpublished. The next chapter (14) describes the coagulation disturbance in liver disease and the chapter thereafter (15) gives an account of haemophilia and Christmas disease in the West of Scotland. These studies are unpublished. Chapter (16) consists of a report on the neurological complications which have been seen in haemophilia and Christmas disease. This has recently been published jointly with Dr. S.G. McAlpine (Scot. med. J. 1956, 1, 270). Chapter (17) describes female relatives of haemophiliacs with a coagulation disturbance, due to antihaemophilic globulin deficiency. These females were thought to be heterozygous for the defect. This is unpublished. Chapter (18) describes a patient with a profound coagulation disturbance in

association with hyperglobulinaemia. This also is unpublished. Circulating thromboplastin inhibitors are described in Chapter (19). An unpublished case, with an atypical circulating anticoagulant, is described. This thromboplastin inhibitor has properties different from those of any previously reported case. The concluding five chapters deal with therapeutic aspects of haemorrhagic disorders - dental extraction in haemophilia, and Christmas disease (20) (unpublished); "in vitro" survival of coagulation components in bank blood and fresh frozen plasma (21) (unpublished); "in vivo" survival of antihaemophilic globulin and Christmas factor (22) (unpublished); effect of vitamin K preparations on "hypoprothrombinaemia" induced by dicumarol and tromexan (23) (published jointly with Dr. Alexander Brown - Brit. med. J. 1952, 1, 512); action of vitamin K_1 (24) (unpublished).

Occasionally patients, or specimens from patients, have been referred for opinion. These patients have sometimes been reported in the literature by those physicians or pathologists referring the patients. Where this is so, it has been indicated in the text.

The work covers investigations into haemostatic disorders as encountered in clinical practice. Where my studies have contributed little which is original, the subject, despite its possible importance, may have been dealt with very briefly. As a consequence some rare haemorrhagic diseases have been reported in greater detail than others which are common. Thrombocytopenia, for instance, is probably the most common of the acquired haemorrhagic diseases. It has been described relatively briefly, in comparison, for example, with the account of circulating thromboplastin inhibitors - a rare condition. The reason for this is that my investigations into thrombocytopenia have contributed little which is original.

The investigations reported in the thesis are not described in the chronological order in which the work was done. The second last chapter, for example, represents the first piece of experimental work in this field. The order followed has been in accordance with the plan outlined above.

This type of investigational work has difficulties common to other forms of biological research. The results are frequently comparable only within the individual experiment carried out over a short time

interval. The activity of the solutions being tested changes rapidly. The results of to-day's experiment are not necessarily comparable with those of to-morrow's. Throughout the work only human blood has been used. This has obviated the difficulty of comparison with experiments on animals.

LIST OF AUTHOR'S PUBLISHED WORK ON BLOOD COAGULATION AND HAEMORRHAGIC DISORDERS

(1) Effect of vitamin K preparations on hypoprothrombinaemia induced by dicumarol and tromexan.
A. S. Douglas and A. Brown. Brit. med. J. 1952, 1, 412.

(2) Anticoagulants and anticoagulant therapy: a review.
A. Brown and A. S. Douglas. Glasg. med. J. 1952, 33, 225.

(3) Christmas disease.
R. Biggs, A. S. Douglas, R.G. Macfarlane, J.V. Dacie, W. R. Pitney, C. Merskey, J.R. O'Brien. Brit. med. J. 1952, ii, 1378.

(4) The formation of thromboplastin in human blood.
R. Biggs, A.S. Douglas, R.G. Macfarlane. J. Physiol. 1953, 119, 89.

(5) The initial stages of blood coagulation.
R. Biggs, A.S. Douglas, R.G. Macfarlane. J. Physiol. 1953, 122, 538.

(6) The action of thromboplastic substances.
R. Biggs, A.S. Douglas, R.G. Macfarlane. J. Physiol.1953, 122, 554.

(7) The consumption of some components involved in physiological blood coagulation.
A.S. Douglas and R. Biggs. Glasg. med. J. 1953,34,329.

(8) The measurement of prothrombin in plasma.
R. Biggs and A. S. Douglas. J. clin. Path. 1953, 6, 15.

(9) The thromboplastin generation test.
R. Biggs and A. S. Douglas. J. clin. Path. 1953, 6, 23.

(10) Christmas disease.
A. S. Douglas. Med. Pr. 1954, 70.

(11) Mode of action of coumarin drugs.
A. S. Douglas. Brit. med. Bull. 1955, 11, 39.

(12) The coagulation defect caused by tromexan therapy.
A. S. Douglas. Clin. Sci. 1955, 14, 601.

(13) Hypoprothrombinaemia in the newborn.
A. S. Douglas and P. Davies.
Arch. Dis. Childh. 1955, 30, 509.

(14) Modern concepts of blood coagulation and its disorders.
A. S. Douglas. Glasg. med. J. 1955, 36,238.

(15) Factor V consumption during blood coagulation.
A. S. Douglas. Brit. J. Haemat. 1956, 2, 153.

(16) Antihaemophilic globulin consumption during blood coagulation.
A. S. Douglas. Blood, 1956, 11, 423.

(17) The action of heparin in the prevention of prothrombin conversion.
A. S. Douglas. J. clin. Invest, 1956, 35, 533.

(18) Neurological complications of haemophilia and Christmas disease.
A. S. Douglas, S. G. McAlpine. Scot. med. J.,1956, 1, 270.

LIST OF ABBREVIATIONS

Throughout the thesis certain abbreviations have been used. This was required particularly in the construction of many of the tables in the appendix of techniques and results.

Frequently the symbols for seconds and minutes have been omitted. In the thromboplastin generation technique the clotting time of the high-spun normal plasma substrate is in seconds. The clotting time of fibrinogen substrates is also recorded in seconds, except where the time was over 180 seconds (3 minutes) when it is recorded as 3+. A clotting time over 3 minutes represents a negligible amount of thrombin. One-stage clotting times are recorded in seconds and whole blood clotting times in minutes.

Volumes are in ml. unless otherwise stated.

Abbreviations

ad.	= adsorbed
ads.	= adsorbed
A.H.G.	= antihaemophilic globulin
al.	= alumina
alum.	= alumina
alumina plasma adsorbed plasma $Al(OH)_3$ plasma	= plasma treated with aluminium hydroxide.

A.R. = analar reagent

brain = brain thromboplastin

C.F. = Christmas factor

C = control

con. = control

cons. = consumption

consump. = consumption

chemicals - these are frequently abbreviated to their formulae, e.g., $CaCl_2$ = calcium chloride.

dilut. = diluted, dilution

Drugs

P.A.S. = para amino salicylic acid

I.N.H. = isoniazid hydrochloride

N.A.B. = neoarsphenamine

B_{12} = vitamin B_{12}

B.D.H. = British Drug Houses

V = factor V

VII = factor VII

glob. = globulin

H.S. = high spun

Hb = haemoglobin

haem. = haemophilic

haemoph. = haemophilic

incub. = incubate

K_1 = vitamin K_1

L.S.	= low spun
M.S.E.	= Measuring & Scientific Equipment
N	= normal
nor.	= normal
Owren %	= referable to Owren's technique
pro.	= prothrombin
plates.	= platelets
P.C.V.	= packed cell volume
P.D.	= prothrombin deficient
P.D.P.	= prothrombin deficient patient
pat.	= patient
P.T.A.	= plasma thromboplastin antecedent
P.T.C.	= plasma thromboplastin component
plas.	= plasma
Q	= Quick
Q %	= percentage on one-stage clotting time test of Quick and related saline dilution curve.
Quick	= refers to one-stage clotting time
R.B.C.	= red cell count
R.V.V. + Lec.	= Russell's viper venom plus lecithin.
Tro.	= Tromexan
T.K.	= thrombokinase thromboplastin
Xmas	= Christmas
µg.	= microgrammes

Part I. Introduction

CHAPTER I

HISTORY OF BLOOD COAGULATION

CONTENTS.

The eras of blood coagulation research.

Pre-Classical Era	Prior to 1904.
Era of the Classical Theory	1904-1943
Post-Classical Era	1943-

Pre-Classical Era (Prior to 1904)

Initiation of blood coagulation.

Thrombin.

Fibrinogen.

Prothrombin.

Tissue extracts.

Calcium.

Classical Era (1904-1943)

The Classical Theory.

Adsorption of prothrombin or inorganic precipitates.

Extrinsic or tissue thromboplastin.

Intrinsic or blood thromboplastin.
(1) Defective prothrombin consumption.
(2) Platelets.
(3) Antihaemophilic globulin.

Quick's one-stage "prothrombin" test.

Post-Classical Era (1943-1956).

Re-assessment of the one-stage "prothrombin" test.

Alternative theories of blood coagulation.

Inhibitory mechanisms.

(1) antithrombin.

(2) antithromboplastin.

Heparin

Fibrinolysis.

In 1935 W. H. Howell wrote "Clotting is the most striking and best known property of blood. It is indeed a remarkable phenomenon. One can realise that nature had a difficult problem to solve in creating a circulating medium for the animal body that would remain entirely liquid while in the blood vessels, but would promptly set into a gel as soon as the vessels were wounded and the blood was in danger of being lost." Very many workers have attempted to unravel the chemical and physical mechanisms responsible for this sudden change of condition from a sol to a gel at the moment of coagulation. There have been many theories postulated to explain the changes resulting in clot formation; these cross and overlap, and abound with a terminology peculiar to any one group of workers in the field, rendering it difficult for those who have not made a special study of the problem to disentangle the same phenomena with different designations.

The eras of blood coagulation research.

The history of the development of our knowledge of blood clotting can usefully be divided into three eras by the so-called Classical Theory of Morawitz. This theory was propounded by Morawitz in 1904 and held as a reasonable scheme to explain the relevant experimental phenomena until 1943. The history of blood clotting can be considered therefore under the following three headings:-

(a) Before the Classical Theory (Pre-Classical Era) — Prior to 1904

(b) The era of the Classical Theory (Classical Era) — 1904-1943

(c) After the Classical Theory (Post-Classical Era) — 1943-

Pre-Classical Era (Prior to 1904)

It is generally believed that Aristotle was one of the first to ascribe the coagulation of blood to the presence of fibrous material. Little further attention was given to the problems of blood coagulation until the seventeenth century when Willis (1659) recognized that serum could be separated from the fibrous clot and Malpighi (1666) described the thread-like nature of the structure of the clot which was called fibrin. Ruysch (1707) produced serum from whole blood by agitation with twigs to which the fibrin adhered. The earliest experimental work of real importance was that of Hewson (1771). Hewson's discoveries can be enumerated as follows:-

(1) He was the first to appreciate that the non-cellular elements of the blood played a major role in coagulation. Using blood with a rapid red cell sedimentation rate, he skimmed off the plasma before clotting had occurred and demonstrated that the clear plasma coagulated. He made a similar observation by ligating a vein and entrapping blood between his ligatures; when the cellular elements had settled he drew off the plasma which then clotted.

(2) He demonstrated that vascular endothilium was remarkable in being able to maintain blood in a fluid state.

(3) He developed the first methods for keeping blood fluid outside the body. He showed that coagulation may be inhibited by cold or by the addition of neutral salts. The salt used was sodium sulphate.

(4) He demonstrated that the coagulation of blood drawn from the body was not due to cooling, cessation of movement or exposure to air.

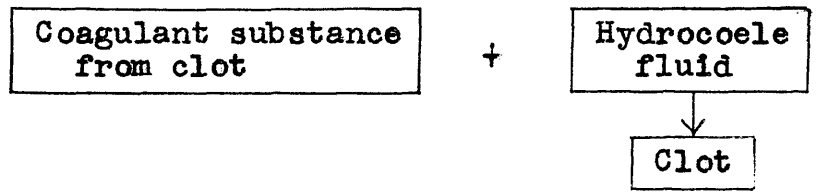
Hewson's observations were forgotten until attention was drawn to them later by Davy (1839) and Gulliver (1846).

Initiation of coagulation.

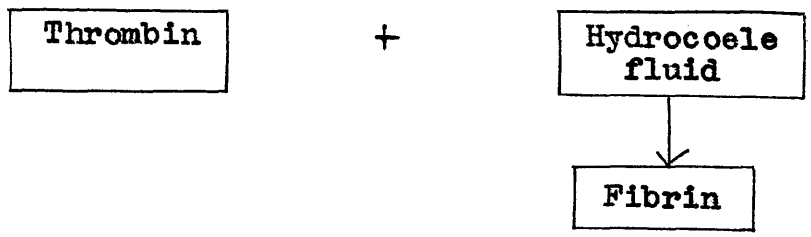
Hewson demonstrated that the removal of blood from the vascular system caused coagulation to occur. This phenomenon was investigated by Richardson (1858) who postulated on the results of his experiments that there was an ammonia compound present in the body which kept the blood fluid, and that on its escape the blood coagulated. He believed that haemorrhagic disease was due to the accumulation of this ammonia compound. On the basis of this hypothesis he used ammonia therapy for the management of intravascular thrombus formation. Lister (1858, 1863) disproved the ammonia theory and concluded rightly, that contact with a foreign surface was the mechanism which initiated coagulation.

Thrombin: The first of the factors concerned in blood coagulation was discovered as a consequence of the work of Dr. Andrew Buchanan (1798-1882). Buchanan was the first

Professor of Physiology in the University of Glasgow. Buchanan (1835, 1845) discovered that the washings from freshly formed clot would coagulate hydrocoele or ascitic fluid. Freshly formed serum also possessed this property. He believed that the active substance in the washing from the clot acted upon a precursor of the fibrin and resulted in coagulation. Buchanan's observations could be written



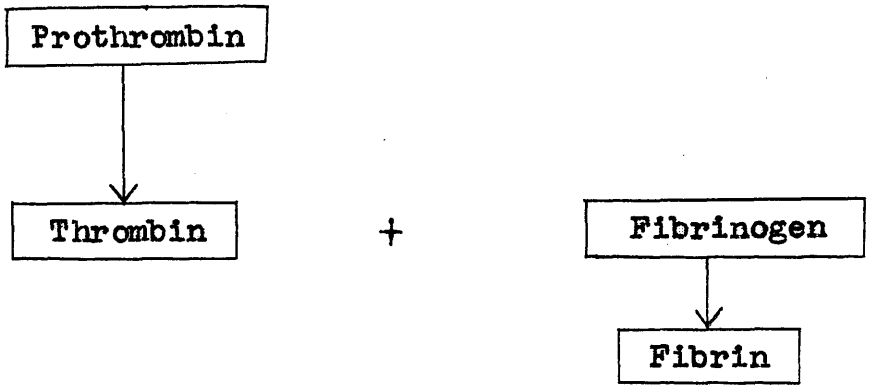
Alexander Schmidt of Dorpat in Germany, unaware of Buchanan's investigations, independently made similar observations in 1861. He used the term "fibrin ferment" or "thrombin" to describe the coagulant activity contained in the fresh serum or in the washings from fresh clot. (Schmidt 1861, 1862, 1893).



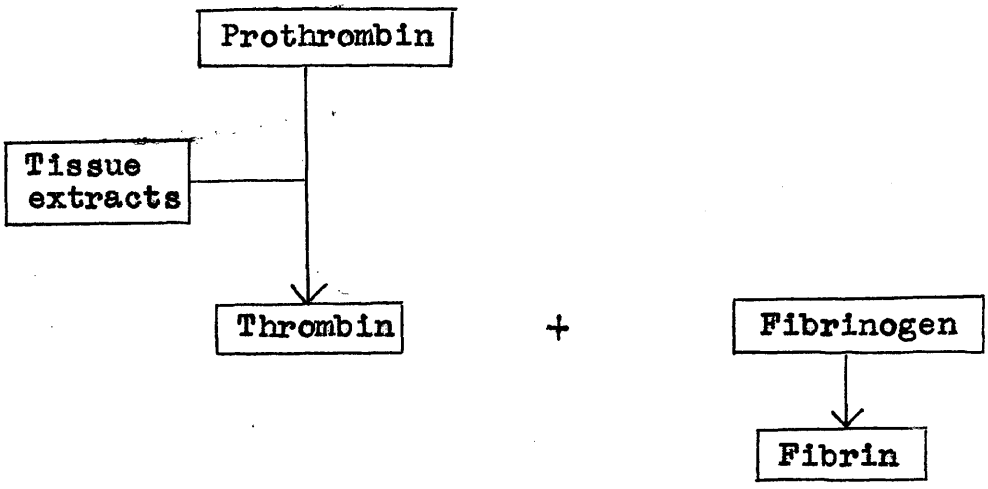
The term thrombin has secured a permanent place in coagulation nomenclature.

Fibrinogen: Dennis (1887) prepared, by saturation of plasma with sodium chloride, a precipitate which when redissolved underwent spontaneous coagulation, indicating that there was a soluble precursor of fibrin in the plasma. This precursor of fibrin was called fibrinogen. Hammarsten (1879) made an important contribution by studying the preparation of fibrinogen. His method consisted of fractionation by the addition of sodium chloride and freeing this component from other plasma proteins by repeated precipitations. Schmidt demonstrated that solutions of fibrinogen could be coagulated by the action of thrombin.

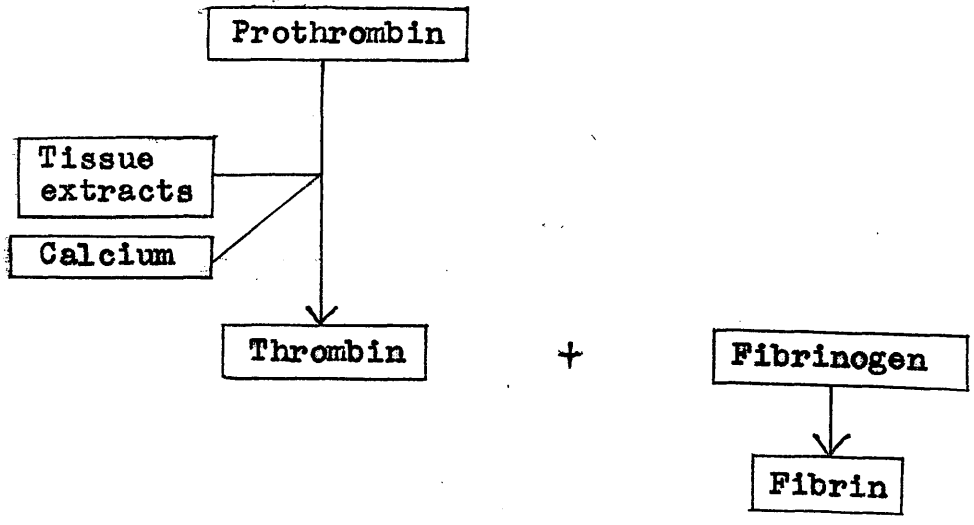
Prothrombin: Schmidt made a preparation of thrombin from fresh serum by alcohol precipitation, but the application of the same technique to plasma failed to produce any thrombin. He demonstrated that thrombin was not present in the circulating blood and postulated that it was formed from an inactive precursor by the shedding of blood. This precursor of thrombin was called prothrombin by Pekelharing (Quick 1951).



Tissue extracts: Buchanan demonstrated that the freshly formed blood clot contained an agent capable of coagulating hydrocoele fluid; he believed that the white corpuscles of the clot were the active component and went on to demonstrate that various tissues had coagulant properties. It is now realised that the action of the fresh clot is due to thrombin, which is not derived from white cells, but the observation of the coagulant action of tissue was one of importance. When blood was collected and part delivered to a tube containing tissue extract and part to a clean tube, the blood in the tissue extract tube was observed to coagulate in a few seconds whereas in the clean tube it took several minutes to coagulate. Tissue extracts, therefore, were found to have marked coagulant property which differed from the thrombin effect in that they were unable to clot fibrinogen. Tissue extracts were believed to accelerate the conversion of prothrombin to thrombin.



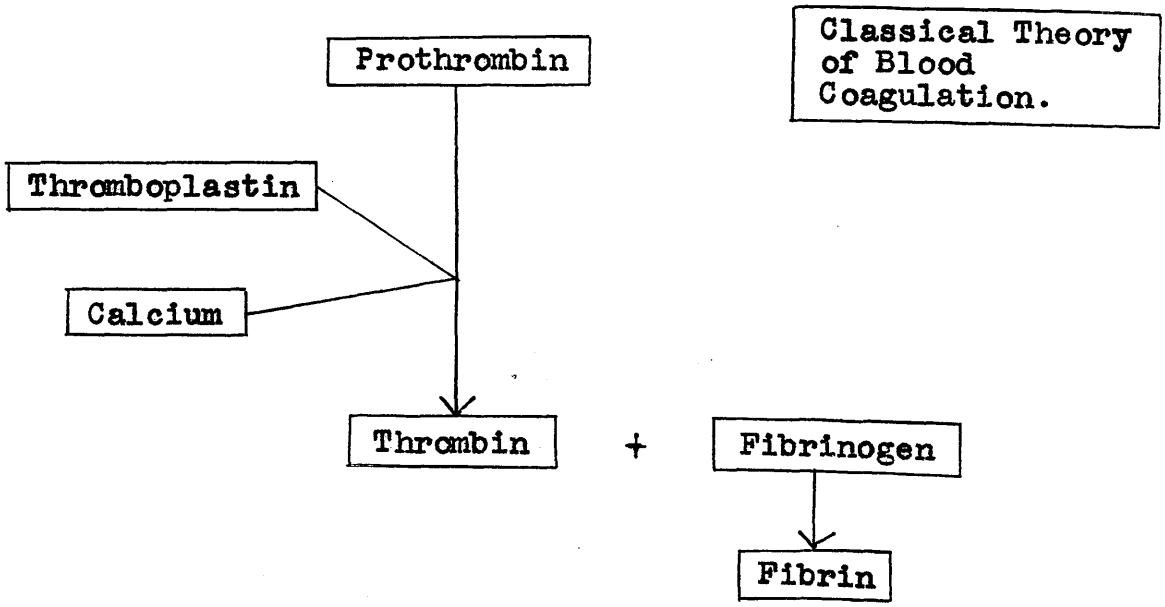
Calcium: In 1890 Arthur and Pagès observed that calcium precipitants inhibited coagulation, an effect which could be reversed by the readdition of calcium. Calcium was not required in the thrombin-fibrinogen reaction and it was concluded that it was needed in the reactions preceding thrombin formation.



Classical Era (1904-1943)

The Classical Theory.

In 1904 Morawitz collected the information obtained from the experiments outlined above and postulated his theory, which has become known as the Classical Theory. This stood unchallenged for almost forty years. Morawitz's theory states that prothrombin is converted to thrombin under the influence of thromboplastin and calcium. The thrombin then interacts with fibrinogen to produce fibrin. At the time of introduction of his theory Morawitz used the term thromboplastin in reference to tissue extracts believing that this was the only requirement together with calcium needed for the conversion of prothrombin to thrombin. We realise now that this interpretation of thromboplastic activity is too narrow, but allowing for this Morawitz theory is still valid.



Morawitz appreciated that in the circulating blood prothrombin, calcium and fibrinogen were present and he suggested that the maintenance of the normal intravascular fluid state of the blood was due to the absence of active thromboplastic activity. It was believed that on injury thromboplastic activity was derived from the wounded tissue or from the disintegration of platelets.

Research during the Classical Era was mainly devoted to the development of our knowledge of the components of the Classical Theory.

Prothrombin: It was not until many years after Morawitz postulated his Classical Theory that prothrombin was accepted as a real entity in the coagulation system; it was a hypothetical substance when first proposed. There have been many hypothetical components of the coagulation system which have not withstood the advance of sound experimental observation, but prothrombin has been generally accepted. The best early observations on prothrombin were made by Bordet and Delange (1914). They added to plasma an insoluble inorganic precipitate (calcium phosphate), which was subsequently removed by centrifugation; the supernatant failed to clot on the addition of tissue extract and calcium. They were able to elute from the calcium phosphate a substance, which when added to the supernatant would restore normal coagulation.

This provided reasonable proof of the existence of prothrombin.

Plasma Fractionation by Adsorption on Inorganic Precipitates.

The work of Bordet and Delange introduced important techniques to laboratory research on blood coagulation. The addition of calcium phosphate to plasma was used by them as a method of fractionation by adsorption of a specific coagulation component on the inorganic precipitate. This technique has been used extensively during the work described in this thesis, the adsorbing inorganic precipitate being aluminium hydroxide (alumina). Certain coagulation factors are adsorbed on the inorganic precipitate and removed by centrifugation while others are left in the supernatant. The experiments of Bordet and Delange also introduced an important principle to blood coagulation research in that clotting can be prevented by the removal of a factor and the defect corrected by its restoration. Later workers (Nolf 1945, Owren 1947, Fantl and Nance 1948, Ware and Seegers 1948) used the property of adsorption of prothrombin upon inorganic precipitates as the basis for methods of preparation of prothrombin.

Thromboplastin: The term thromboplastin in the Classical Theory was used by Morawitz in particular reference to the coagulant activity of tissue extracts. It is now

appreciated that such tissue extracts form only a part of the mechanism involved in the conversion of prothrombin to thrombin. The development of thromboplastic activity in which tissue is an essential component is referred to, throughout this thesis, as extrinsic thromboplastin. During the era of the Classical Theory only very limited attention was paid to the blood's own thromboplastin mechanism (intrinsic thromboplastin), though it was appreciated that such a mechanism probably did exist. Blood collected without tissue contamination clots under the influence of this intrinsic thromboplastin system.

Extrinsic Thromboplastin: As mentioned above, blood, which is collected by venepuncture and in part delivered to a clean tube, and in part to a tube containing tissue extract, takes several minutes to coagulate in the clean tube, whereas it requires only a few seconds in the tissue extract tube. This accelerating effect of tissue on coagulation is due to the extrinsic thromboplastin system.

The chemical nature of this tissue factor has been investigated. There is some measure of agreement that a lipoid is at least a part of the reacting principle. Some have said that this is a lecithin, others that it is a cephalin. It is probable that thromboplastic activity is enzymatic in nature accelerating thrombin formation from

prothrombin but not affecting the eventual amount formed. Proteolytic enzymes have been shown to form thrombin from prothrombin. Macfarlane and Pilling (1946) and Macfarlane (1947) demonstrated that soya bean trypsin inhibitor, which is antiproteolytic, inhibits the action of thromboplastin.

Intrinsic Thromboplastin.

Thrombocytopenia and Platelets.

Morawitz believed that there was thromboplastic activity in platelets and that the coagulation of blood in contact with wound surfaces was the consequence not only of the thromboplastic action of tissue but also of platelets. He believed that this was released from platelets when they came in contact with the foreign surfaces of the wound. Various contemporary workers had postulated that platelets were concerned with coagulation. Hayem (1877), Bizzozero (1882) and Burker (1904) all believed that platelets had some role to play in coagulation. Delezenne (1897) demonstrated that the removal of thrombocytes from goose blood by centrifugation made it incoagulable. There were some who believed that platelets provided prothrombin but this was disproved by the experiments of Bordet and Delange (1912), Eagle (1925), Mills (1927) and Ferguson (1936).

In 1911 Bordet and Delange made important observations on the action of platelets; they showed that very little

thrombin is formed in serum from plasma freed from platelets and that the serum contained much prothrombin. After clotting of normal blood serum contained only traces of prothrombin; prothrombin consumption therefore was defective where there was a deficiency of platelets (Bordet and Delange 1912). These observations were of fundamental importance; they were the first observations of importance into the blood's own thromboplastin (intrinsic thromboplastin). Prothrombin conversion to thrombin is dependent on the presence of thromboplastin. When the thromboplastic activity of the blood is normal, the prothrombin is converted completely to thrombin by the end of one hour after withdrawal of the blood, if it is maintained at 37° C. Where there is considerable prothrombin left after one hour at 37° C. there is defective development of thromboplastic activity. This is the basis of the well-known procedure called the prothrombin consumption test. Defective prothrombin consumption is a non-specific indication of failure of intrinsic thromboplastin. The demonstration by Bordet and Delange of defective prothrombin utilisation in thrombocytopenia provided good evidence that platelets were required for intrinsic thromboplastin formation.

Haemophilia and anti-haemophilic globulin. Hopff introduced the term haemophilia in 1837. Ancell in 1839-1840

described it as an hereditary condition, affecting the males of a family but capable of transmission by the female. Wardrop (1835) considered that the disease was due to a deficiency of the coagulating powers of the blood. Liston (1838-1839) demonstrated that there was deficient fibrin formation in the blood. Ancell (1839-1840) recognized the beneficial effects of blood transfusion in the condition. Weil (1906) was the first to recognise that the addition of a small quantity of normal plasma to the haemophilic plasma corrected this defect. Addis (1911) demonstrated in vitro that 20 per cent of normal plasma restored the clotting time of haemophilic plasma. He then showed that a fraction of normal plasma, prepared by acid precipitation of the globulins with subsequent removal of fibrinogen, was able to correct the haemophilic defect. It could have been deduced from this experiment that a clotting factor lacking in haemophilia was present in the globulin fraction of normal plasma. To Fursky in 1924 goes the credit for demonstrating that normal plasma deprived of platelets was as good as whole blood in shortening the clotting time of haemophilic blood. This confirmed that the defect in haemophilia was related to the plasma rather than the platelets. Pohle and Taylor (1937) produced a concentrated "globulin material" which reduced the coagulation time in haemophilia.

Brinkhous (1939) demonstrated defective prothrombin consumption in haemophilia. This was valuable evidence that the defect in haemophilia was an inability to form intrinsic thromboplastin. Since the missing plasma component is a globulin, it has been called anti-haemophilic globulin (A.H.G.). It was believed in view of the defective prothrombin consumption that A.H.G. was needed for blood thromboplastin formation.

As thrombocytopenia and haemophilia both had defects characterised by inability to convert prothrombin to thrombin it was appreciated that platelets and antihaemophilic globulin (A.H.G.) were likely to be essential components of blood thromboplastin. Platelets can be prepared by the differential centrifugation of plasma and plasma fractions prepared containing A.H.G. When these were incubated together, however, no appreciable thromboplastin activity was developed. This system was recognized therefore to be incomplete. The work described in this thesis is concerned in large part with the application to clinical medicine of those aspects of blood coagulation relating to the recent developments of our knowledge of intrinsic thromboplastin.

Quick's one-stage "prothrombin" test.

In 1935 Quick introduced his well known and valuable test. This test was based on the Classical Theory and was

theoretically sound at the time of its introduction. The principle of the test was that the addition to plasma of an excess of tissue extract and calcium would leave only one limiting factor, prothrombin which could therefore be measured. For this to be true it had to be assumed that the speed of thrombin formation under these conditions is proportional only to the amount of prothrombin present. It is now appreciated that the test is a measure of the rate, not the amount of prothrombin conversion. Nevertheless it has led to the elucidation of many aspects of blood coagulation and is still the most commonly used method for the control of anticoagulant therapy with the coumarin drugs. A prolonged clotting time by the one-stage test is popularly, but erroneously, referred to as due to "hypoprothrombinaemia". The tissue extract in popular use in this test is an acetone-dried extract of rabbit or human brain.

Post-Classical Era (1943-1956).

The developments in our understanding of blood coagulation since 1943 are intimately related to the subject matter of this thesis and will be considered in detail in the succeeding chapters. The discovery in 1943 by Owren of a patient with a prolonged one-stage "prothrombin" time, not due to deficiency of prothrombin revealed a discrepancy in the Classical Theory and led to the discovery of factor V.

(Owren 1947). A further discrepancy in the one-stage test led soon thereafter to the discovery of yet a further coagulation factor - factor VII.

Other Theories of Blood Coagulation.

In order to follow some reasonable sequence in this chapter, I have purposely followed the basis of the Classical Theory. This gives the false impression of continued progress along one line of thought. There were however many very different theories during this period which added to the confusion of the contemporary workers. These additional theories give some appreciation of the difficulties which may beset the coagulation worker and an account of them has been given by Biggs and Macfarlane 1953. Only a few are mentioned here.

Howell's Theory (Howell 1935).

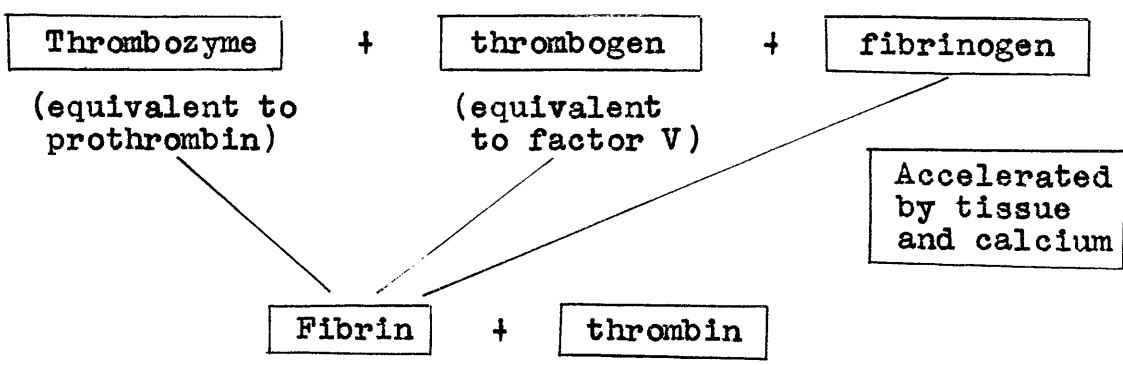
This theory stated that the occurrence of coagulation was due to the removal of an inhibitor. Howell believed that prothrombin was converted to thrombin by the action of calcium alone and that the fluidity of the circulating blood was due to the prothrombin being in combination with heparin. Thromboplastin inactivated the heparin and permitted the activation of prothrombin to thrombin by calcium. This theory has been disproved by the observation that preparations

of prothrombin cannot be converted to thrombin under the influence only of calcium and brain extract. (Nolf 1945, Owren 1947, Ware and Seegers 1948 and Milstone 1948).

The Theory of Nolf.

The work of Nolf is worthy of special mention. It covers almost the whole era of the classical theory from 1908-1945. His work, which was probably the most progressive of its time, received little recognition because of the complexity of some of his writings and experimental details. It was not until the classical era was on the wane that his experiments and concepts received general recognition.

In 1908 he recorded his views in the following equation:-



In 1945 Nolf restated his views in more modern terms. By the treatment of plasma with $Ca_3(PO_4)$ he could remove his thrombozyme leaving behind thrombogen and fibrinogen. The thrombogen of Nolf corresponds to the factor V of Owren.

The Theory of Bordet

Bordet (1920) observed that bird blood collected into paraffin lined containers clotted more slowly than when it was delivered into untreated glass surfaces. This suggested that something present in the blood was activated by contact. Bird and rabbit plasma freed from most cells by centrifuging in paraffin lined containers clotted very slowly on the addition of calcium, and after coagulation much serozyme (prothrombin) remained in the serum. On the addition of thromboplastin (cytozyme) the serum formed thrombin at greater speed than did the corresponding plasma (Bordet and Delange, 1912, Bordet 1920).

Bordet's theory may be summarised:-

$$\text{Pro-serozyme} \xrightarrow{\text{contact. } Ca^{++}} \text{serozyme}$$

$$\text{Serozyme + cytozyme} \longrightarrow \text{Thrombin}$$

$$\text{Thrombin + fibrinogen} \longrightarrow \text{Fibrin}$$

Bordet's experiments were of value in that he demonstrated some change to be occurring as a consequence of contact with a foreign surface and as a sequel to coagulation itself.

Robb-Smith has recently ably reviewed many of the historical aspects of research into blood coagulation (Robb-Smith 1955).

Inhibitory Mechanisms.

In addition to the system for the formation of fibrin there is evidence of mechanisms for the prevention of fibrin formation and for its removal when it has been deposited on intravascular sites. In respect to the prevention of fibrin formation there is a powerful antithrombin, and probably an antithromboplastin. The fibrinolytic mechanism for the removal of fibrin appears to be as complex as that for its formation.

Antithrombin. When thrombin formation in whole blood is studied it disappears rapidly following fibrin formation. (Schmidt 1892). Solutions containing thrombin coagulate preparations of fibrinogen more rapidly than they do oxalated or citrated plasma (Morawitz 1905, Mellanby 1909). This antithrombin activity of plasma is present in the albumin fraction (Quick 1938); prothrombin can be obtained from plasma freed from antithrombin by dilution and acidification at PH5.

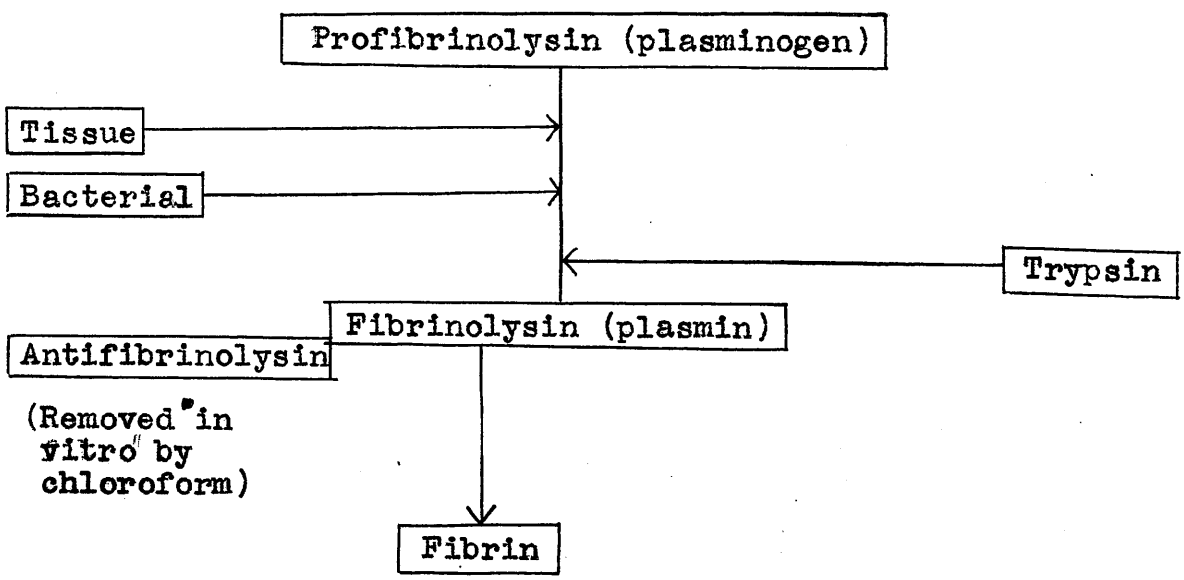
Antithromboplastin: When the progress of blood thromboplastin formation is followed it will be seen to decrease in potency with the passage of time. Whether this is a measure of the instability of the thromboplastin itself or to the existence of a separate inhibitor system is not known.

Heparin. The discovery of heparin by McLean in 1916 led to further study of the inhibitors of blood coagulation.

Heparin interferes with the action of thrombin and fibrinogen. This activity is dependent on a co-factor, present in the albumin fraction of plasma. In a system containing isolated thrombin and fibrinogen the heparin is relatively inactive in the prevention of the thrombin-fibrinogen reaction (Howell & Holt 1918, Mellanby 1935, Quick 1938). Howell also suggested that heparin interfered with the reactions preceding thrombin formation. No appreciable amount of heparin has been isolated from normal blood and the normal antithrombin activity is believed to be independent of heparin or its cofactor. (Lyttleton 1954).

Fibrinolysis. In the normal process of haemostasis and the repair of wounds the fibrin deposited is not a permanent structure. It serves merely as a temporary method of preventing blood loss and of providing a supporting framework for the growth of fibrous tissue. When this permanent form of repair is adequate the fibrin is removed. Similarly fibrin, which is deposited intravascularly in thrombus formation can be removed. The blood itself contains a fibrinolytic system which when fully activated is very powerful and capable of dissolving fibrin in a few minutes. By recently developed methods it can be shown that normal blood contains some active fibrinolysin. This fibrinolytic activity may be much increased "in vivo" by physiological stimuli

such as exercise or pathologically, for example, after injury or in chronic parenchymatous liver disease. It is believed that the active enzyme fibrinolysin has an inactive precursor profibrinolysin. The activation of profibrinolysin to fibrinolysin can be carried out "in vitro" by the addition of tissue or of certain bacterial enzymes particularly streptokinose and streptodornase. It can apparently also be activated by removal of an inhibitor system, by treatment with chloroform or other organic solvents. Trypsin is capable of activating the system and is itself proteolytic to fibrin.



REFERENCES

Addis, T. (1911). The pathogenesis of hereditary haemophilia. J. Path. Bact. 15,427.

Ancell, H. (1839-40). Course of lectures on the physiology and pathology of the blood and other animal fluids. Lancet, 2,836.

Arthrûs, M. and Pagès, C. (1890). Nouvelle Théorie chimique de la coagulation du sang. Arch. Physiol. Norm et Path. 2,739.

Birzzozero, J. (1882). Ueber einem neuen Formbestandtheil des Blutes und dessen Rolle bei der Thrombose und der Blutgerinnung. Virchows Arch. 90,261.

Bordet, J. (1920). Considerations sur les théories de la coagulation du sang. Ann. Inst. Pasteur 34,561.

Bordet, J. and Delange L. (1912). La coagulation du sang et la genèse de la thrombine. Ann. Inst. Pasteur 26,657.

Bordet, J. and Delange L. (1913). Sur la nature du cytozyme. Recherches sur la coagulation du sang. Ann. Inst. Pasteur 27,341.

Bordet, J. and Delange L. (1914). Analyse et synthèse du process de la coagulation. Ann. et bull. Soc. Roy. de Sc. méd. et nat. de Bruxelles 72,87.

Brinkhous, K. M. (1939). A study of the clotting time in haemophilia: the delayed formation of thrombin. Amer. J. med. Sci. 198,509.

Buchanan, A. (1835-36; 1845). On the coagulation of the blood and other fibriniferous liquids.
Lond. Med. Gaz. 18,50, 1835-36.
Lond. Med. Gaz. 1,617.

Bürker, K. (1904). Blutplattchen und Blutgerinnung, Pfluger's Arch. f.d. Ges. Physiol., 102,36.

Davy, J. (1839). Researches, physiological and anatomical. Smith Elder, London.

Delezenne, C. (1897). Recherches sur la coagulation du sang chez les oiseaux. Arch. Physiol. Norm. Path. 5e ser 9,333.

Denys, J. (1887). La Cellule, 3,445. Quoted by Tocantins 1938.

Eagle, H. (1935). Studies on blood coagulation. I. The role of prothrombin and of platelets in the formation of thrombin. J. gen. Physiol. 18,531.

Fantl, P. and Nance, M. H. (1948). The physiological activation of prothrombin. Med. J. Aust. 1,128.

Ferguson, J. H. (1936). Experimental analysis of the coagulant activation. Amer. J. Physiol. 117,587.

Gulliver, G. (1846). The works of William Hewson, F.R.S., edited with an introduction and notes by George Gulliver, F.R.S. Sydenham Society, London.

Hammersten, O. (1879). Über das Fibrinogen. Pflug. Arch. 19, 563.

Hayem, G. (1877) Mem. Soc. Biol. Paris, 29, 97. Note sur les caractères et l'evolution des hématoblastis chez les ovipares.

Hewson, W. (1771). An experimental inquiry into the properties of the blood. Cadell, London.

Hopff, F. (1828). Uber die Haemophilie, oder die erbliche Anlage zu tödtlichen Blutungen. Becker, Würizburg.

Howell, W. H. (1935). Physiol. Rev. 15, 435. Theories of blood clotting.

Howell, W. H. and Holt, E. (1918). Two new factors in blood coagulation - heparin and pro-antithrombin. Amer. J. Physiol. 47, 328.

Lister, J. (1858). On a case of spontaneous gangrene from arteritis and on the causes of coagulation of blood in diseases of the blood vessels. Edinb. med. J. 3, 893.

Lister, J. (1863). On the coagulation of the blood. Croonian Lecture Proc. Roy. Soc. 12, 580.

Liston, J. (1838-39). Haemorrhagic idiosyncrasy. Lancet 2, 137.

Lyttleton, J. W. (1954). The antithrombin activity of human plasma. Biochem. J. 58, 8.

Macfarlane, R. G. (1947). The action of soya-bean trypsin inhibitor as an antithromboplastin in blood coagulation. J. Physiol. 106, 104.

Macfarlane, R. G. and Pilling, J. (1946). Anticoagulant action of soya-bean trypsin inhibitor. Lancet, 1, 888.

Malpighi, M. (1666). De viscerum structura exercitatio anatomica: accedit dissertatio ejusdem de polypo cardis. Martyn, London.

McLean, J. (1916). The thromboplastic action of cephalin. Amer. J. Physiol. 41, 250.

Mellanby, J. (1909). The coagulation of the blood. J. Physiol. 38, 27.

Mellanby, J. (1935). Heparin and blood coagulation. Proc. Roy. Soc. Lond. B. 116, 1.

Mills, C. A. (1927). The role of the platelets in blood clotting. Chinese J. Physiol. 1, 235.

Morawitz, P. (1904). Die Chemie der Blutgerinnung Ergebn Physiol. 4, 307.

Nolf, P. (1945). Le plasma phosphate reactif de la coagulation. Arch. Int. Pharmacodyn et Ther. 70, 5.

Owren, P. A. (1947). The coagulation of blood. Investigation on a new clotting factor. Acta. med. Scand. Suppl. 194.

Pohle, F. J. and Taylor F.H.L. (1937). The coagulation defect in haemophilia. The effect in haemophilia of intramuscular administration of a globulin substance derived from normal human plasma. J. clin. Invest. 16, 741.

Quick, A. J. (1935). The prothrombin in haemophilia and in obstructive jaundice. J. biol. Chem. 109, lxxiii.

Quick, A. J. (1938). The normal antithrombin of the blood and its relation to heparin. Amer. J. Physiol. 123, 712.

Quick, A.J. (1951). The physiology and pathology of haemostasis. Henry Kimpton, London.

Robb-Smith, A.H.T. (1955). The Rubicon: changing views on the relationship of thrombosis and blood coagulation. Brit. med. Bull. 11,70.

Ruysch, F. (1707). Thesaurus anatomicus. Woltus. Amsterdam.

Schmidt, A. (1861). Ueber den Faserstoff and die Urachen seiner Gerinnung. Arch. Anat. Physiol. p. 545.

Schmidt, A. (1862). Weiteris Ueber den Faserstoff und die Urachen seiner Gerinnung. Arch. Anat. Physiol. p. 428.

Schmidt, A. (1893). Zut Blutlehre. Leipzig.

Wardrop, J. (1835). On blood letting; an account of the curative effects of the obstruction of blood; with rules for employing both local and general blood letting in the treatment of disease. Baillière, London.

Ware, A.G. and Seegers, W.H. (1948). Plasma accelerator globulin; partial purification, quantitative determination and properties. J. biol. Chem. 172, 699.

Weil, P. F. (1906). Etude de sang chez les haemophilis. Bull. Soc. Méd. Hôp. Paris, 23, 1001.

Willis, T. (1659). Diatribae dual medico-philosophicae, quarum prior agit de fermintatione sine de motu intèstino particularum in quovis corpore, altera de fibribus sine de motu earundum in sanguine animalium, his accessit dissertatio epistolica de urinus. Roycroft, London.

CHAPTER 2

THROMBIN - FIBRINOGEN REACTION

CONTENTS.

All experimental observations in blood coagulation depend on the reaction of thrombin with fibrinogen and the resultant formation of a fibrin clot. This is the only end point in the whole field of research on blood clotting and through this small window the coagulation worker is attempting to view the complicated reactions which form our present concepts of blood coagulation. The further away from this end point at which any reaction may be occurring the more difficult it is to study.

Thrombin can be defined as the substance, derived from the prothrombin of plasma, which clots fibrinogen to form fibrin. Thrombin is formed from prothrombin by a complicated chain reaction, which is at present only partially understood.

In dealing with the thrombin-fibrinogen reaction at this stage the final result is being described, before dealing with the reactions which precede it. This is inevitable, however, the thrombin-fibrinogen reaction being the only available indicator system.

Fibrinogen.

Fibrinogen is defined as a globulin which interacts with thrombin to produce a visible fibrin network or clot. It has a molecular weight of 400,000 and the molecule is 3-4

times larger than the other plasma globulins. It is destroyed by heating to 47° C.

Fibrinogen is prepared from plasma by precipitation with salts, alcohol or ether. Different laboratories have used different species of plasma for their preparations of fibrinogen - some use human plasma while others use bovine plasma. The purity of the preparations has varied and has probably been in part responsible for contradictory results in different laboratories.

The method of preparation of fibrinogen used throughout this work has been that of Jaques (1943) and the details are given in the appendix (page 555). Human plasma was used throughout for the preparation of the fibrinogen. It has been shown by Avery and Monroe (1948) that this is relatively the purest form of fibrinogen when compared with other methods in common usage. This preparation of fibrinogen is free from other known coagulation factors except antihaemophilic globulin (the missing component in haemophilia) and possibly profibrinolysin (concerned in the fibrinolytic mechanism).

Measurement of Fibrinogen: This was carried out by the micro-Kjeldahl technique and the details are given in the appendix (page 580). This is the only chemical assay used throughout this work - the other procedures all being biological. The method is dependent on the assumption that

fibrinogen is converted quantitatively into fibrin by thrombin. The fibrin is then carefully collected on a glass rod, washed so far as possible in saline to remove other proteins and assayed as nitrogen by the micro-Kjeldahl technique.

Thrombin - may be defined as the active coagulant of fibrinogen, which appears in plasma during its coagulation. It is destroyed by heating to 60° C. but has a remarkable resistance to acetone.

Thrombin is prepared by separation of prothrombin from antithrombin and the subsequent activation of this prothrombin to thrombin gives a solution from which the thrombin can be prepared. Seegers et al (1950) have prepared thrombin from prothrombin by dissolving the latter in 25% citrate. (A similar experiment is described in the appendix page 6). Using this method no extraneous factors were added and the product was said therefore to be "pure". This product when tested electrophoretically was found to consist of three components. Thus there is some doubt about the chemical purity of even the best preparations of thrombin. The preparation of prothrombin used by Seegers in these experiments, it is now appreciated, contained at least two other coagulation components.

The preparations of thrombin used in this present work

Figure (1)

Figure [illegible]

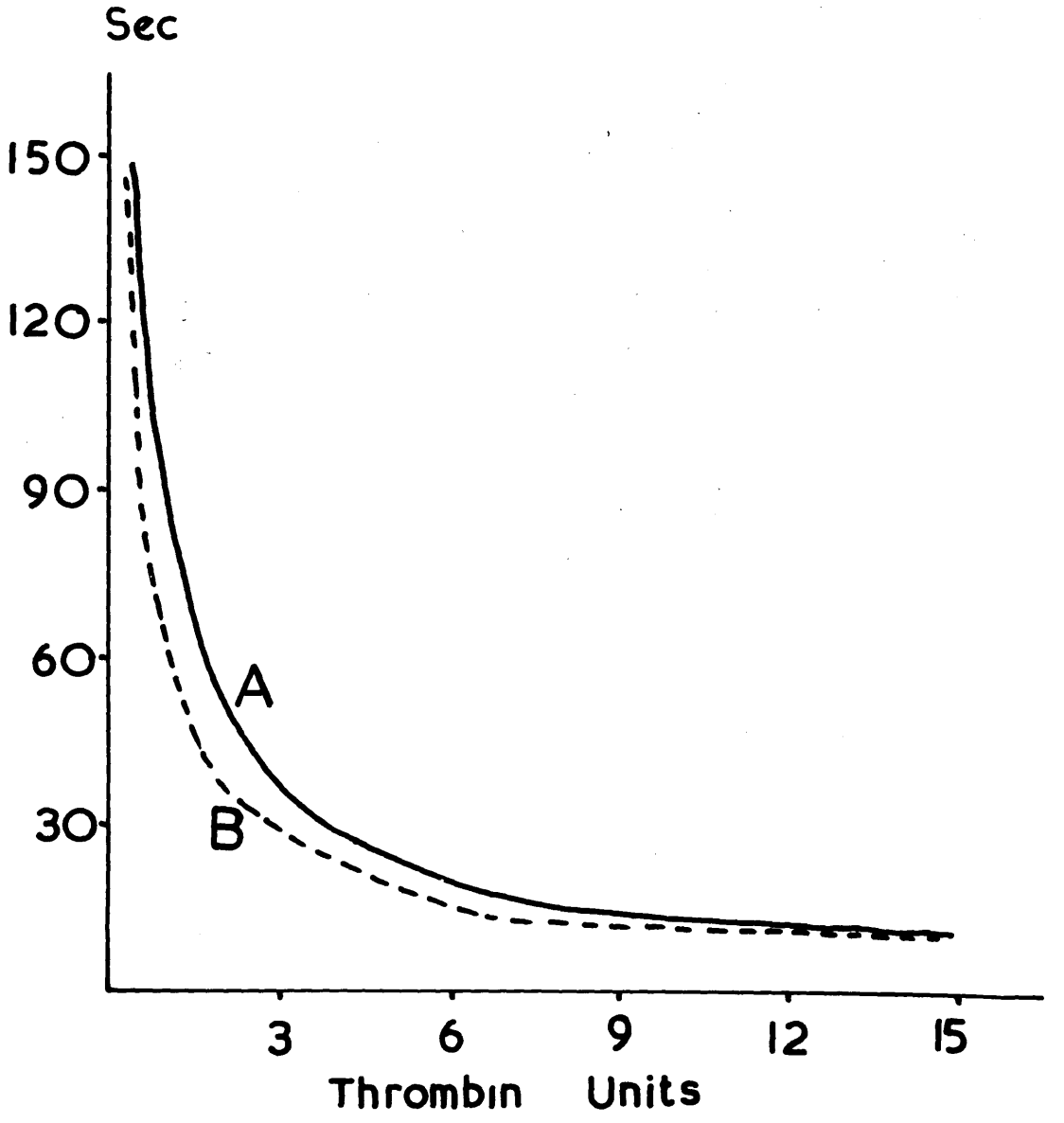
Thrombin-fibrinogen dilution curve.

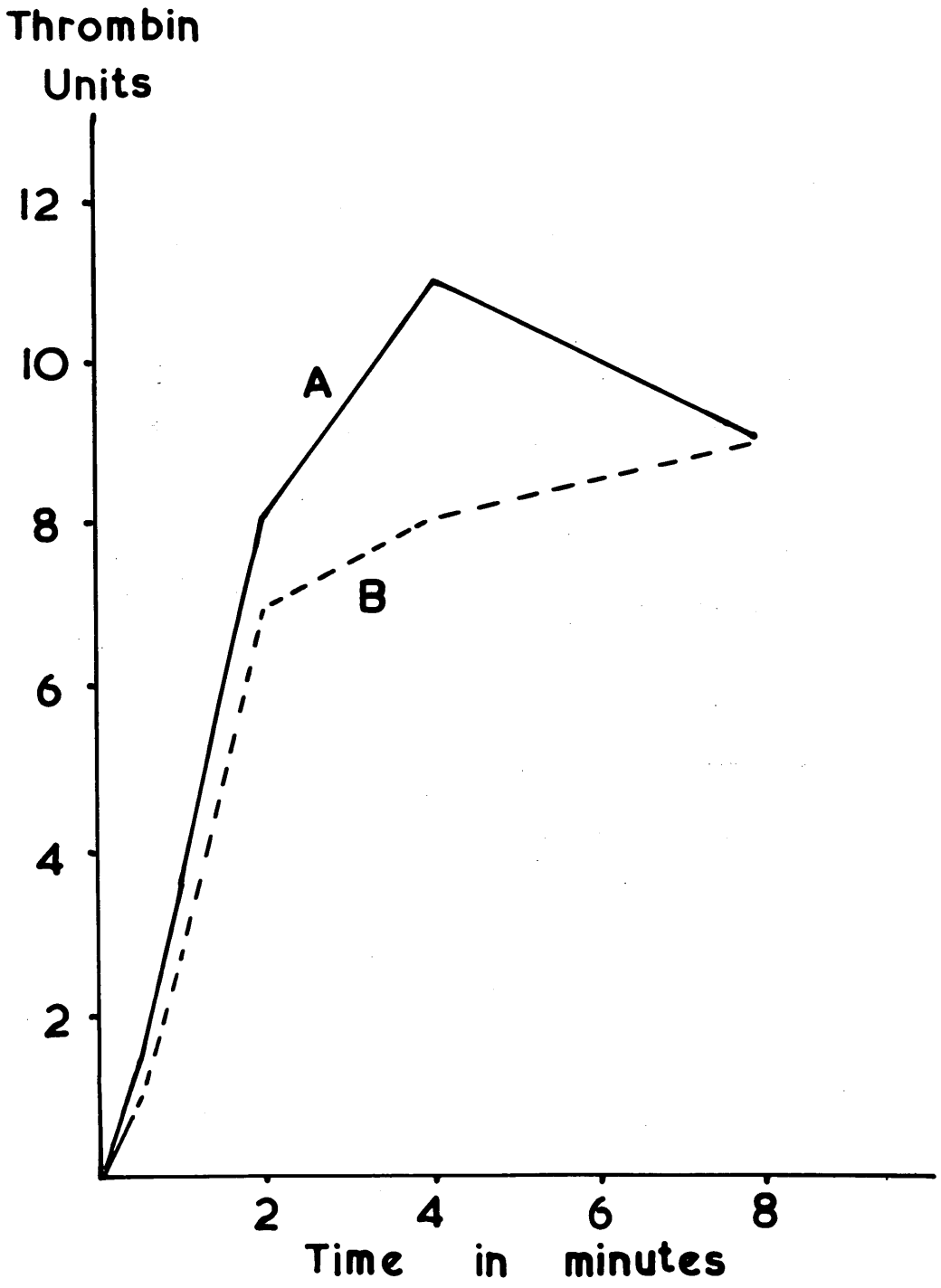
Ordinate - clotting time of fibrinogen in seconds.

Abscissa - thrombin units.

0.4 ml. amounts of fibrinogen were clotted with various concentrations of thrombin and the clotting times recorded. The batches of fibrinogen were found usually to "fit" curve A. Occasionally this has not been so and curve B represents a separate curve prepared for one particular batch of fibrinogen.

In the accompanying figure is shown an example of the differing result, which may arise from a set of clotting times of fibrinogen, when read off the curves A and B. In this experiment the progress of thrombin formation from prepared prothrombin was being studied. This illustrates the importance of using the thrombin-fibrinogen dilution appropriate to the batch of fibrinogen in use.


Sec
150
120
90
60
30
A
B
3
6
9
12
15
Thrombin Units


Thrombin
Units
12
10
8
6
4
2
A
B
2
4
6
8
Time in minutes

have been shown to contain traces of thromboplastic activity but the preparations are adequately pure for most observations. The details of the method used for the preparation of thrombin are given in the appendix (page 556).

Interaction of thrombin and fibrinogen. Thrombin-fibrinogen Dilution curve.

When increasing concentrations of thrombin are added to fibrinogen in an antithrombin-free system the clotting time shortens proportionately and it can be shown that

$$C.T. \propto \frac{1}{T}$$ Formula (1)

C.T. = Clotting Time

T. = Conc. of thrombin.

This means that if we have twice the concentration of thrombin the clotting time of the fibrinogen is halved. In figure 1 a curve is shown which is prepared by clotting 0.4 amounts of fibrinogen with 0.1 amounts of varying strengths of thrombin. This can be expressed as a straight line relationship by plotting the clotting time against the reciprocal of the concentration of thrombin. (See Figure 2). This line does not pass quite through the zero point as no matter how concentrated the thrombin, it still requires some time to clot. At low concentrations of thrombin the relationship is also unreliable because the process of

Figure (2)

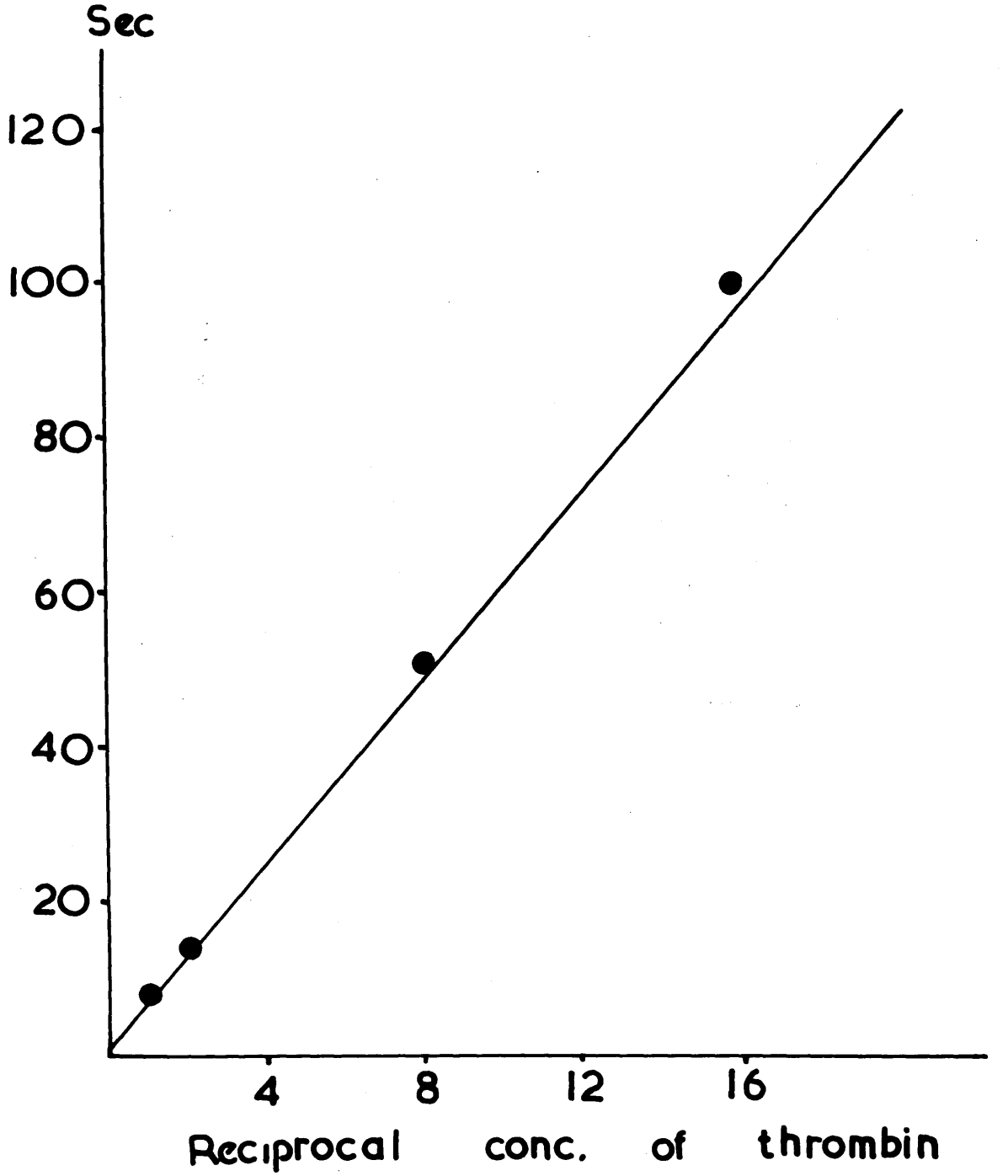
Figure (1)

Thrombin-fibrinogen dilution curve.
(Thrombin concentration as a reciprocal).

Ordinate - clotting time of fibrinogen in seconds.

Abscissa - concentration of thrombin as a reciprocal.

0.4 ml. amounts of fibrinogen were clotted with various concentrations of thrombin and the clotting times recorded. It will be seen that when the clotting times of the fibrinogen are plotted against the reciprocal of the concentration of thrombin, there is a straight line relationship.


Sec
120
100
80
60
40
20
4
8
12
16
Reciprocal conc. of thrombin

coagulation is more protracted and no clear end point can be defined. The relationship expressed above is only true within certain limits. It is found in practice however that the range is adequate to cover most experimental observations. Astrup and Darling (1941) have demonstrated that impure preparations of fibrinogen do not obey this relationship and therefore only "pure" preparations should be used.

Principles in the assay of prothrombin.

The measurement of prothrombin is dependent on the assay of the thrombin, which can be produced from the prothrombin. In such a technique the thrombin is developed from the prothrombin in an incubation mixture and the thrombin assayed, by transferring at intervals aliquots into fibrinogen, the clotting time of which is determined. Such a technique is described as a two-stage method. In a one-stage method the fibrinogen is in the incubation mixture and is clotted by the thrombin as it develops.

It has been shown by Biggs (1951) that, while working in an antithrombin free system, when prothrombin is converted to thrombin in the two-stage technique, then the amount of thrombin formed as represented by the minimum clotting time of the fibrinogen is directly proportional to the amount of prothrombin present.

i.e. $P \propto T$ Formula (2)

where P is the concentration of prothrombin

and T is the concentration of thrombin formed.

From formulae (1) and (2) it follows that

$$P \propto \frac{1}{C.T.}$$

in the two-stage test free from antithrombin.

Thus:

$$\frac{\text{Minimum clotting time of normal}}{\text{Minimum clotting time of abnormal}} = \frac{\text{Concentration of prothrombin in abnormal}}{\text{Concentration of prothrombin in normal}}$$

e.g. in an experiment where prothrombin has been freed from antithrombin by preparation of the globulin fraction

Minimum clotting time of normal = 12 secs. = 10.4 thrombin units.

Minimum clotting time of abnormal = 18 secs. = 6.8 thrombin units.

$$\text{Thus } \frac{12}{18} = \frac{\text{Unknown}}{100}$$

$$\text{Unknown} = \frac{12}{18} \times 100$$

$$= 66\%.$$

or expressed in thrombin units (see below).

$$\frac{\text{Thrombin units of unknown}}{\text{Thrombin units of normal}} = \frac{6.8}{10.4} = 66\%.$$

The nature of thrombin-fibrinogen reaction is complicated and imperfectly understood. The reaction has been studied using whole plasma or purified fibrinogen. When whole plasma is used substances other than fibrinogen are affecting the reaction, for example the variability in the antithrombin content. On the other hand when purified fibrinogen is used it may bear little relationship to the true reaction in plasma.

Using purified thrombin and fibrinogen unfortunately the reaction is variable. Many solutions of fibrinogen deposit a white sediment on standing in the cold. This readily redissolves on warming and has been called profibrin (Apitz 1938). This cold-insoluble substance reacts much more rapidly to thrombin than does the supernatant solution (Owren 1947). If different preparations of fibrinogen contain a variable amount of this substance their reaction to thrombin will naturally be variable. Many other factors influence the speed of the reaction between thrombin and fibrinogen. Concentration of fibrinogen may cause some change in reactivity particularly towards dilute thrombin. Temperature influences the reaction, the optimum being at 37° C. Fibrinogen from different species behaves differently to the same thrombin. The reverse however is not true in that it appears that the thrombin of different species has the same reactivity with the

one species of fibrinogen.

Thrombin units.

Thrombin is usually measured in units which are defined by the speed of clotting of fibrinogen. The definition of a thrombin unit has varied from one laboratory to another. Owren (1947) in his work on factor V - "The coagulation of Blood - Investigations on a New Clotting Factor" - defines a thrombin unit, "one thrombin unit has been defined in this study as equal to the amount of thrombin which in a volume of 1 cc. containing 0.10 per cent fresh profibrin-free human fibrinogen at a temperature of 37° C., pH 7.3 and sodium chloride concentration 0.154m, causes coagulation in 15 seconds."

This absolute definition of a thrombin unit enables thrombin solutions of constant strength to be prepared but in practice the errors from one laboratory to another must necessarily be considerable. As discussed above, a difficulty which sometimes arises, is that the reactivity of one batch of fibrinogen, as compared with another, may vary considerably. The preparations of fibrinogen used throughout this work in general fitted the curve A shown in figure / . In the case of one of the batches of fibrinogen a new curve had to be drawn (see B of figure /). It is thus difficult or even impossible to define a thrombin unit which will have

the same meaning to every laboratory.

Early in this work there was available a preparation of human thrombin of stated unitage per mg. This was adopted as the basis from which subsequent standardisations were made. The original calibration of the thrombin was made using a certain batch of thrombin topical (Roche) which was stated to contain at least 1000 units of thrombin per cc. of solution.

Every new batch of fibrinogen was tested against the existing batch of thrombin and vice versa. It was essential therefore not to allow the preparations of thrombin and fibrinogen to become exhausted together. With each test the clotting time was plotted against the reciprocal of the concentration of thrombin to ensure that these points fell on a straight line which passed near zero though not necessarily through it, e.g. Figure 2 . It was necessary also to fix one point on the existing thrombin-fibrinogen dilution curve to determine whether the new points fitted the old curve. On one occasion only with a new batch of fibrinogen, this was not so; a thrombin-fibrinogen dilution curve was made, individual to this preparation of fibrinogen (Curve B - figure /).

Summary.

(1) The relationship of the clotting time of fibrinogen

by thrombin is described. Over a certain range, the clotting time is proportional to the reciprocal of the concentration of thrombin.

(2) The measurement of thrombin in terms of thrombin units and the preparation of a thrombin-fibrinogen dilution curve are described. The use of this for assay of thrombin is demonstrated.

(3) The amount of thrombin formed represented by the minimum clotting time of fibrinogen, in an anti-thrombin free system, is directly proportional to the amount of prothrombin present.

(4) Details of the methods used for the preparation of fibrinogen and thrombin and for the measurement of fibrin are given in the appendix.

(5) The clotting of fibrinogen with thrombin is the final stage in blood coagulation and it is the only stage which can be observed directly. All reactions prior to the thrombin-fibrinogen reaction are inferred from the rate of coagulation under varying circumstances.

REFERENCES

Apitz, K. (1938). Pathologische physiologie der blutgerinnung. Kolloid Zeit. 85, 196.

Astrup, T., and Darling, S. (1941). Preparation and purification of thrombin. Acta Physiol. Scand. 2, 22.

Avery, A., and Monroe, F.L. (1948). Chemical and electrophoretic studies of fibrinogen prepared by various methods. Arch. Biochem. 16, 33.

Biggs, R. (1951). Prothrombin deficiency. Blackwell's Scientific Publications, Oxford.

Jaques, L.B. (1943). The reducing properties of fibrinogen. Biochem. J. 37, 344.

Owren, P.A. (1947). The coagulation of blood. Investigations on a new clotting factor. Acta med. Scand. Suppl. 194.

Seegers, W.H., McClaughry, R.I., and Fahey, J.L. (1950). Some properties of purified prothrombin and its activation with sodium citrate. Blood. 5, 421.

CHAPTER 3

DISSOLUTION OF THE CLASSICAL THEORY

CONTENTS.

(3) Synonyms of factor VII.
Serum prothrombin conversion accelerator.
Co-thromboplastin.
Proconvertin and convertin.
Serum factor of Jacox.
Free prothrombin.

CHAPTER 3

THE DISSOLUTION OF THE CLASSICAL THEORY

Since the clotting time of fibrinogen is proportional to the amount of thrombin present and since prothrombin is believed to be quantitatively converted to thrombin methods have been devised to measure prothrombin.

In 1935 Quick introduced the one-stage test as a measure for prothrombin. The theory of blood coagulation at the time was the Classical Theory and the test on this basis theoretically sound. It was assumed that when an excess of tissue thromboplastin and calcium was added to plasma there was only one limiting factor prothrombin, which could then be measured. According to the Classical Theory the speed of thrombin formation in the one-stage test is controlled only by the concentration of prothrombin. The difficulties in the application of this test as a measure for prothrombin will be discussed later. For the present it is the purpose merely to consider how certain discrepancies in the one-stage test led to the development of much of our recent knowledge of the prothrombin complex. The dissolution of the Classical Theory marked also the dissolution of Quick's one-stage test as a measure for prothrombin. The

Figure (3)

Figure (3)

Quick's one-stage test - saline dilution curves.

Ordinate - clotting times of plasma and dilutions of plasma in seconds.

Abscissa - concentration of plasma ("prothrombin") in saline.

0.1 ml. amounts of plasma or dilutions of plasma were clotted after the addition of 0.1 ml. brain thromboplastin and 0.1 ml. of $^{m}/_{40}$ calcium chloride. a b c d e correspond to different preparations of thromboplastin with corresponding variation in the clotting time of the whole plasma.

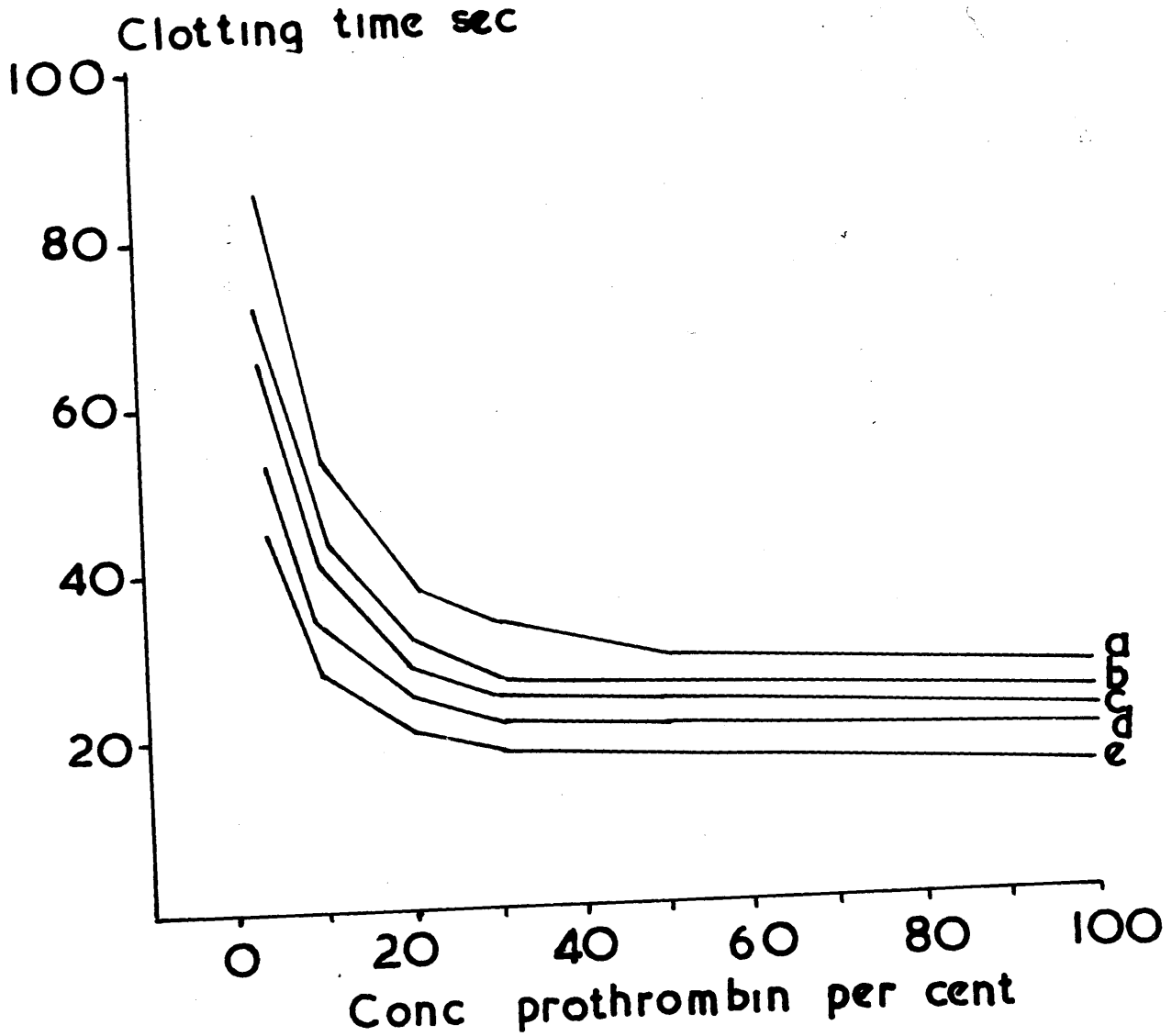

Clotting time sec
100
80
60
40
20
0
20
40
60
80
100
Conc prothrombin per cent
a
b
c
d
e

Figure (4)

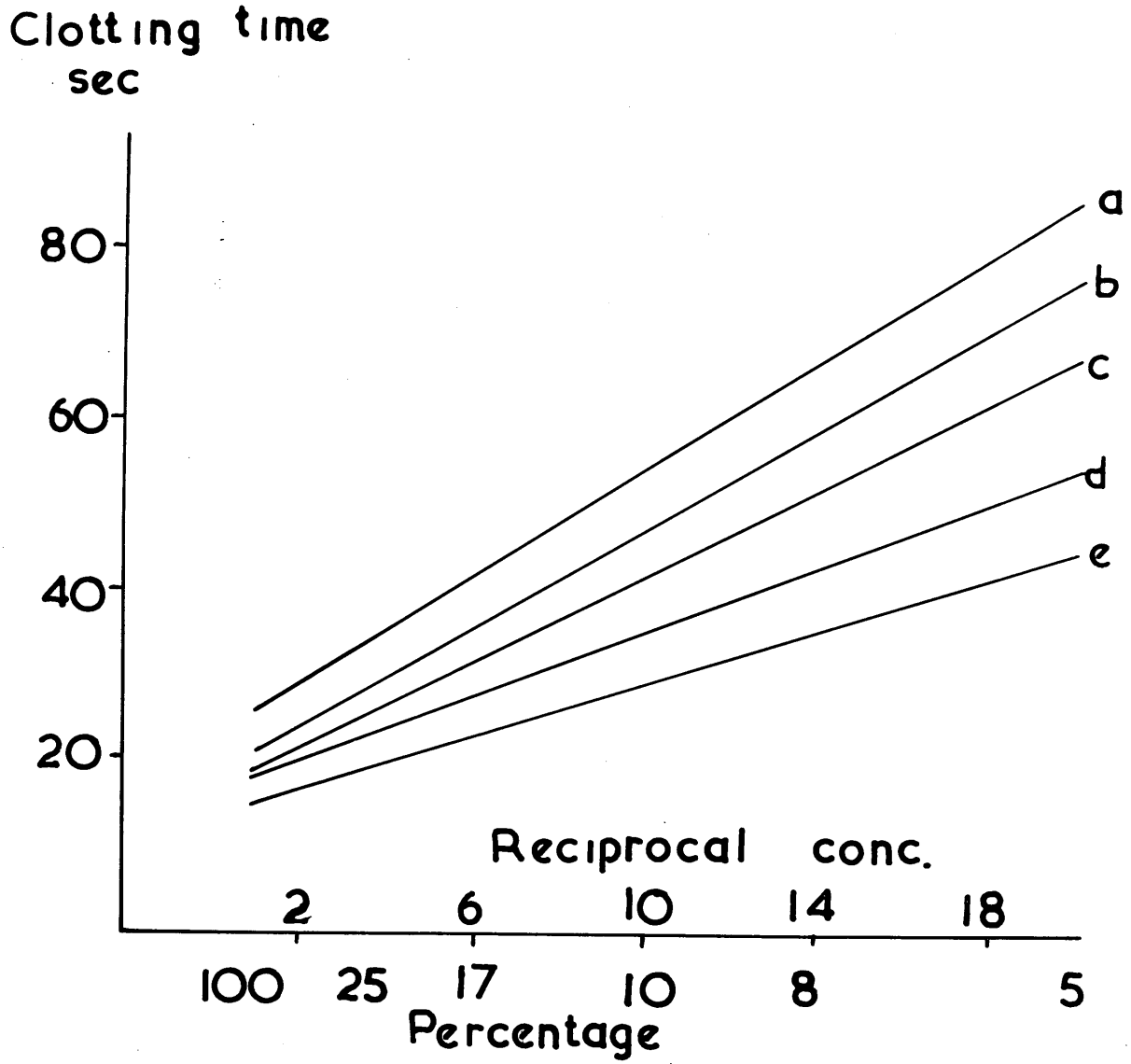
Figure (4)

Quick's one-stage test - saline dilution curves (concentration of plasma ("prothrombin") expressed as a reciprocal).

Ordinate - clotting times of plasma and dilutions of plasma in seconds.

Abscissa - concentration of plasma (prothrombin) in saline expressed as a reciprocal.

The data used in this figure is the same as that in figure (3).


Clotting time
sec
80
60
40
20
a
b
c
d
e
Reciprocal conc.
2
6
10
14
18
100
25
17
10
8
5
Percentage

technical details of Quick's one-stage "prothrombin" test as used in this work are given in the appendix.

The preparation of a saline dilution curve is also described; Figures 3 and 4 illustrate the dilution curves prepared using acetone dried human brain thromboplastin. The series of curves is required because the prepared thromboplastin may vary, so that the clotting time of the control plasma differs from one day to the next. The dilution curves shown are applicable only to acetone dried human brain. The results for example using saline extract of human brain cannot be read from these curves. Even though the clotting times of the normal plasma are the same with the two thromboplastins the result with an abnormal plasma or a specified dilution of the normal may be different, e.g.

Plasma	- Normal	Abnormal	12.5% dilution
Acetone dried brain	- 15	30	26
Phenol-saline brain	- 15	46	37

Other similar examples are shown in the appendix page 662.

At the end of the Classical era the following conditions were known to give a prolongation of the one-stage clotting time by Quick's technique.

(a) in the new born infant on the third and fourth days and in haemorrhagic disease of the newborn.

(b) in some cases of jaundice, particularly obstructive jaundice.

(c) in some cases of steatorrhoea.

(d) after the administration of certain drugs particularly dicumarol and salicylates.

Quick's test for the measurement of prothrombin was introduced in 1935. In the following year Warner, Brinkhous and Smith (1936) described the two-stage method of prothrombin assay. In this technique the same reagents were mixed as in the one-stage test of Quick, namely plasma, tissue extract and calcium. The development of thrombin in this incubation mixture was followed by transferring aliquots to fibrinogen and recording the clotting time of the fibrinogen. The prothrombin content was estimated by comparing the thrombin generated from the test plasma with that from a normal plasma. The discrepancy between the assays by the one-stage method and the two-stage technique raised the possibility that the Classical Theory was incomplete.

Factor V. It was not until 1942 that the inadequacy of the Classical Theory became obvious when Owren investigated a patient with haemorrhagic disease, the feature of significance being a prolongation of the one-stage clotting time (Owren 1947); according to the Classical theory this patient would be said to lack prothrombin. As described in Chapter / prothrombin can be removed by adsorption on

inorganic precipitates. Normal plasma so treated has a much prolonged one-stage clotting time or may even be incoagulable. Normal plasma so treated, was able to correct the defect in Owren's case. The work consequent upon this experimental observation established that plasma contained a previously unrecognised coagulation component which was named factor V by Owren (1947). The concept at the time of discovery was that this new factor was an accelerator of prothrombin conversion - i.e. it entered the reaction after prothrombin conversion had started. It was required for the conversion of prothrombin when this was occurring under the influence of tissue thromboplastin. It was not adsorbed by inorganic precipitates and therefore was present in the supernatant after adsorption.

Factor VII. When dicumarol or any of the related drugs are administered therapeutically, there is a prolonged one-stage clotting time by Quick's test. When the factor V content of dicumarol plasma is assayed, there is no significant reduction in the factor V. According to the theory the prolonged one-stage clotting time should therefore have been due entirely to a deficiency of prothrombin. In 1948, Owen & Bollman reported that the addition of one part of normal serum to nine parts of dicumarol plasma caused a marked

shortening of the one-stage clotting time. Serum contains little prothrombin and thus the possibility of yet a further coagulation component had to be considered. This property of serum is ascribed to its content of factor VII.

These three components of the prothrombin complex (prothrombin, factors V and VII) require detailed consideration.

Prothrombin.

Prothrombin may be defined as the precursor of thrombin and has been extensively investigated during the last decade. When it was first named, prothrombin was a hypothetical substance, but it has since been accepted as a real entity in the coagulation system.

Preparation of prothrombin.

In order to study the properties of prothrombin attempts were made early in the Classical Era to separate prothrombin from other plasma proteins. In 1914 Bordet and Delange prepared prothrombin from bird plasma by adsorption on tricalcium phosphate and release with carbon dioxide. Mellanby (1930) prepared prothrombin by diluting oxalated beef plasma with water and precipitating at pH 5.3. The prothrombin was then selectively dissolved from the globulin precipitate by brief treatment with lime water. A product of considerable

activity was prepared by Seegers, Smith, Warner and Brinkhous (1938). In their method the prothrombin, after precipitation from diluted plasma at pH 5.3 was adsorbed on $Mg(OH)_2$. The prothrombin was liberated from the adsorbent by carbon dioxide. Seegers and his colleagues have since made a particular study of the preparation of prothrombin; their most recent technique is described by Seegers (1952). This more recent technique is similar to the 1938 method with the additional procedure that the prothrombin is fractionated with concentrated ammonium sulphate and finally precipitated from aqueous solution near the isoelectric point. The product is fairly stable after lyophil drying but the stability varies from one batch to the next. Seegers and his colleagues have made a detailed study of the separation of prothrombin but, it is now appreciated that, even their best preparations are likely to contain considerable amounts of two other coagulation components.

The method of preparation used in this work is described in detail in the appendix. In principle it depends on the adsorption of the prothrombin from human plasma on aluminium hydroxide and its subsequent elution with phosphate buffer. The details of the method of preparation of aluminium hydroxide are also given in the appendix. Prothrombin prepared in this way is freed from factor V and antihaemophilic globulin,

fibrinogen and antithrombin.

Units of prothrombin.

The amount of prothrombin present in a preparation is sometimes measured in terms of the number of units of thrombin that can be formed from it under optimal conditions. It is then said that a unit of prothrombin is that amount of prothrombin which is capable of forming one unit of thrombin. Ware and Seegers (1948) and Owren (1947) have adopted this terminology.

Activation of prothrombin to thrombin by concentrated sodium citrate.

Seegers, McClaughry and Fahey (1950) describe an experiment designed to show that thrombin can be derived from prothrombin in the absence of other coagulation factors. A solution of prothrombin was made in 25% sodium citrate and the appearance of thrombin tested by transferring aliquots into fibrinogen. A similar experiment is described in the appendix (page 611). Seegers, McClaughry and Fahey concluded from this that prothrombin must contain all the structural material needed for thrombin. It is now appreciated, however, that their preparations of prothrombin were not pure and must have contained at least two other coagulation factors (factor VII and the recently recognized Christmas factor).

Factor V.

The term factor V is used for the coagulation component mentioned above and now to be discussed in more detail, as Owren who used this term made an important study of its properties. He subsequently renamed this factor "accelerin" and a hypothetical precursor as "pro-accelerin". The evidence that there is a precursor form of this factor is insufficient and it is proposed to retain Owren's original term "factor V". Owren's contribution to this study was outstanding and serves as a model of careful experimental observation in this field. For this reason his work will be described in some detail.

Owren's investigations of his patient.

In April 1943, Owren investigated his case - a 29 year old female suffering from a haemorrhagic diathesis since the age of 3½ years. The outstanding feature of this case was a prolonged one-stage clotting time - 70-80 seconds as compared with a normal of 15-20 seconds. Owren removed prothrombin from normal plasma by adsorption. Aluminium hydroxide and magnesium hydroxide were used as adsorbing agents. Passage of plasma through a Seitz filter was also used by Owren for the removal of prothrombin. A mixture containing 90 per cent of his patient's plasma and 10 per cent of normal prothrombin-free human plasma had a one-stage

clotting time of 32 seconds. A similar proportion of adsorbed ox plasma reduced the time to 18 seconds and of adsorbed guinea-pig plasma to 24 seconds. The plasmas used had been very thoroughly adsorbed in that they were incoagulable on the addition of brain extract and calcium. A mixture containing 80 per cent of the patient's plasma and 20 per cent of prothrombin-free normal plasma was sufficient to produce a normal one-stage clotting time of 16 seconds.

Owren made a preparation of prothrombin by adsorption on $Mg(OH)_2$ and liberation with carbon dioxide. He added his preparation of prothrombin to his patient's plasma and to the "adsorbed" normal plasma. The one-stage clotting time of his patient's plasma was little altered whereas that of the "adsorbed" normal plasma was completely corrected. Owren then prepared prothrombin from his patient's plasma with $Mg(OH)_2$; the patient's prothrombin behaved with normal adsorbed plasma as well as normal prothrombin, in its ability to correct the one-stage clotting time. Owren added tissue extract and calcium chloride to the patient's plasma and studied the speed of thrombin formation by adding aliquots at intervals to fibrinogen and recording the clotting times. The thrombin formation from the patient's plasma was very slow as compared with that from normal plasma. By the addition of adsorbed normal plasma to the patient's plasma

the speed of thrombin formation was restored to normal.

From these investigations Owren concluded that in addition to tissue extract and calcium another component was needed for the conversion of prothrombin to thrombin at normal speed. As this was in addition to the four factors in the classical theory - fibrinogen (I), prothrombin (II), tissue extract (III) and calcium (IV) he named this new factor as V.

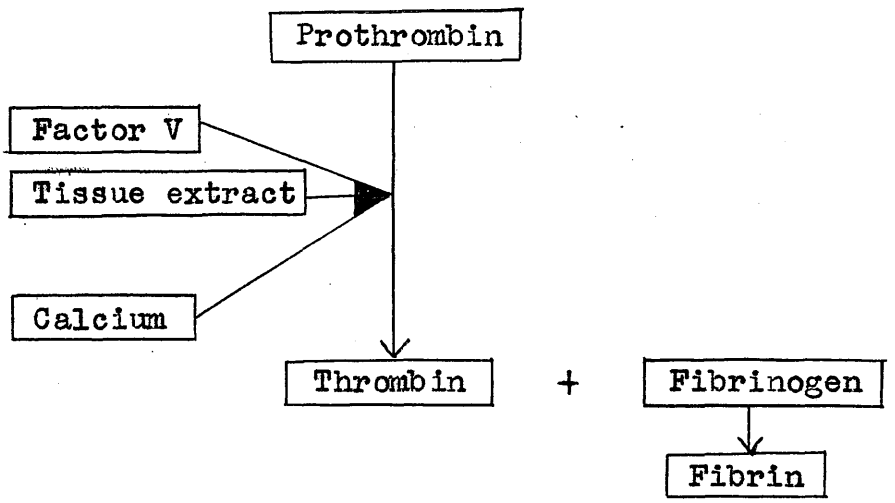
Isolation of factor V.

Owren investigated methods for the preparation of his factor V from plasma. He obtained the maximum yield by precipitation with saturated ammonium sulphate in the 33-50 per cent fraction or by acid precipitation using 1 per cent acetic acid between pH 5.0 and 5.5 in plasma, diluted 1/10 with distilled water. The plasmas used were rendered free from prothrombin by adsorption before commencing the preparation of the factor V. The ammonium sulphate saturation method yielded a fibrinogen-free precipitate as distinct from the acid precipitation where fibrinogen was still present. Dried preparations of factor V remained active for prolonged periods but in solution it was labile, the rate of inactivation varying with the method of storage.

Activation of prothrombin by factor V, brain and calcium.

Owren prepared prothrombin by adsorption on $Mg(OH)_2$ and liberation by carbon dioxide under pressure. This preparation of prothrombin occasionally contained traces of factor V, but was free from antithrombin. The prothrombin was then activated in the presence of varying concentrations of factor V. Owren concluded from this experiment that the velocity of thrombin formation rises with increasing concentration of factor V up to a certain limit. Further increase of factor V beyond this limit is without influence.

As a consequence of Owren's discovery the Classical Theory of blood coagulation had to be amended to read as follows:-



Preparation of Factor V.

The method used throughout this study is one of two techniques described by Owren. It is given in detail in

the appendix. The principle is to remove prothrombin by adsorption on $Al(OH)_3$ and to obtain a precipitate containing factor V, between 33-50 per cent saturation with ammonium sulphate. The addition to the adsorbed plasma of a half volume of saturated ammonium sulphate (33 per cent saturation) precipitates fibrinogen and antihaemophilic globulin. This is removed by spinning and a further half volume of ammonium sulphate added. This further precipitate contains factor V. It is dissolved in a volume of saline equivalent to the original volume of plasma and dialysed against citrate-saline to remove the ammonium sulphate. The citrate-saline contains nine parts of 0.85 per cent sodium chloride and one part of 3·8 per cent sodium citrate.

Measurement of factor V.

Two methods have been used throughout this thesis.

Method (1).

This is a modification of that used by McClaughry and Seegers (1950) and is described by Douglas and Biggs(1953). The fraction containing factor V is prepared from the normal plasma and the test specimen under identical circumstances. Each plasma is treated with the same amount of alumina for the same duration of time. The factor V fraction from each is made as described above by ammonium sulphate precipitation

Figure (5)

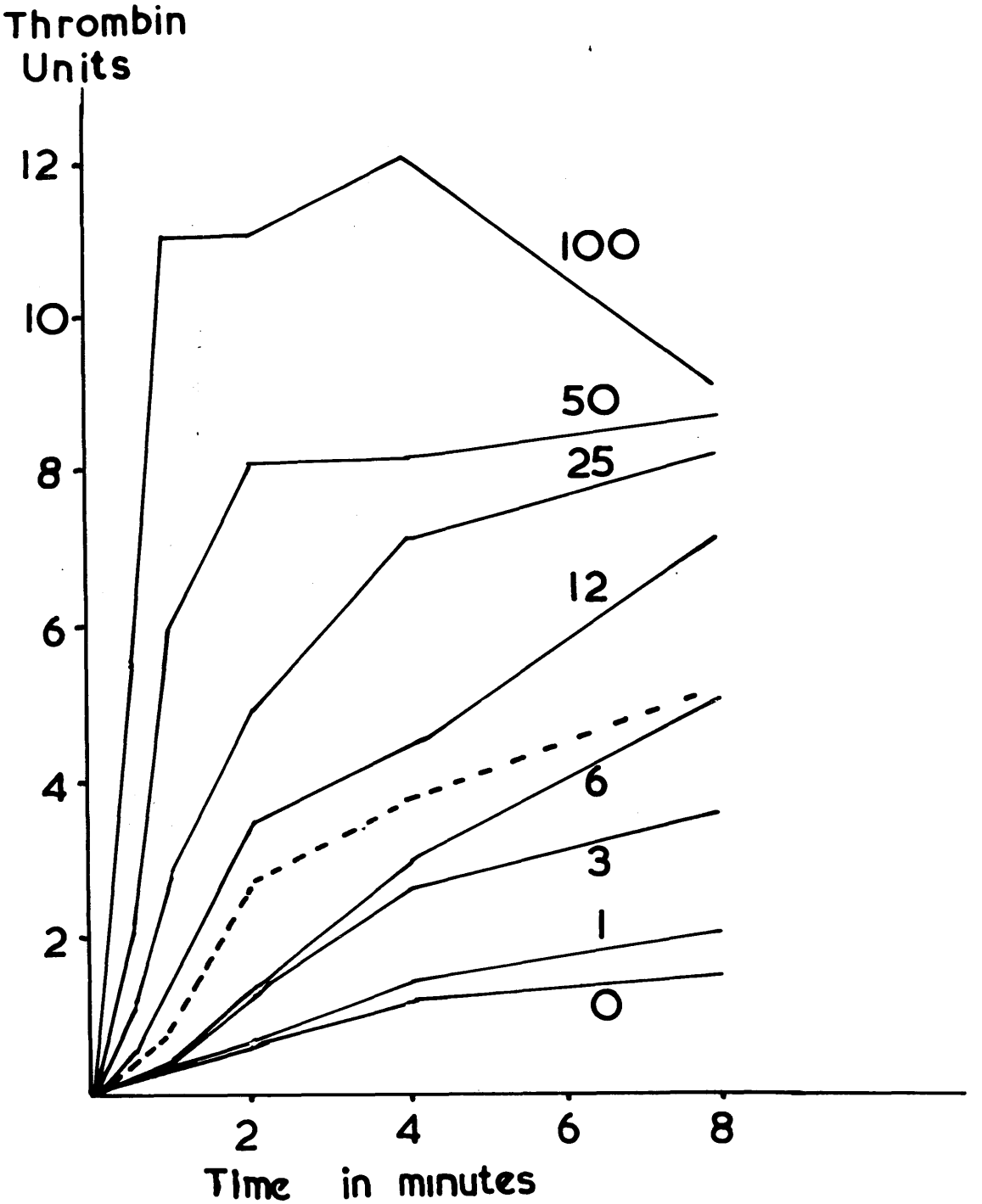
Figure (5)

Factor V assay by the activation of isolated prothrombin.

Ordinate - Thrombin units.

Abscissa - Time in minutes after recalcification of the incubation mixture.

The factor V fraction is prepared from a normal and the test specimen. "Pure" prothrombin is then activated by brain and calcium and various concentrations of factor V from the normal (continuous lines) - 100% to 0% and from the test specimen (discontinuous line). The test specimen is at the same concentration as the 100% normal. The test specimen contains between 6 and 12 per cent of factor V as compared with the control specimen.


Thrombin
Units
12
10
8
6
4
2
100
50
25
12
6
3
1
0
2
4
6
8
Time in minutes

and dialysed for the same length of time. Both preparations of factor V are then diluted one in twenty, with saline. In the test system the one in twenty dilution of the normal is called 100% of factor V and from this dilutions of factor V are made from 50% to 1·6% by doubling dilutions. The test specimen is examined at the original dilution of one-in-twenty. Prothrombin is then activated by brain extract and calcium in the presence of the preparations of factor V. An example is shown in figure 5 (see also appendix page). The continuous lines show the speed and amount of thrombin produced under the influence of varying concentrations of factor V. The unknown at one-in-twenty is shown as a discontinuous line and contains between 6 per cent and 12 per cent of factor V. The dilutions of factor V are kept in a refrigerator which is adjacent to the bench on which the water bath is situated. If the factor V has to be kept on the bench it should be placed in a bath of melting ice.

In later years the method was modified so that only the fifth and sixth minute points were taken. The time involved in the assay was thus reduced. There was thereby less chance of changes in the reactivity of reagents used between the start and the finish of the experiment.

Figure (6)

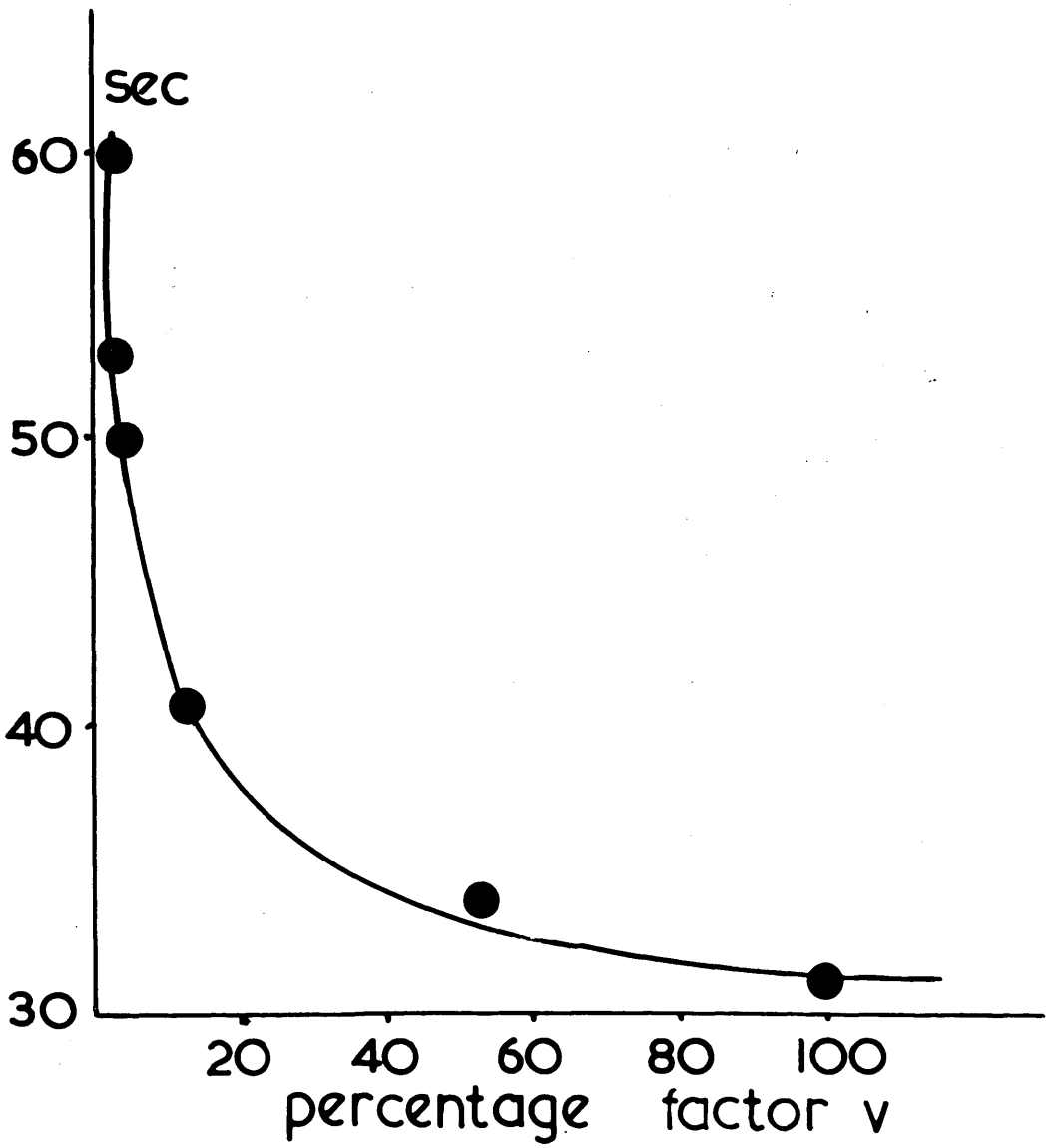
Figure (6)

Factor V assay by the correction of stored oxalated plasma.

Ordinate - clotting time of plasma - mixtures of normal and stored oxalate plasma.

Abscissa - percentage of fresh normal plasma in the stored oxalate plasma.

Mixtures were made of fresh normal plasma in stored oxalate plasma and the one-stage clotting times recorded. These clotting times were then plotted against the concentration of factor V.


sec
60
50
40
30
20
40
60
80
100
percentage factor v

Method (2).

Normal blood was collected into 0.1 m. sodium oxalate on the day before the factor V assay was to be made (9 parts of blood and 1 part of oxalate). The plasma was collected by high speed centrifugation and placed in a water bath at 37° C. overnight. This caused very marked prolongation of the one-stage clotting time due to loss of factor V. This factor V deficient plasma is used for the assay. Mixtures are made containing one part of normal plasma and nine parts of factor V deficient plasma and doubling dilutions of this mixture made into factor V deficient plasma. A mixture is also prepared containing one part of the test plasma and nine parts of the factor V deficient plasma. One-stage clotting times are done on these mixtures. Using the specimens containing the varying amounts of normal a curve can be drawn to be used for the assay - see figure 6 .

Other factors probably identical with factor V - Thrombogen.

Much other work supports Owren's findings. In 1908, 1928 and 1945 Nolf claimed that his thrombozyme (prothrombin of other workers) when isolated from plasma by adsorption on $Ca_3 (PO_4)_2$ could not be activated to thrombin by tissue thromboplastin and calcium chloride alone, but required a substance from the adsorbed plasma which he called "thrombogen". It is probable that Nolf's thrombogen is identical with

factor V.

Labile Factor.

In 1943 Quick observed that his one-stage test progressively lengthened when oxalated plasma was stored. Quick found that a mixture of equal parts of stored human oxalated plasma and plasma from a dog under the influence of dicumarol had a one-stage clotting time at least as good as normal. He further observed that aluminium hydroxide adsorbed little of this new factor. The factor which Quick observed to disappear in stored oxalated plasma was named by him as the "labile factor". Quick's labile factor is believed to be the same as Owren's factor V. Citrated plasma as distinct from oxalated plasma does not show this phenomenon to nearly the same extent. The factor is much more labile in the presence of oxalate than it is in the presence of citrate. (Fahey, Ware and Seegers 1948). Quick and Stefanini (1948) described the method for the measurement of the labile factor which is dependent on the ability to correct the prolonged one-stage clotting time of stored oxalated plasma. Method (2) described above is a modification of this technique.

Observations of Munro & Munro (1947).

These workers found that raising the pH of plasma to

10.5 altered its one-stage clotting time. This defect could be corrected by the addition of alumina-treated plasma. This phenomenon is probably caused by the destruction of factor V by change of pH.

Accelerator Globulin; Prothrombin accelerator

Ware and Seegers (1948 a & b) described accelerator globulin (ac-globulin) and Fantl and Nance (1946, 1948) described prothrombin accelerator; these factors are believed to be identical with factor V. Both these groups of workers showed that prothrombin, prepared by adsorption on inorganic precipitates and elution, required their respective factor for rapid conversion to thrombin.

The synonyms for factor V may be summarized as follows:

Coagulation components probably identical with factor V

Proaccelerin and accelerin (re-named by Owren 1951)

Labile factor (Quick 1943)

Accelerator of prothrombin conversion (Fantl & Nance 1946)

Plasma accelerator globulin (Ware & Seegers 1948)

Properties of Factor V

The material prepared by ammonium sulphate precipitation of alumina adsorbed normal plasma is free from prothrombin, other coagulation factors adsorbed on alumina, and most of the fibrinogen, antihaemophilic

Figure (7)

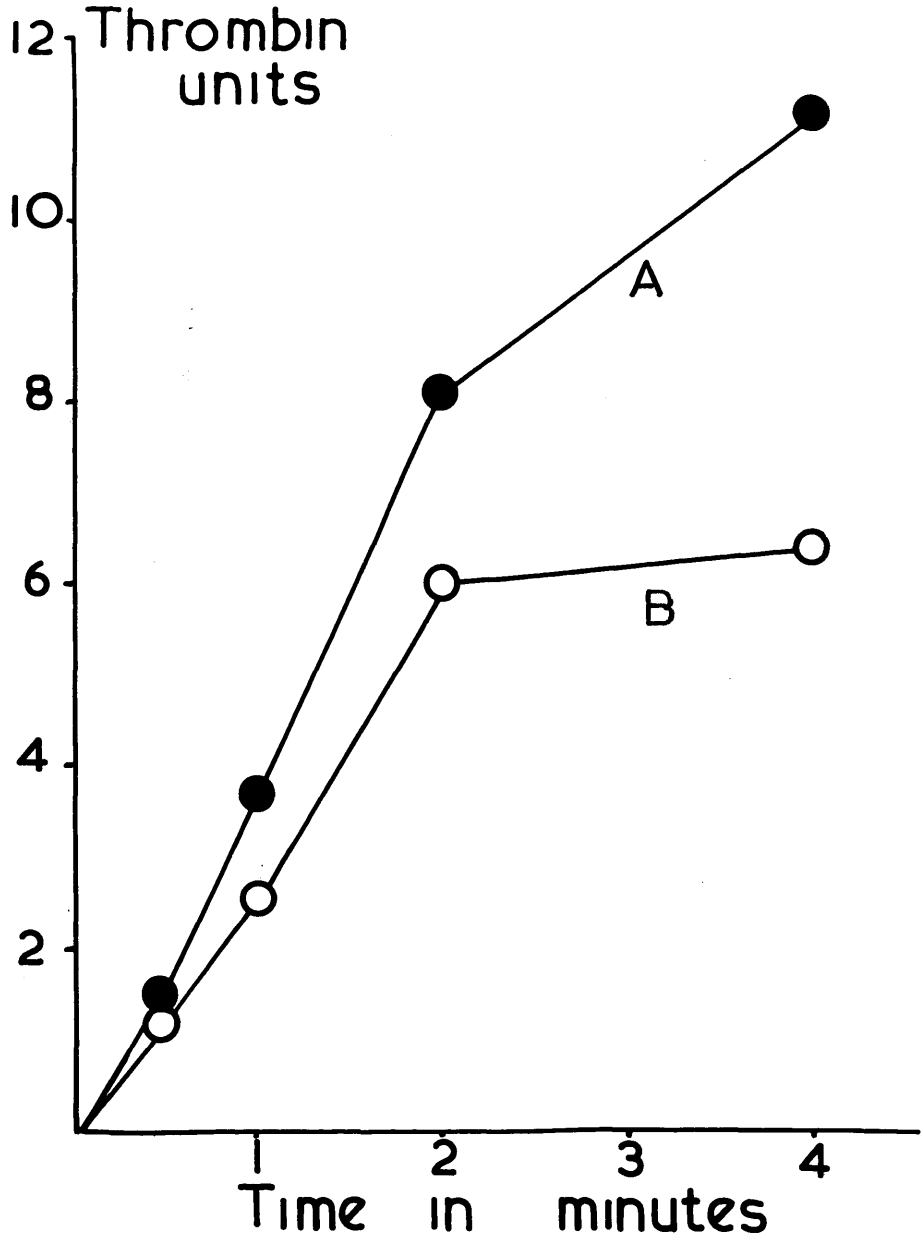
Figure (7)

Lability of factor V activity in solution.

Ordinate - Thrombin units.

Abscissa - Time in minutes after recalcification of incubation mixture.

This experiment shows the impaired ability to activate prothrombin by the same preparation of factor V, after its storage at bench temperature for three hours. ●—A—● represents the activation by the fresh factor V and O—B—O is the result from the same specimen after 3 hours at bench temperature.


12
Thrombin units
10
8
6
4
2
A
B
1
2
3
4
Time in minutes

globulin and antithrombin. It is obvious however that the factor V is not pure in any other sense. Factor V when prepared is unstable, which complicates its use in experimental work. For example if an experiment takes 3 hours to complete and the factor V, left at room temperature on the bench, may give different results at the end of the experiment from those at the start. In Fig. 7 is shown the results obtained by fresh factor V and by the same preparation kept for 3 hours at room temperature, in their comparative ability to activate prothrombin. Curve A is the activation by the fresh factor V and Curve B is the result by the 3 hours' specimen. (See page 613 of the appendix).

Role of factor V in blood coagulation.

It is not proposed to discuss this finally at the present time as it has to be considered in relation to blood thromboplastin. For the present it has been shown that this factor V is required for the conversion of prothrombin at optimal speed under the influence of tissue extract and calcium. According to Owren this factor enters the reactions involved in prothrombin conversion after these have started. In this concept factor V is an accelerator of prothrombin conversion. Biggs, Douglas and Macfarlane (1953) have produced evidence to suggest that the factor V is involved in reaction with tissue extract before prothrombin conversion begins.

Occurrence of factor V.

Factor V is present in different amounts in the plasma of different species. The concentration decreases in the following order: rabbit > dog > cat > cow > rat > guinea pig > man > chicken > turtle. Human plasma therefore has a relatively small supply in comparison with other animals. (Seegers and Ware 1949). This is mentioned to illustrate the difficulty of interpretation of the human coagulation mechanism by animal experiments. For this reason human blood and plasma products are used throughout this work. For example factor V is completely consumed during the clotting of human blood, but bovine serum still has much factor V after coagulation is complete (Alexander, Goldstein and Landwehr 1951). This failure of bovine blood to consume all its factor V probably caused confusion to Seegers and his co-workers when they claimed that serum contains a coagulation component serum ac-globulin - an active form of their plasma ac-globulin. Suspensions of platelets and platelet extracts have an activity resembling factor V when tested in a system containing prothrombin brain thromboplastin and calcium. This activity cannot be washed off platelets (Ware, Fahey and Seegers 1948). A similar observation is shown in Figure 8 and described in detail in the appendix.

Figure (8)

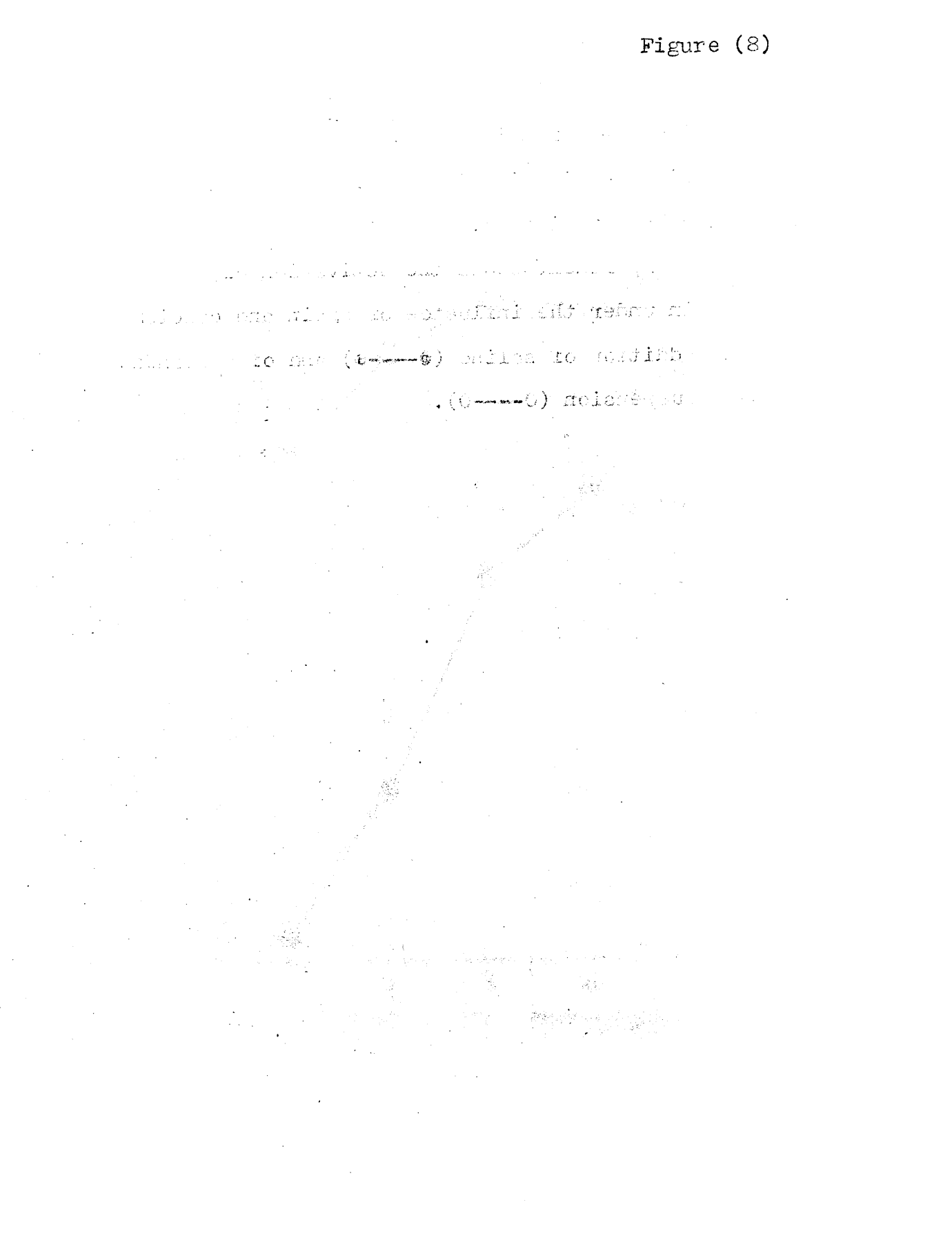


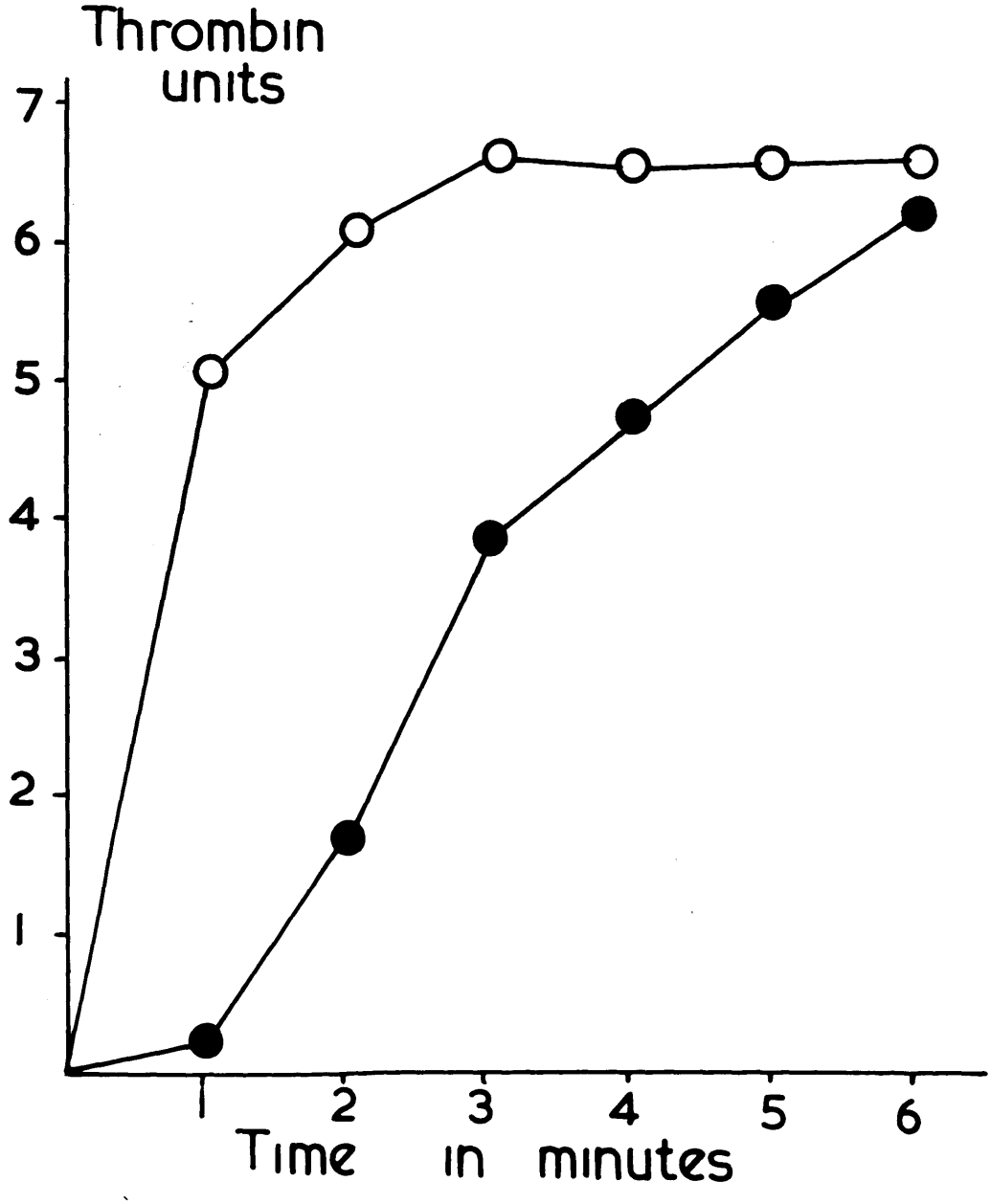
Figure (8)

Factor V activity of platelets.

Ordinate - Thrombin units.

Abscissa - Time in minutes after recalcification of the incubation mixture.

This experiment shows the activation of prothrombin under the influence of brain and calcium with the addition of saline (●——●) and of a washed platelet suspension (O——O).


Thrombin
units
7
6
5
4
3
2
1
1
2
3
4
5
6
Time in minutes

Incidence of factor V deficiency.

Factor V deficiency is rare in clinical practice. It occurs as an inherited constitutional abnormality or as an acquired defect complicating parenchymatous hepatic disease (Owren 1949, Stefanini 1950). It has been reported by Koller et al (1950), to cause a haemorrhagic state complicating scarlet fever.

There have been 46 cases (involving 13 families) of constitutional deficiency of factor V reported - so called parahaemophilia. Inheritance is from one generation to the next both sexes being affected. An incompletely dominant gene is the most likely method of transmission (Owen & Cooper 1955)

Factor VI or serum accelerator globulin.

Owren believes that factor V activity changes to an active form during clotting which he originally called factor VI. In his most recent terminology factor V corresponds to pro-accelerin and factor VI to accelerin. Similarly Seegers and his colleagues believe that their plasma ac-globulin changes from the inactive form to the active serum ac-globulin during clotting. The experimental evidence quoted in support of these concepts may have more than one interpretation and the question is left at that for the present. The terms are mentioned as they are not infrequently used in the literature on blood coagulation.

Factor VII.

Sweet clover disease in cattle and its treatment with serum.

Thirty years ago Schofield in Alberta in Canada and Roderick in North Dakota were studying haemorrhagic sweet clover disease in cattle (Schofield 1924; Roderick 1931; Link 1943-44). Roderick established that the prothrombin activity of the blood was reduced and that the extent of the diminution was parallel to the severity of the haemorrhagic state. The prothrombin was estimated by Howell's acetone method (Howell and Cekada 1926); this technique involved the precipitation by acetone, of the prothrombin freed from antithrombin. This method was sound in principle. Roderick observed that serum from unaffected cattle had considerable therapeutic value in the treatment of the condition. The following is a quotation from Roderick's paper:- "the writer found that the intravenous injection of freshly defibrinated bovine blood from a normal animal which was not fed on sweet clover resulted in the prompt recovery of many animals which already showed the effects of serious haemorrhage." "The results were equally favourable whether the blood is injected within 10 minutes after it is drawn or if several hours elapse before transfusion. Normal citrated blood is likewise effective."

It is widely accepted that serum, which has stood for

several hours contains no appreciable amounts of prothrombin. Why then did serum have a therapeutic effect on the management of sweet clover disease?

The effect of normal serum on the one-stage clotting time of dicumarol plasma. Discrepancies in the assay of prothrombin.

Owen and Bollman reported on the "Prothrombin Conversion Factor of Dicumarol Plasma". They administered dicumarol to dogs and performed Quick's one-stage test on the plasma and compared the results with those obtained by the two-stage test of Warner, Brinkhous and Smith (1936). It is now appreciated that neither of these methods gives a true assay of prothrombin, but nevertheless some important conclusions were drawn.

(a) At certain times after the administration of dicumarol the two-stage test gave a much higher level of prothrombin than did the one-stage test.

(b) Mixtures of 9 parts of dicumarol plasma and 1 part of normal plasma caused a greater rise in prothrombin percentage as estimated by the one-stage test than the theoretical rise should have been.

(c) One part of normal serum, free from prothrombin, mixed with nine parts of dicumarol plasma was also effective in shortening the one-stage clotting time.

Macmillan (1948) made similar observations. The experiments of Mawson 1949 indicated that there might be an

abnormality in dicumarol plasma other than the deficiency of prothrombin.

The most significant of these observations was the finding that normal serum which contains no prothrombin was able to shorten the one-stage clotting time of dicumarol plasma. This was very suggestive evidence that there was a defect in dicumarol plasma other than the deficiency of prothrombin.

Factor VII. The property of normal serum to correct the one-stage clotting time of dicumarol plasma is referred to as its factor VII activity; the term factor VII was introduced by Koller, Loeliger, and Duckert who made an instructive study of its properties. Ox plasma was filtered through a Seitz filter with a specified amount of asbestos and a preparation of prothrombin made from the filtrate by adsorption on barium sulphate and elution. This prothrombin was freed from factor V by the barium sulphate adsorption but even on the addition of optimal amounts of factor V, brain thromboplastin and calcium could not be activated satisfactorily to form thrombin. The factor VII was shown to be necessary for the formation of thrombin at optimal speed but did not increase the amount of thrombin formed. Koller et al describe several other features of factor VII.

(a) its concentration in plasma and in serum is the same.

(b) it is relatively stable on storage.

(c) separation of prothrombin from factor VII. The authors state that if human or bovine plasma is filtered slowly through an asbestos filter (containing 20% asbestos) and then through another filter containing 30% asbestos,- the filtrate is free of factor VII but still contains some prothrombin (up to 3% if human and 10-20% if bovine) in terms of the prothrombin content of normal human plasma.

(d) The test systems used for the study were:

Measurement of factor VII.	0.1 cc. 1/10 test plasma	
	0.1 cc. asbestos filtered ox plasma (see above)	(Factor V (Prothrombin (Fibrinogen (No factor VII.
	0.1 cc. m/40 $CaCl_2$	
	(Limiting factor = factor VII)	
Measurement of prothrombin.	0.1 cc. 1/10 test plasma.	
	0.05 cc. ox plasma treated with tricalcium phosphate	(Factor V (Fibrinogen
	0.1 cc. oxalated human serum or preparation of factor VII.	
	0.1 cc. m/40 $CaCl_2$	
	(Limiting factor = prothrombin)	

(Koller, Loeliger, Duckert, 1951)

Synonyms of Factor VII.

The term factor VII has been adopted throughout the thesis in consequence of the experimental work mentioned above.

Other investigators have described experimental phenomena, which can probably be explained as due to the coagulation component described by Koller, Loeliger and Duckert. In consequence certain synonyms of factor VII may be found in the literature.

(a) S.P.C.A. (Serum prothrombin conversion accelerator)
Alexander and co-workers (Boston U.S.A.)

(b) Co-thromboplastin.
Mann and co-workers. (Mayo Clinic U.S.A.)

(c) Proconvertin and convertin.
Owren and co-workers. (Oslo, Norway).

(d) Serum factor of Jacox.
(Jacox, U.S.A.)

(e) Free prothrombin.
(Quick, U.S.A.)

Serum prothrombin conversion accelerator (S.P.C.A.)

(Alexander, B., de Vries A. and Goldstein, R. 1949 a.b.c.d.
(Alexander, B., Goldstein R. and Landwehr, G. 1950, 1951)
(Alexander & Landwehr (1949) De Vries, Alexander and Goldstein, 1949)

The essential observation made was as follows:-

"Plasma prothrombin activity" was measured; plasma was treated with an inorganic precipitate barium sulphate. The supernatant plasma contains factor V but not prothrombin. A mixture was made containing nine parts of barium sulphate-treated plasma and one part of normal plasma.

0.1 cc. normal plasma
0.9 cc. $BaSO_4$ plasma

The mixture is used as in Quick's one-stage test.

0.1 cc. of above
0.1 brain thromboplastin
0.1 m/40 $CaCl_2$

The mixture containing one part of normal plasma and nine parts of $BaSO_4$ plasma is called 100% prothrombin activity. Normal plasma is further diluted with $BaSO_4$ plasma and a curve of prothrombin activity computed.

Serum prothrombin activity.

The test serum was oxalated and the prothrombin activity determined in the same way.

0.1 ml serum)
0.9 ml $BaSO_4$ plasma) were mixed

0.1 ml of above
0.1 ml Brain thromboplastin
0.1 ml m/40 $CaCl_2$

In one example this proved to be 10%. The S.P.C.A. activity of this serum was estimated as follows:-

0.05 ml normal plasma (100%)
0.05 ml oxalated test serum (10%)
0.9 ml $BaSO_4$ plasma

Theoretically this contains 55%
Observed value = 120%

Enhancement 120 - 55 = 65%

$$\% \text{ Enhancement} = \frac{65}{55} \times 100$$

$$= 118\%$$

This property of enhancement is referred to as due to the S.P.C.A. content of the serum.

The results on this example were:-

Oxal Plasma	Serum	Saline	$BaSO_4$ plasma	Clotting time
0.05	0	0.05	0.90	41.4"
0	0.40	0	0.60	55"
0.05	0.05	0	0.90	24.6"

(Volumes in ml)

Alexander and his colleagues described the properties of S.P.C.A. It is adsorbed from serum by barium sulphate or a Seitz filter and is reduced in concentration after the administration of the coumarin drugs.

Co-thromboplastin (Mann (1949), Mann, Barker and Hurn (1951), Mann and Hurn (1951, 1952), Mann, Hurn and Barker, (1951), Mann, Hurn and Magath (1947), Mann, Mann and Bollman (1950)).

Hurn, Barker and Mann (1947) observed that dicumarol plasma had a higher prothrombin content when measured by a two-stage test than when examined by Quick's one-stage test. While using their two-stage technique they observed a striking initial delay in thrombin formation in dicumarol plasma (Hurn and Mann 1947). This suggested to them that a preliminary reaction was occurring prior to prothrombin conversion (Mann 1949).

The test system employed to study this preliminary reaction was as follows:

1 cc. of brain and calcium were incubated at room temperature with

0.5 cc. of 1/100 plasma or serum (test or control)

At 3 minute intervals:

0.15 of above added to 0.05 of 1/25 defibrinated plasma and one minute later 0.2 of fibrinogen added and clotting time recorded. The observed clotting times were measures of the amounts of thrombin formed in one minute from the defibrinated plasma under the influence of the thromboplastin previously treated.

The amount of thrombin formed at one minute was large as compared with that from the untreated thromboplastin. The thrombin generated from the prothrombin in the plasma incubated with the brain and calcium was unrecordable and therefore the one minute yield under the conditions of the test was said to be a measure of "co-thromboplastin". The clotting time at one minute is transformed into thrombin units. The measure of percentage co-thromboplastin activity is then as follows:-

$$\frac{\text{One minute thrombin yield of unknown}}{\text{One minute thrombin yield of control}} \times \frac{\text{Dil.of unknown}}{\text{Dil.of control}} \times 100$$

= Percentage co-thromboplastin activity.

Co-thromboplastin can be prepared from serum in which it is stable and free from prothrombin. It is adsorbed by calcium phosphate and eluted with sodium citrate. Co-thromboplastic activity is reduced following the administration of the coumarin drugs and this conversion defect can be corrected by the co-thromboplastin preparation from normal serum. The coumarin drugs produce a greater depression of co-thromboplastin than of prothrombin. The name co-thromboplastin was chosen because the authors believe that their factor reacts with tissue thromboplastin before prothrombin enters the reaction.

Proconvertin (Owren and co-workers).

Owren, P.A. (1947, 1951), Owren and Aas (1951), Owren and Bjirkelund (1949).

Owren was probably first to appreciate that there was in addition to factor V, a further coagulation component needed for the conversion of prothrombin to thrombin in the presence of brain and calcium. In 1947 he called this co-factor V. Prothrombin and co-factor V were separated by fractional adsorption with Seitz filters, because the co-factor V was more easily adsorbed than prothrombin. Prothrombin purified in this way, showed slow incomplete thrombin formation by the addition of thromboplastin, factor V and calcium. By the addition of co-factor V the thrombin formation proceeded rapidly. Co-factor V was renamed proconvertin.

These experiments are essentially the same in principle as those described by Koller.

Observations of Jacox (1949) (Jacox 1949, Jacox and Bays 1949 1950)

Jacox (1948) observed that if brain and serum were incubated together then there was an increase in thromboplastic activity; this increase occurs rapidly, comes to a maximum and then declines. The mixture can be reactivated by the further addition of brain. This thromboplastic activity was tested by the ability to clot normal plasma when added together with calcium.

Inactive and free prothrombin (Quick 1943, Quick and Stefanini 1949)

In 1943 Quick found that when he mixed stored oxalated human plasma (with a prolonged one-stage clotting time) with dicumarol plasma the coagulation time of the mixture was normal. Quick stated that the prolongation of the clotting time of the stored oxalated human plasma was due to the disappearance of "labile factor" which he argues is identical with Owren's factor V.

Quick and Stefanini (1949) found that when normal oxalated plasma is stored in glass, the prothrombin time as determined by the one-stage procedure steadily increases, but when a small quantity of adsorbed plasma which is rich

is added
in factor V, the mixture will have a prothrombin time as low as 8 seconds which is shorter than that of fresh plasma which is 12 seconds. If the plasma is stored in silicone coated tubes it likewise loses its factor V activity but when adsorbed plasma is added, the prothrombin time is restored to only 12 seconds.

Quick says that the increase in prothrombin activity, which occurs in glass but not in silicone is not attributable to the labile factor but appears to arise from an actual increase of prothrombin itself. Quick and Stefanini have postulated that plasma contains prothrombin in a precursor state in addition to active prothrombin. For the conversion of the precursor a rough surface such as glass is required. Quick believes that in the one-stage test free or reactive prothrombin is determined, whereas in the two-stage procedure most of the inactive prothrombin (called prothrombogen) is converted to the active or free state. He believes that his one-stage test measures free prothrombin and that the two-stage measures total prothrombin (prothrombinogen and free prothrombin). Quick measures total prothrombin by the following technique; blood is collected into silicone tubes, chilled and then centrifuged at high speed to render it free from platelets. As soon as coagulation occurs the one-stage prothrombin time is determined as in Quick's prothrombin

test supplying a source of fibrinogen. This gives a measure of the total prothrombin. Quick has found that in dicumarol therapy the free prothrombin drops promptly but it requires a number of days before the total is significantly reduced.

The similarity between factor VII and Quick's free prothrombin is striking as is the similarity between his prothrombinogen and prothrombin.

S U M M A R Y

Prothrombin.

(a) this is the precursor of thrombin.

(b) it is prepared by adsorption on inorganic precipitates followed by elution; even the best preparations must contain other coagulation components.

(c) these preparations of prothrombin can be activated to thrombin by concentrated sodium citrate without any additional components being present.

Factor V.

(a) a short description is given of Owren's patient; this patient had a prolonged one-stage clotting time, which was reduced by the addition of normal plasma from which the prothrombin had been adsorbed by an inorganic precipitate. Eluates from this inorganic precipitate after treatment of normal plasma, failed to correct the abnormality in this patient. These observations led to the discovery of factor V.

(b) preparations of prothrombin cannot be activated to thrombin by brain thromboplastin and calcium unless factor V is present.

(c) factor V, free from other known coagulation components, can be prepared from normal adsorbed plasma by ammonium sulphate precipitation between 33 and 50% saturation.

(d) measurement of factor V.

The methods employed in this thesis are:-

(1) by comparing the activation of prothrombin by factor V prepared from the test and control specimens.

(2) by the ability to correct the one-stage clotting time of stored oxalated plasma.

(e) the synonyms of factor V are discussed - thrombogen, labile factor, accelerator globulin, prothrombin accelerator.

Factor VII

The term factor VII was introduced by Koller et al (1951); the important experimental observations suggesting this further coagulation factor may be enumerated as follows:-

(a) the addition of normal serum to dicumarol plasma produces a shortening of Quick's one-stage test.

(b) mixtures of dicumarol and normal plasma produce a higher percentage prothrombin as read off Quick's dilution curve than would be expected from the addition of the two.

(c) various two-stage methods record more prothrombin than the one-stage test in dicumarol plasma.

(d) reaction of dicumarol plasma to differing thromboplastins gave a different percentage of prothrombin. This was particularly so when Russel's viper venom plus lecithin was used as compared with brain.

(e) in a mixture of 1 part of normal plasma and 9 parts of $BaSO_4$-treated plasma the addition of normal serum produced a shortening of the one-stage clotting time. Such a system is not deficient in factor V. Dicumarol serum was less powerful than normal serum in this property.

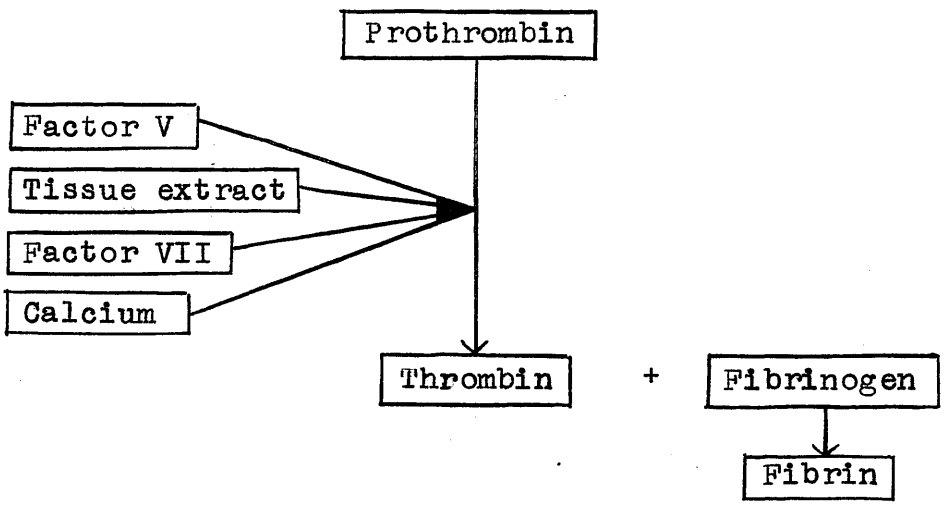
(f) If Brain, M/40 $CaCl_2$ and Serum are incubated together and then added to dicumarol plasma (with a long one-stage clotting time) collected during the early days of therapy there is a very marked shortening of the clotting time. This is much greater than the addition of these entities without pre-incubation. This inter-action of brain and serum is homologous and species specific.

(g) plasma, passed through a Seitz filter with specified asbestos content will contain prothrombin which cannot be activated properly in the presence of factor V, brain and calcium chloride but requires the presence of a factor present in normal serum (factor VII).

(h) the property of normal serum in the observations described above is found in serum free from prothrombin and can be adsorbed by Seitz filters, $Ca_3(PO_4)_2$, $BaSO_4$ or $Al(OH)_3$. It can be eluted from these with citrate-saline or phosphate buffer.

(i) dicumarol plasma and serum are deficient in this property as compared with normal plasma and serum.

The following diagram summarises the concept of blood coagulation, which resulted from the dissolution of the Classical Theory as outlined in Chapter 3.



REFERENCES

Alexander, B., and Landwehr, G. (1949). Evolution of a prothrombin conversion accelerator in stored human plasma and prothrombin fractions. Amer. J.Physiol. 159,322.

Alexander, B., Vries, D. de and Goldstein, R. (1949).
(a) A factor in serum which accelerates the conversion of prothrombin to thrombin. Blood. 4,272.

(b) II. Its evolution with special reference to the influence of conditions which affect blood coagulation. Blood. 4, 739.

(c) III. Its relationship to the coagulation defect of thrombocytopenic blood. Blood. 4, 747.

(d) V. The coagulation defect in haemophilia with particular reference to the conversion of prothrombin to thrombin and the evolution of the prothrombin conversion accelerator. Blood. 4, 752.

Alexander, B., Goldstein, R., and Landwehr, G. (1950). The prothrombin conversion accelerator of serum (S.P.C.A.): its partial purification and its properties compared with serum ac-globulin. J. clin. Invest. 39, 881.

_____(1951)

The labile factor of prothrombin conversion; its consumption under normal and pathological conditions affecting blood coagulation. J. clin. Invest.30, 252.

Biggs, R., Douglas, A.S., and Macfarlane, R. G. (1953). The action of thromboplastic substances. J. Physiol. 122, 554.

Bordet, J. and Delange, L. (1914). Analyse et synthese du processes de la coagulation. Ann. et Bull. Soc. Roy. de Sc. med. et nat. de Bruxelles. 72, 87.

Douglas, A.S. and Biggs, R. (1953). The consumption of some components involved in physiological blood coagulation. Glasg. med. J. 34, 329.

Fahay, J.L., Ware, A.G., and Seegers, W.H. (1948). Stability of prothrombin and ac-globulin in stored human plasma as influenced by conditions of storage. Amer. J. Physiol. 154, 122.

Fantl., P., and Nance, M.H. (1946). Acceleration of thrombin formation by a plasma component. Nature,158,708.

Fantl.,P., and Nance, M.H. (1948). The physiological activation of prothrombin. Med. J. Aust. 1, 128.

Howell, W.H., and Cekada, E.B. (1926). Cause of delayed clotting of haemophilic blood. Amer. J. Physiol.128,500.

Hurn, M., Barker, N., and Mann, F. (1947). Variations in prothrombin and antithrombin following the administration of dicumarol. Amer. J. clin. Path. 17,712.

Hurn, M., and Mann, F.D. (1947). The determination of prothrombin (2-stage method) and antithrombin using commercially available reagents. Amer. J. clin. Path. 17, 741.

Jacox, R.F. (1949). Studies on the activation of a serum 'prothrombin converting factor'. J. clin. Invest. 28, 492.

Jacox, R.F., and Bays, R.P. (1949). Studies on the 'thrombin' effect of fresh serum. Proc. Soc. exp. biol. Med. N.Y. 70,587

Jacox, R.F., and Bays, R.P., (1950). The relationship of plasma, prothrombin concentration and the degradation rate of serum prothrombin converting factor during dicumarol therapy. Blood. 5, 313.

Koller, F., Gasser, C., Krusi, G., and Muralt, G. de (1950). Purpura fulminans nach charlach mit Faktor V- Mangel und antithrombinuberschurs. Acta. Haemat, 4, 33.

Koller, F., Loeliger, A., and Duckert, F. (1951). Experiments on a new clotting factor (Factor VII). Acta Haemat. 6, 1.

Link, K.P. (1943-1944). The anticoagulant from spoiled sweet clover hay. The Harvey Lectures Series, 39,162.

McClaughry, R.I., and Seegers, W.H. (1950). Prothrombin, thromboplastin, ac-globulin and platelet accelerator quantitative inter-relationships. Blood, 4, 303.

MacMillan, R.L. (1948). Observations on the mechanism of action of dicumarol. Science.108, 416.

Mann, F.D. (1949). Co-thromboplastin assay. Amer. J. clin. Path. 19, 861.

Mann, F.D., Barker, N.W., and Hurn, M. (1951). The effect of dicumarol on co-thromboplastin, a factor in blood concerned with the conversion of prothrombin to thrombin. Blood, 6, 838.

Mann, F.D., and Hurn, M. (1951). Co-thromboplastin, a probable factor in coagulation of blood. Amer. J.Physiol. 164, 105.

Mann, F.D., and Hurn, M. (1952). Species specificity of thromboplastin: Role of co-thromboplastin reaction. Proc. Soc. exp. biol. Med.

Mann, F.D., Hurn, M., and Barker, N.W. (1951). Platelets and coagulation defect caused by dicumarol. Amer.J. clin. Path. 21, 814.

Mann, F.D., Hurn, M., and Magath, T.B. (1947). Observations on the conversion of prothrombin to thrombin. Proc.Soc. exp. Biol. Med. N.Y. 66, 33.

Mann, F.D., Mann, J.D., and Bollman, J.L. (1950). The coagulation defect of vitamin K deficiency compared with that caused by dicumarol. J. lab. clin. Med.36,234.

Mawson, C.A. (1949). The use of Russell viper venom and lecithin as thromboplastin in the estimation of prothrombin. J. lab. clin. Med. 34, 458.

Mellanby, J. (1930). Prothrombase - its preparation and properties. Proc. roy. Soc. London, S.B.107, 271.

Munro, M.P., and Munro, M.L. (1947). The reversible inactivation of prothrombin: a factor responsible for its partial reactivation. Amer. J. Physiol. 150,409.

Nolf, P. (1908). Contribution a l'étude de la coagulation du sang. Les facteurs primordiaux, leur origin. Arch. int. Physiol, 6, 1.

Nolf, P. (1928). Quoted by Pickering (1928), page 130.

Nolf, P. (1945). Le plasma phosphate, réactif de la coagulation. Arch. int. Pharmacodyn.et Ther. 70,5.

Owen, C.A., and Bollman, J.L.(1948). Prothrombin conversion factor of dicumarol plasma. Proc. Soc.Biol. Med. N.Y. 67, 231.

Owen, C.A. and Cooper, T. (1955). Parahaemophilia. Arch. intern. Med. 95, 194.

Owren, P.A. (1947). The coagulation of blood. Investigations on a new clotting factor. Acta. med. Scan. Suppl.194.

Owren, P.A. (1949). The diagnostic and prognostic significance of plasma prothrombin and factor V levels in parenchymatous hepatitis and obstructive jaundice. Scand. J. clin. lab. Invest. 1,131.

Owren, P.A.(1951). Proconvertin, the new clotting factor. Scand. J. clin. lab. Invest. 3, 168.

Owren, P.A., and Aas, K. (1951). The control of dicumarol therapy, and the quantitative determination of prothrombin and proconvertin. Scand. J. clin. lab. Invest, 3, 201.

Owren, P.A., and Bjirkelund, C.J. (1949). A new previously unknown clotting factor. Scand. J. clin. lab.Invest. 1, 162.

Pickering J. W. (1928). Monographs of Medical and Surgical Science. Heinemann, London. The Blood Plasma in Health and Disease.

Quick, A.J. (1935). The prothrombin in haemophilia and in obstructive jaundice. J. biol. Chem.109,33.

Quick, A.J. (1943). On the constitution of prothrombin. Amer. J. Physiol. 140, 212.

Quick, A.J., and Stefanini, M. (1948). The concentration of the labile factor of the prothrombin complex in human, dog and rabbit blood: its significance in the determination of prothrombin activity. J. Lab. clin. Med. 33, 819.

Quick, A.J., and Stefanini, M. (1949). The concentration of component A in blood, its assay and relation to the labile factor. J. Lab. clin. Med. 34, 973.

Roderick, L.M. (1931). Blood coagulation in sweet clover disease of cattle. Amer. J. Physiol. 96, 413.

Schofield, F.W. (1924). Damaged sweet clover. The cause of a new disease in cattle simulating haemorrhagic septicaemia and blackleg. J. Amer. vet. med. Assn. 64, 553.

Seegers, W.H., McClaughry, R.I., and Fahey, J.L.(1950). Some properties of purified prothrombin and its activation with sodium citrate. Blood, 5, 421.

Seegers, W.H. (1952). The purification of prothrombin. Recordof Chemical Progress.

Seegers, W.H., Smith, H.P., Warner, E.D., and Brinkhous,K.M. (1938). Purification of prothrombin. J. biol. Chem. 123, 751.

Stefanini, M.(1950). The haemorrhagic diathesis of liver dysfunction and obstructive jaundice. International Society of Haematology. Third International Congress, Cambridge, p. 484.

Vries, A. de, Alexander, B., and Goldstein, R. (1949). A factor in serum which accelerates the conversion of prothrombin to thrombin. I. Its determination and physiologic and biochemical properties. Blood. 4, 247.

Ware, A.G., Fahey, J.L., and Seegers, W.H. (1948). Platelet extracts, fibrin formation and interaction of purified prothrombin and thromboplastin. Amer. J. Physiol.154,140.

Ware, A.G., and Seegers, W.H. (1948a). Serum ac-globulin - formation from plasma ac-globulin. Role in blood coagulation: partial purification; properties and quantitative determination. Amer. J. Physiol. 152,567.

Ware, A.G., and Seegers, W.H. (1948b). Plasma accelerator globulin: partial purification quantitative determination and properties. J. biol. Chem. 172, 699.

Ware, A.G. and Seegers, W.H. (1948). Studies on prothrombin: Purification, inactivation with thrombins and activation with thromboplastin and calcium. J. biol. Chem. 174, 565.

Warner, E.D., Brinkhous, K.M., and Smith, H.P. (1936). Quantitative study on blood clotting: Prothrombin fluctuations under experimental conditions. Amer. J. Physiol. 114, 667.

Part II. The Components of Blood Thromboplastin

CHAPTER 4

BLOOD THROMBOPLASTIN

CONTENTS.

CHAPTER 4

BLOOD THROMBOPLASTIN

The investigations of Biggs, Douglas and Macfarlane (1953 a.b.c.) resulted in a new understanding of blood thromboplastic activity. Much of the work described in this thesis is the application of these advances to problems of defective haemostasis in clinical practice.

When blood is withdrawn by clean venepuncture and half delivered to a clean test tube and half delivered to a test tube containing some tissue extract the blood in the clean tube takes 5-10 minutes to coagulate whereas in the tissue extract tube it clots in a few seconds. Until recent years it had been assumed from this that the tissue thromboplastin was a powerful mechanism and that the blood thromboplastin was weak, if present at all. Some believed that the long clotting time of whole blood was due to minimal contamination with tissue. By changing of syringes and collection of a second specimen after discarding the first collection, significant admixture with tissue thromboplastin can be avoided. It was likely therefore that blood did contain a thromboplastin system of its own. Since blood remains fluid within the vessels it was inferred that this intrinsic or blood thromboplastin was very feeble or did not exist in an active form.

The existence of an inactive precursor of blood thromboplastin was postulated by Collingwood and MacMahon (1912) who called it "prothrombokinase" and believed that it was derived from platelets. Macfarlane (1942) suggested that a plasma factor and a lipoid reacted together on contact with a foreign surface to produce active thromboplastin. Quick (1947) and Brinkhous (1947) both carried out experiments suggesting that thromboplastin was produced by the interaction of the platelets with "antihaemophilic globulin", the plasma factor lacking in haemophilia. Quick (1947) has called this plasma factor "thromboplastinogen" believing it to be the precursor of thromboplastin and that it is activated by platelets.

A number of attempts have been made in the past to prepare thromboplastin from human blood; the results of these all suggested that blood thromboplastin was weak. The platelets were found by Ware, Fahey and Seegers (1948) to contain "only a small amount of thromboplastin if any". Antihaemophilic globulin alone has no thromboplastic action. By mixing platelets with antihaemophilic globulin Ferguson (1949) and Shinowara (1951) were able to produce thromboplastic activity but it was considerably less than that of tissue extracts. This finding was apparently in keeping with the long normal clotting time of whole blood of 5-10 minutes as

Figure (9)

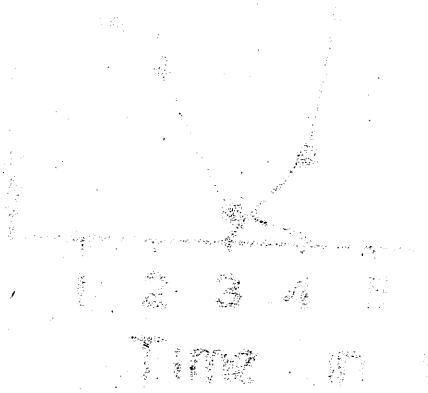


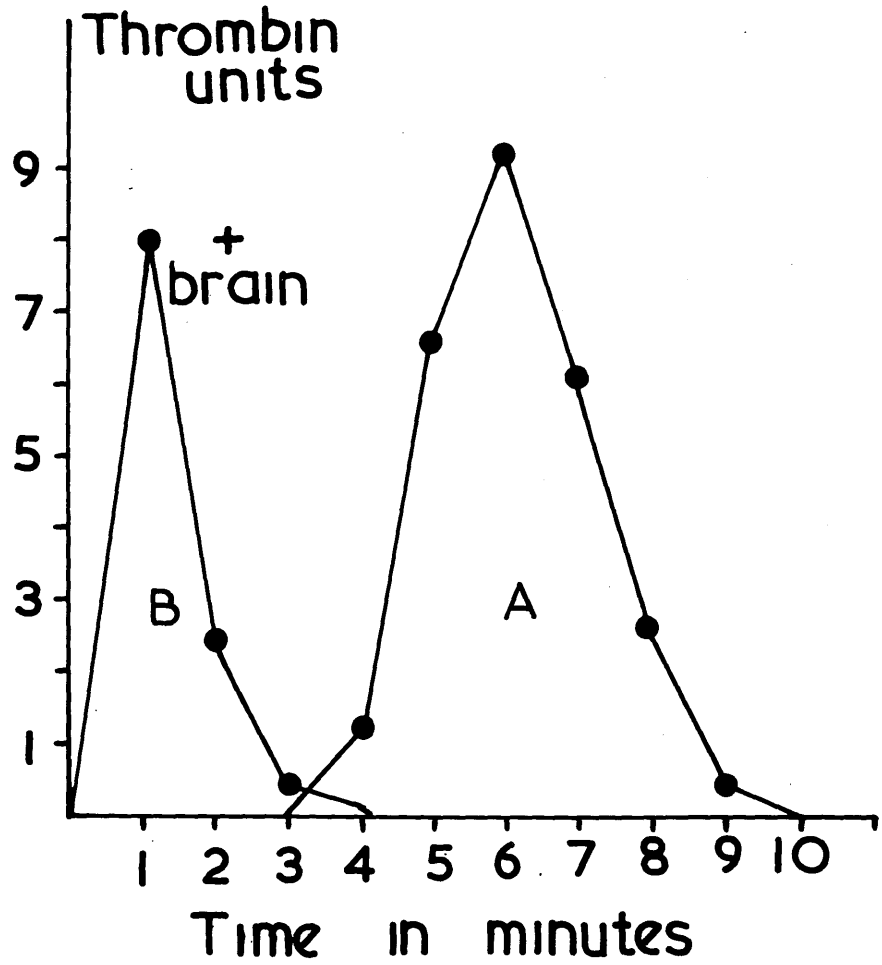
Figure (9)

Thrombin generation from whole blood with and without added brain.

Ordinate - Thrombin units.

Abscissa - Time in minutes after delivery of the whole blood to the centrifuge tubes.

This experiment shows the generation of thrombin from whole blood delivered to glass centrifuge tubes in one of which there was some brain thromboplastin. The presence of the brain reduces the delay in the formation of the thrombin from the whole blood.


Thrombin units
9
7
5
3
1
+ brain
B
A
1 2 3 4 5 6 7 8 9 10
Time in minutes

compared with 12-15 seconds when an optimal amount of tissue thromboplastin is added to the blood. If a preparation of brain thromboplastin is diluted and a series of clotting times recorded with these dilutions, it can be deduced that the normal blood clotting time of 5-10 minutes is the equivalent of a tissue thromboplastic activity of not more than a 1/100,000.

Thrombin Generation from whole blood.

When whole blood is placed in a glass tube and the concentration of thrombin therein is determined at intervals by transferring aliquots into fibrinogen, during the first few minutes no thrombin appears; then there is a sudden generation of thrombin which causes rapid clotting and a steeply rising thrombin concentration followed by a decline in concentration as inactivation by antithrombin overtakes the rate of thrombin formation. These observations were described by Macfarlane and Biggs (1953). An experiment based on these lines is shown in Figure 9 given in detail in the appendix, page 616 . Curve A shows the formation of thrombin from the blood under the influence of its own thromboplastin. Curve B represents the results when the 0.2 saline in A was replaced by 0.2 brain extract.

These observations do not support the old concept of the presence of a feeble thromboplastin slowly producing thrombin

over a period of minutes sufficient eventually to cause coagulation; they suggest rather that thromboplastin does not appear until just before clotting. Since the rate of thrombin generation once started is almost as rapid in untreated blood as it is when the optimal amount of tissue extract is added, it seems likely that the intrinsic blood thromboplastin once formed is as potent as the tissue preparation. From these observations it could be concluded that the relatively long clotting time of whole blood was likely to be due to a delay in the formation of blood thromboplastin not to the weakness of this once formed. The short clotting time produced by tissue extracts was likely to be due to the avoidance of delay in its action.

In consequence of these observations an attempt was made to prepare this blood thromboplastin from reagents derived from blood. To obtain conclusive results these components had to be freed from prothrombin as the activity of the product was tested by its ability to clot plasma. It was important that the activity of any incubation mixture was not attributable to thrombin formed in it.

At the outset, it was likely that two components of this blood thromboplastin were platelets and the plasma factor missing in haemophilia. When normal clotted blood is maintained at 37° C. in a water bath the prothrombin

disappears over the following hour so that the serum after one hour contains only trace amounts of prothrombin - that is the prothrombin is completely consumed. It is believed that this conversion of prothrombin to thrombin is the consequence of satisfactory blood thromboplastin formation. Where there is failure to form blood thromboplastin then there is failure to convert prothrombin to thrombin i.e. the consumption of prothrombin is defective. In thrombocytopenia and haemophilia the consumption of prothrombin is defective.

Platelets. In 1912 Bordet and Delange found that the plasma of birds freed from platelets became incoagulable. Rabbit plasma collected into paraffin coated containers and freed from platelets by centrifuging clotted very slowly and in the resulting serum there was much residual prothrombin even when fibrin formation was complete. Later workers (Quick, 1947, Brinkhous, 1947, Soulier 1950 and Merskey 1950) have confirmed that there is defective prothrombin consumption in thrombocytopenia.

Platelet suspensions can be prepared by differential centrifugation. Plasma is collected into graduated centrifuge tubes where the surfaces have been treated with silicone. The technique for the siliconing of glassware is described in the appendix. The blood is collected into citrate, in the standard proportions - 1 ml. of 3.8% sodium citrate and

9 ml. of blood. This blood is centrifuged at 500 r.p.m. for 30 minutes and the supernatant platelet-rich plasma separated. This plasma is then centrifuged in silicone-treated containers at 5000-10,000 revs. for 5-10 minutes in the high speed attachment of the centrifuge. The platelet free plasma is decanted and the "plug" of platelets washed twice in saline with resuspension aided by wooden applicator sticks. The preparation of platelet suspensions is described in greater detail in the appendix, page 592. When this suspension of platelets is used in place of brain extract in the one-stage test there is no significant thromboplastic activity.

Antihaemophilic Globulin.

Haemophilic plasma has a normal one-stage clotting time. The reaction with a high concentration of tissue extract is normal. The whole blood clotting time is often prolonged as is the recalcification time. Prothrombin consumption has been shown to be defective by Brinkhous (1939), Quick (1947), Soulier (1950) and Merskey (1950). From this it was concluded that there was something lacking from haemophilic blood which was needed for intrinsic thromboplastin formation. It was suggested at one time that there was some abnormality of the platelets which prevented their normal function in haemophilia but there is no evidence to support

this. Brinkhous (1947) and Quick (1947) found that haemophilic platelets and normal platelets were equally effective in the correction of the abnormal prothrombin consumption of high-spun haemophilic plasma provided a small quantity of normal high-spun plasma was also added. The missing plasma factor in haemophilia has been called antihaemophilic globulin (AHG).

It was recognised therefore that two components were probably required for the production of blood thromboplastin-platelets and the missing plasma factor in haemophilia. Quick (1951) believes that the missing factor in the plasma of haemophiliacs is the precursor of thromboplastin - thromboplastinogen - and that this is activated by a factor derived from the disintegration of platelets against a foreign surface. Brinkhous (1947) believes that the platelets contain thromboplastin and their breakdown is caused by a plasma factor which is activated on contact and which causes a breakdown of platelets. He believes the plasma factor to be a thrombocytolysin.

Failure of AHG and platelets to form thromboplastin.

A preparation of antihaemophilic globulin was prepared by adsorbing normal plasma with aluminium hydroxide. This is obviously a very crude preparation of antihaemophilic globulin as it contains in addition factor V and fibrinogen

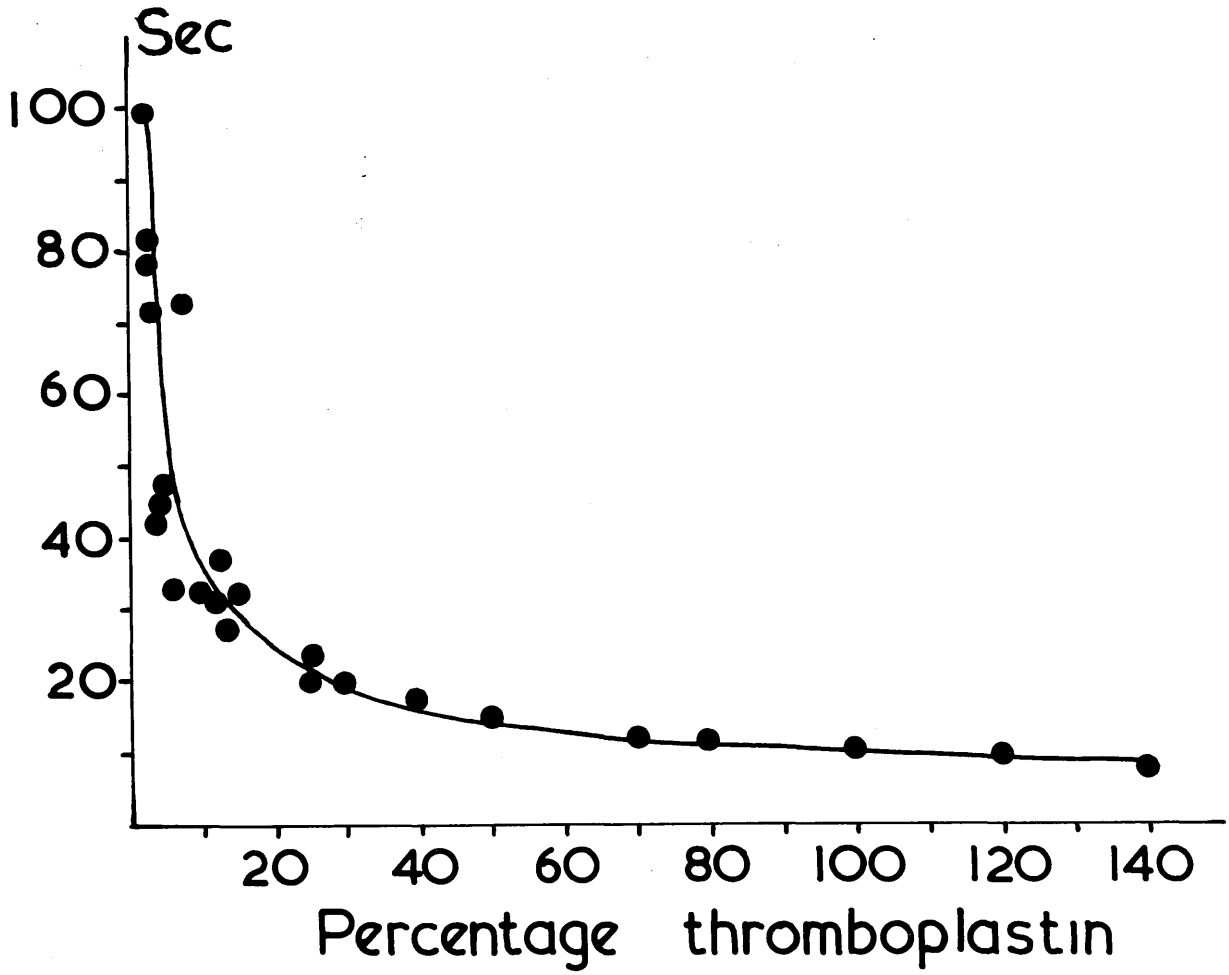
Figure (10)

Blood thromboplastin dilution curve.

Ordinate - clotting times of high spun normal plasma.

Abscissa - percentage of blood thromboplastin.

This figure shows the results from diluting several preparations of blood thromboplastin and clotting 0.1 ml. amounts of normal high spun plasma with these dilutions.


Sec
100
80
60
40
20
20
40
60
80
100
120
140
Percentage thromboplastin

and antithrombin. One ml. of normal plasma was treated with 0.1 ml. of alumina and the mixture incubated at 37° C. in a water bath for exactly three minutes. It was then immediately centrifuged to remove the alumina and the supernatant decanted into 4 ml. of saline. This was used as the preparation of AHG - i.e. adsorbed normal plasma diluted one-in-five with saline. A platelet suspension was prepared as described in the appendix (page 592). Equal parts of the alumina treated plasma, the platelet suspension, saline and m/40 calcium chloride were incubated together and at minute intervals 0.1 ml. of this incubation mixture transferred to 0.1 ml. of normal H.S. plasma which was simultaneously recalcified with 0.1 ml. of m/40 calcium chloride. The clotting times of the plasma were then noted.

Experiment (a)

	Minute Intervals	(1)	(2)	(3)	(4)	(5)	(6)
0.3 $Al(OH)_3$ plasma 1/5) 0.3 platelets) 0.3 saline) 0.3 m/40 $CaCl_2$)		120"	65"	57"	57"	55"	50"

The clotting time of a preparation of brain thromboplastin tested in the same system would be 12"-15". It can be appreciated therefore that the incubation mixture used in this experiment did not contain the reagents required to form a powerful thromboplastin. In chapter 3 an account was given

Figure (11)

Figure (11)

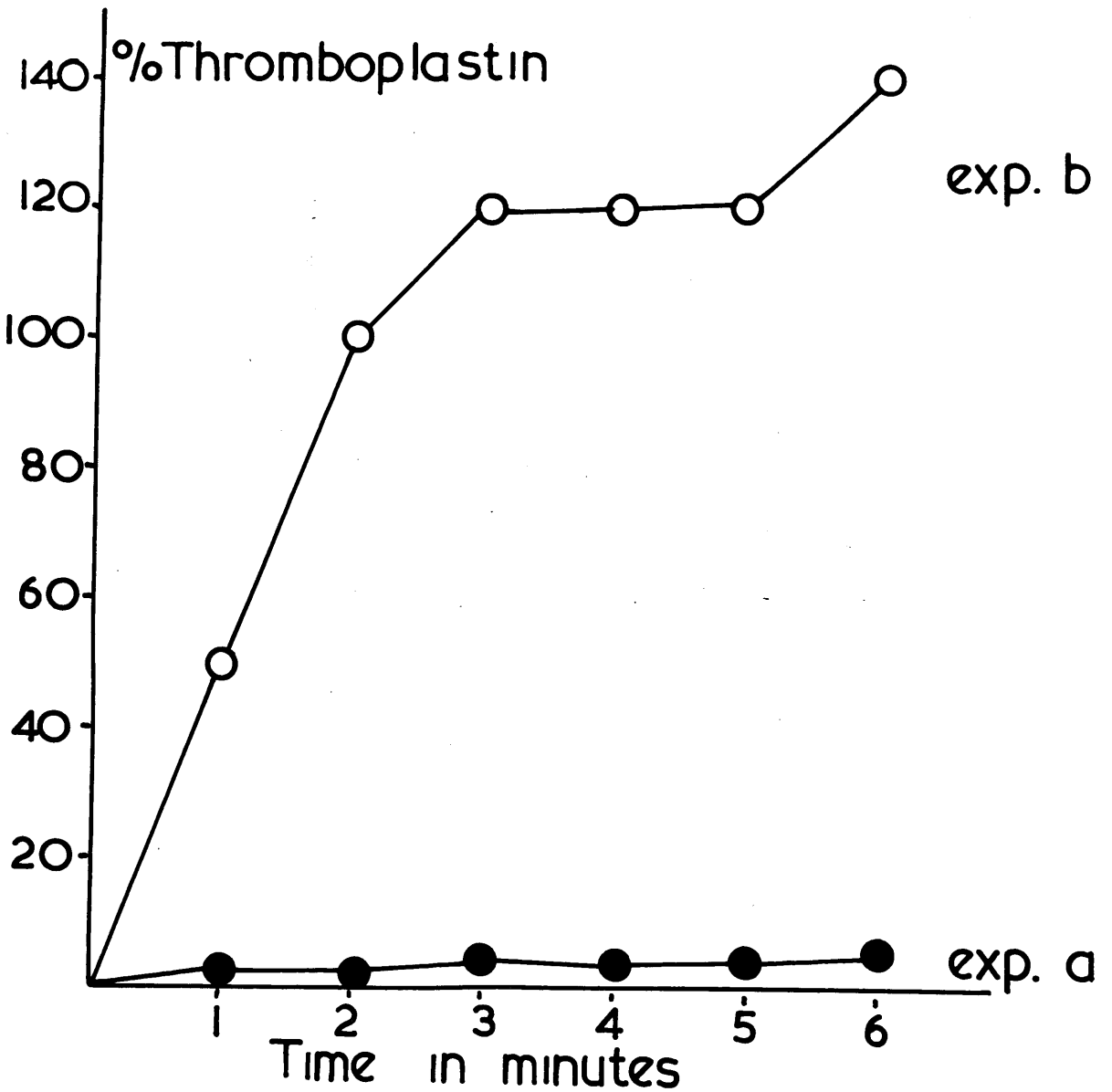
Formation of blood thromboplastin.

Ordinate - Percentage blood thromboplastin.

Abscissa - Incubation time in minutes after addition of calcium.

This figure shows (1) the failure of formation of blood thromboplastin from normal adsorbed plasma and platelets (exp. a. ●——●)

(2) the formation of a powerful thromboplastin from normal adsorbed plasma, platelets and normal serum (exp. b. O——O).


%Thromboplastin
140
120
100
80
60
40
20
exp. b
exp. a
1
2
3
4
5
6
Time in minutes

of factor VII - a clotting factor which is adsorbed on alumina and is present in normal serum not being consumed during clotting. Factor VII is needed for the optimal speed of conversion of prothrombin to thrombin under the influence of tissue extract and calcium. Mann (1949) and Jacox (1949) have suggested that factor VII is needed for the action of tissue extract in the conversion of prothrombin. Mann (1949) used the term co-thromboplastin to describe factor VII.

Since the system containing adsorbed normal plasma, platelets and calcium was shown to be incomplete it was decided to add a source of factor VII. For this reason normal serum was added to the incubation mixture in a dilution of one-in-ten in saline. This serum was obtained from normal clotted blood maintained at 37° C. for one hour. Such serum has been shown not to contain any appreciable amounts of prothrombin, factor V or antihaemophilic globulin (Douglas and Biggs 1953). This addition resulted in the formation of a very powerful thromboplastin - tested in the same system as mentioned above.

Experiment (b)

	(1)	(2)	(3)	(4)	(5)	(6)
0.3 $Al(OH)_3$ plasma 1/5) 0.3 Platelets) 0.3 Normal serum 1/10) 0.3 m/40 $CaCl_2$)	15"	10"	9"	9"	9"	8"

It might be argued that these short clotting times were

Figure (12)

Figure (12)

Formation of blood thromboplastin.

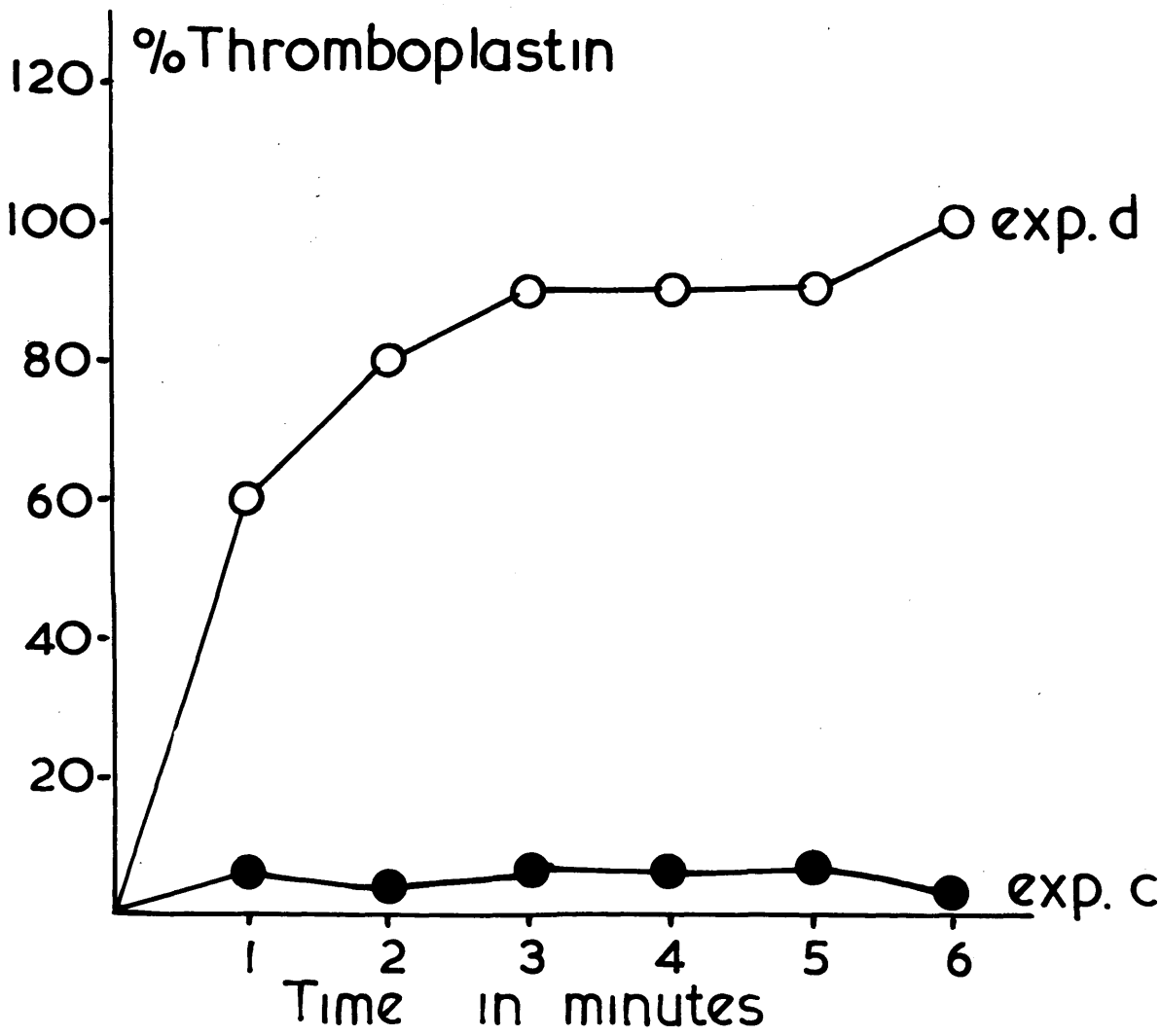
Ordinate - Percentage blood thromboplastin.

Abscissa - Incubation time in minutes after addition of calcium.

This figure shows

(1) the failure of formation of blood thromboplastin from normal adsorbed plasma, platelets and serum which has also been adsorbed with alumina (exp. c. ●——●)

(2) the formation of a powerful thromboplastin from normal adsorbed plasma, platelets and the eluate from alumina adsorption of serum. (exp.d. O——O).


%Thromboplastin
120
100
80
60
40
20
exp. d
exp. c
1
2
3
4
5
6
Time in minutes

the consequence of thrombin contaminating the incubation mixture. The same reagents therefore were incubated together and instead of using 0.1 of high-spun plasma as the substrate, fibrinogen was used; the substrate was 0.4 amounts of fibrinogen.

	(1)	(2)	(3)	(4)	(5)	(6)
0.3 $Al(OH)_3$ plasma 1/5) 0.3 Platelets) 0.3 Normal serum 1/10) 0.3 m/40 $CaCl_2$)	3'+	3'+	3'+	128	110	108

Reference to the thrombin-fibrinogen dilution curve will establish that these clotting times represent only trace amounts of thrombin.

Active fraction of serum is adsorbed by alumina.

Where the normal serum before dilution was treated with alumina it was established that the active principle required from serum for the formation of blood thromboplastin had been removed.

Experiment (c)

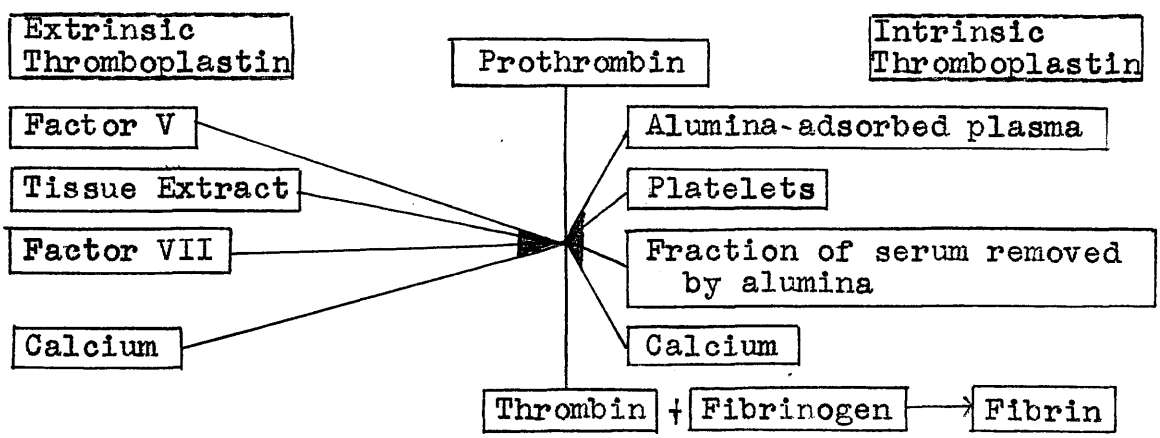
	(1)	(2)	(3)	(4)	(5)	(6)
0.3 $Al(OH)_3$ plasma 1/5) 0.3 Platelets) 0.3 $Al(OH)_3$ serum 1/10) 0.3 m/40 $CaCl_2$)	85	64	48	46	47	45

The alumina-adsorbed fraction of serum was therefore found to be needed in the system for the preparation of blood

thromboplastin. This was confirmed by preparing the eluate from the alumina and testing it in the system. The eluate was made by eluting with a phosphate buffer at pH8 followed by dialysis against citrate-saline. The exact details of this are described in the appendix.

Experiment (d)	(1)	(2)	(3)	(4)	(5)	(6)
0.3 $Al(OH)_3$ plasma 1/5) 0.3 Platelets) 0.3 eluate from alumina) adsorption of serum) 0.3 m/40 $CaCl_2$)	14	12	11	11	11	10

At this stage the scheme of blood coagulation could be represented as follows:-



Factor V and AHG both required.

The alumina-adsorbed fraction of plasma was known to contain factor V and antihaemophilic globulin. These were separated by the ammonium sulphate precipitation of adsorbed

Figure (13)

Figure (13)

Formation of blood thromboplastin.

Ordinate - Percentage blood thromboplastin.

Abscissa - Incubation time in minutes after addition of calcium.

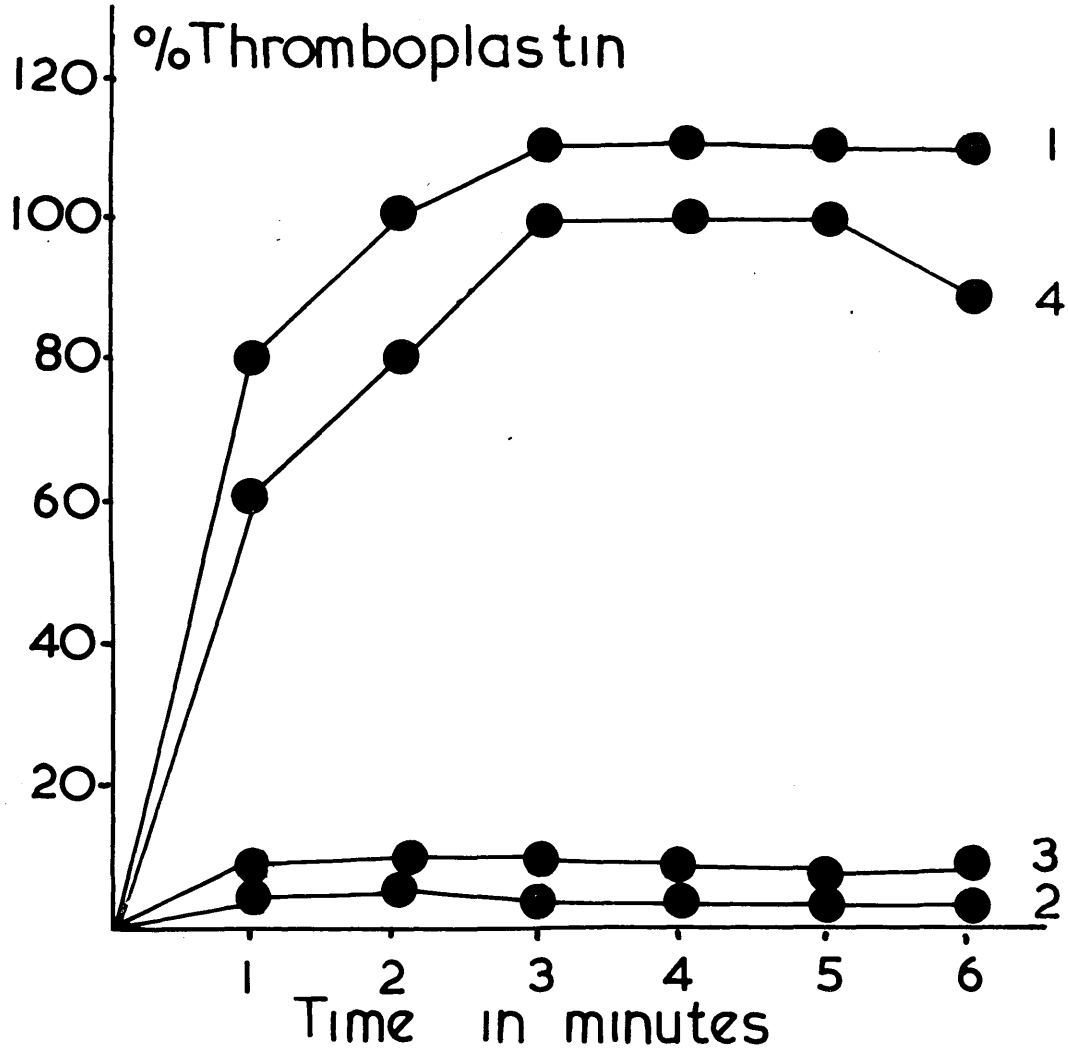
This figure shows

(1) The formation of a powerful thromboplastin from normal adsorbed plasma, platelets and normal serum.

(2) The failure to form thromboplastin when factor V alone replaces the normal adsorbed plasma in the system.

(3) The failure to form thromboplastin when A.H.G. alone replaces the normal adsorbed plasma in the system.

(4) The restoration of satisfactory blood thromboplastin formation when factor V and A.H.G. are both present in place of the normal adsorbed plasma.

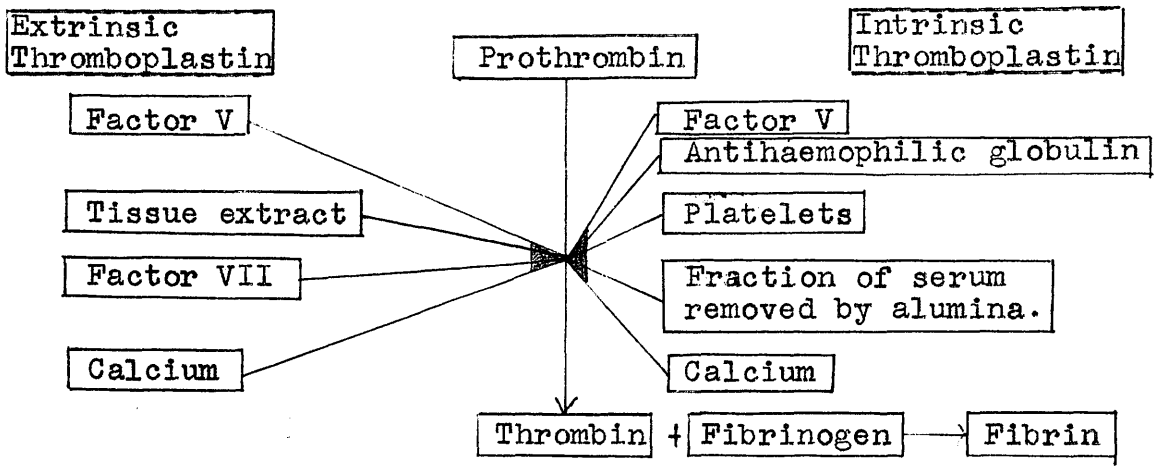

%Thromboplastin
120
100
80
60
40
20
1
4
3
2
1
2
3
4
5
6
Time in minutes

normal plasma and each tested separately in the system as described above. When the $Al(OH_3)$-treated normal plasma is replaced by either factor V or antihaemophilic globulin thromboplastin formation is reduced and delayed. When the two are combined rapid thromboplastin formation is restored. The results of such an experiment were as follows:-

Experiment (e)

		(1)	(2)	(3)	(4)	(5)	(6)
(1)	0.3 $Al(OH)_3$ plasma 1/5 0.3 platelets 0.3 normal serum 1/10 0.3 saline 0.3 m/40 $CaCl_2$	12	10	9	9	9	9
(2)	0.3 Factor V 1/5 0.3 platelets 0.3 normal serum 1/10 0.3 saline 0.3 m/40 $CaCl_2$	85	72	55	55	52	48
(3)	0.3 A.H.G. 1/5 0.3 platelets 0.3 normal serum 1/10 0.3 saline 0.3 m/40 $CaCl_2$	70	64	52	41	40	38
(4)	0.3 Factor V 1/5 0.3 A.H.G. 1/5 0.3 platelets 0.3 normal serum 1/10 0.3 m/40 $CaCl_2$	14	12	10	10	10	11

In consequence of these observations the scheme of blood thromboplastin described above had to be altered to read as follows:-



Measurement of Blood Thromboplastic Activity.

Although plasma thromboplastin was not stable at 37° C. and all attempts to prepare a stable form failed, the potency could be maintained at 4° C. for sufficient time to test various samples prepared and diluted with saline. 0.1 ml. of each dilution together with 0.1 ml. of m/40 $CaCl_2$ were then added to 0.1 ml. of normal substrate. From a number of samples the curve shown in figure 10 was obtained. The curve can be converted to a straight line by plotting the figures on a double logarithmic scale. The dilution curves from various samples of blood thromboplastin used for the construction of the graph in figure 10 are given in the appendix.

Using this graph the results of the experiments (a) (b) (c) (d) and (e) can be represented in figures: 11, 12, & 13.

experiments a-b - figure 11

experiments c-d - figure 12

experiment e - figure 13

It is clear therefore that there are two powerful systems for the conversion of prothrombin to thrombin. The extrinsic thromboplastin system has as an essential component tissue extract, while the intrinsic system is dependent entirely on components derived from the blood itself. Since damage to tissue is an invariable accompaniment of accidentally occurring haemorrhage it is not unreasonable that the extrinsic system is the one which, until recent years, received most attention. Two factors have stimulated recent interest in the blood's own intrinsic system. The first is the demonstration that the blood thromboplastin is very powerful, instead of the former concept of a weak mechanism. The second is the observation by Brown (1951) that a powerful tissue thromboplastin could be prepared from the tissues of a haemophiliac who died of haemorrhage. This brain extract from the haemophiliac was as powerful as that from a normal brain in its ability to clot haemophilic plasma. This stresses the grave results of a failure of the blood thromboplastin system in physiological haemostasis.

In this chapter I have described a few experiments as they represent key observations in our understanding of blood

thromboplastin. The elucidation of the mechanism of blood thromboplastin covered a considerable period of experimental work done in the Department of Clinical Pathology, The Radcliffe Infirmary, Oxford, together with Dr. Rosemary Biggs and Dr. R. G. Macfarlane.

SUMMARY

Evidence is presented that a powerful blood thromboplastin can be prepared by incubating together in the presence of calcium, factor V, antihaemophilic globulin, platelets and the fraction of serum adsorbed by alumina.

REFERENCES

Biggs, R., Douglas, A.S., and Macfarlane, R.G. (1953). The formation of thromboplastin in human blood. J. Physiol. 119, 89.

Biggs, R., Douglas, A.S., and Macfarlane, R.G. (1953). The initial stages of blood coagulation. J. Physiol. 122, 538.

Biggs, R., Douglas, A.S., and Macfarlane, R.G. (1953). The action of thromboplastic substances. J. Physiol. 122, 554.

Bordet, J., and Delange, L. (1912). La Coagulation du sang et la genèsè de la thrombine. Ann. Inst.Pasteur, 26, 655.

Brinkhous, K.M. (1939). A study of the clotting time in haemophilia: the delayed formation of thrombin. Amer. J. med. Sci. 198, 509.

Brinkhous, K.M. (1947). Clotting defect in haemophilia. Deficiency in a plasma factor required for platelet utilisation. Proc. Soc. exp. Biol. N.Y. 66, 117.

Brown, A. (1951). Unpublished observations.

Collingwood, B.J. and MacMahon, M.T. (1912). The anticoagulants in blood and serum. J. Physiol. 45,119.

Douglas, A.S. and Biggs, R. (1953). The consumption of some components involved in physiological blood coagulation. Glasg. med. J. 34, 329.

Ferguson, J.H. (1949). Blood Clotting and Allied Problems. Second Conference of the Josiah Macey Jr. Foundation, p. 211.

Jacox, R.F. (1949). Studies on the activation of a serum prothrombin converting factor. J. clin. Invest. 28, 492.

Macfarlane, R.G.(1942). Vitamin K and the estimation of prothrombin. Proc. Roy. Soc. med. 35, 410.

Macfarlane, R.G. and Biggs, R. (1953). A thrombin generation test. The application in haemophilia and thrombocytopenia. J. clin. Path., 6, 3.

Mann, F.D. (1949). Co-thromboplastin assay. Amer.J. clin. Path. 19, 861.

Merskey, C. (1950). The consumption of prothrombin during coagulation. The defect in haemophilia and thrombocytopenic purpura. J. clin. Path. 3, 130.

Quick, A.J. (1947). Studies on the enigma of the haemostatic dysfunction of haemophilia. Amer. J. med.Sci. 214, 272.

Quick, A.J. (1951). The physiology and pathology of haemostasis. Henry Kempton, London.

Shinowara, G.Y. (1951). Enzyme studies on human blood. The isolation and characterisation of the thromboplastic cell and plasma components. J. Lab. clin. Med. 38,11.

Soulier, J.P. (1950). Study of prothrombin consumption. Practical interest for the diagnosis of haemorrhagic diseases. Acta. med. Scand. 137, 1.

Ware, A. G., Fahey, J.L. and Seegers, W.H. (1948). Platelet extracts, fibrin formation and interaction of purified prothrombin and thromboplastin. Amer. J. Physiol. 154, 140.

CHAPTER 5

DISCOVERY OF CHRISTMAS DISEASE

CONTENTS.

The observation that the coagulation defect in "haemophiliacs" is sometimes mutually correctable.

Investigation on the first two recognized cases of Christmas Disease.

(1) Study by recalcification times.

(2) Study by the thromboplastin generation technique.

- (a) the normal reaction from patient's adsorbed plasma.
- (b) the defective reaction of patient's serum.
- (c) normal platelet function as regards thromboplastin formation.
- (d) the ability of the adsorbed plasma to correct the defect in haemophilic adsorbed plasma.
- (e) comparison of the defective serum reaction with dilutions of normal serum.
- (f) the property of normal serum to correct the abnormality.

(3) Studies on prothrombin consumption.

Investigation of Case 3, including inheritance.

Investigation of Cases 4 and 5.

Investigations to confirm the uniformity of the defect.

Effect of plasma derivatives on the coagulation abnormality.

Comparison of A.H.G. and Christmas factor.

Inheritance.

Assessment of maternal coagulation system.

Other cases reported in the literature.

Problems of terminology.

Christmas factor as a component of blood thromboplastin.

CHAPTER 5

DISCOVERY OF CHRISTMAS DISEASE

Until recent years haemophilia has been defined as a constitutional anomaly of blood coagulation, depending on the hereditary transmission of a sex-linked recessive trait, and having as its feature a lifelong liability to haemorrhage in the affected males. Thus it has been accepted that females are almost exclusively immune from the disease, but capable of transmitting it to their male offspring and that the manifest disease is characterised by the occurrence of bleeding into joints and deep tissues either spontaneously or following minor trauma. The time-honoured laboratory criterion for the diagnosis of haemophilia is a prolongation of the whole blood clotting time. The investigations about to be described have thrown fresh light on this disease and show that the condition formerly covered by the term haemophilia contains two quite different entities, indistinguishable by clinical methods.

Mutual correction of the coagulation defect amongst haemophiliacs.

In 1950 Dr. J.C.F. Poole working in Oxford observed that the plasma of one particular "haemophiliac" (N.J.) was able to correct the calcium clotting time of haemophilic plasma, as well as did normal plasma (Poole 1952). The observation

was repeated in relation to several other haemophilics - the blood from this patient was able to correct the defect in several other haemophiliacs. Dr. J.V. Dacie and Dr. W.R. Pitney working in the Department of Haematology, Post-graduate Medical School, Hammersmith, London, found a further case (S.C.) of "haemophilia" also capable of correcting the defect in several haemophiliacs. This was suggestive evidence that "haemophilia" consisted of two different conditions.

Investigations on the first two recognized cases of Christmas Disease.

It was by the application of the techniques described in Chapter 4 for the study of blood thromboplastin that this problem was further elucidated. Using these techniques these first two cases were investigated by me in the summer of 1952 in the Department of Clinical Pathology, Radcliffe Infirmary, Oxford. This formed one of the most intriguing aspects of the experimental work described in this thesis.

Plasma and serum was obtained from each of these two patients - the Oxford case (N.J.) and the Hammersmith case (S.C.). The patient from the Hammersmith Hospital had the surname of Christmas and as he was one of the first two investigated it was decided to retain this name for the condition, which has been called "Christmas Disease". The

Figure (14)

Figure (14)

Family tree of Christmas disease - case (1). S.C.

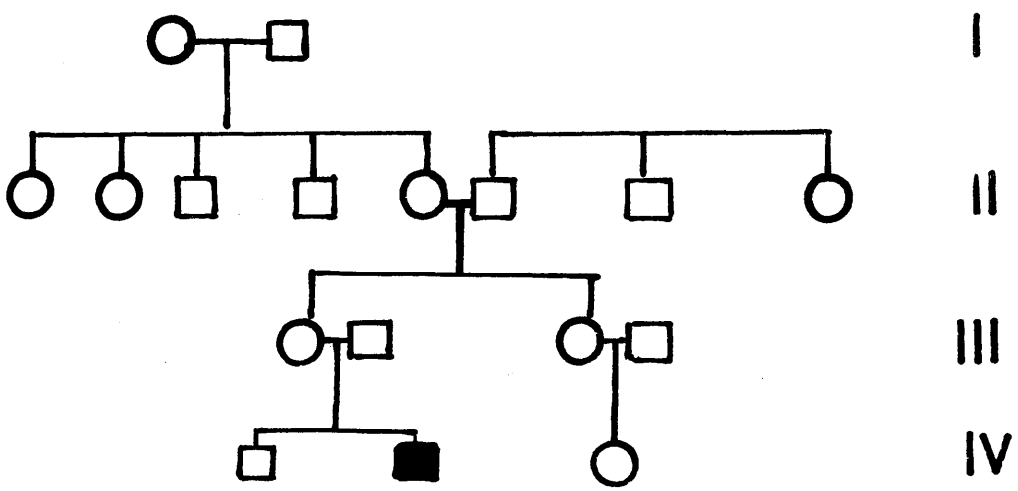
Females represented O

Unaffected males represented □

Affected males represented ■

The family history was negative.

Case 1 S.C.



problem of terminology will be discussed later. As these first two patients were investigated by me, the laboratory findings in them will be described in considerable detail. Three of the other patients reported in the original publication are described in less detail. (Biggs, Douglas, Macfarlane, Dacie, Pitney, Merskey, O'Brien, 1952).

Case Reports.

Case 1. S.C. (aged 5). Male. In this case there was no history of definite haemorrhage in other members of the family (see figure 14). The patient had numerous episodes of haemorrhage with haemarthroses dating from the age of 20 months. He had been transfused on several occasions and it had been considered that this was a successful form of therapy in his case.

Whole blood clotting time (Lee & White) 14'.
(Method (1))
On another occasion 36', 46', 72'.

Prothrombin Consumption index (Merskey) 100%.

One-stage "prothrombin" time - normal.

Case 2. N.J. (aged 7). Male. In this case also there was no definite family history of bleeding. (See figure 15) The patient had suffered numerous haemorrhagic incidents from the age of 3 months, these often necessitating transfusion. When seen in July 1952 he was suffering from a

Figure (15)

Figure (15)

Family tree of Christmas disease - case (2). N.J.

Females represented O

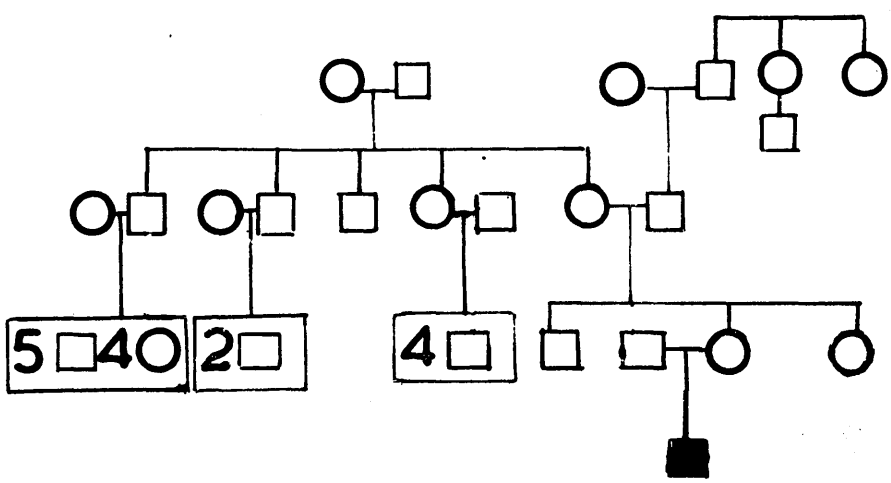
Unaffected males represented □

Affected males represented ■

The figures in front of the symbols indicate the number of brothers or sisters in that particular series of siblings e.g. 40 indicates 4 sisters.

The family history was negative.

Case 2 N.J.



haemarthrosis of the knee.

Whole blood clotting time (Lee & White) 39-72'.

Prothrombin Consumption (Merskey index) 150%.

One-stage "prothrombin" time - normal.

Bleeding time, tourniquet test and platelet count - normal

Study by recalcification times on Cases 1 and 2.

Haemophiliac for comparison with these patients was P.L.

		Clotting time in minutes.
.1 cc. Nor. plasma		2¾ & 2½
.1 cc. Haem. plasma		9¼
.1 cc. Case 1. plasma		15
.1 cc. Case 2. plasma		7¼
.05 Haem. plasma	.05 Nor. plasma	2½
.05 Case 1. plasma	.05 Nor. plasma	3¼
.05 Case 2. plasma	.05 Nor. plasma	2¼
.09 Haem. plasma	.01 Nor. plasma	3
.09 Case 1. plasma	.01 Nor. plasma	4¾
.09 Case 2. plasma	.01 Nor. plasma	2¾
.05 Haem. plasma	.05 Case 1. plasma	4'
.05 Haem. plasma	.05 Case 2. plasma	2¾
.05 Case 1. plasma	.05 Case 2. plasma	9
.01 Case 1. plasma	.09 Case 2. plasma	9¾
.09 Case 1. plasma	.01 Case 2. plasma	9¾
.05 Nor. plasma	.05 alumina plasma	4¼
.05 Haem. plasma	.05 alumina plasma	3¾
.05 Case 1. plasma	.05 alumina plasma	8¼

Conclusions from this.

(1) Cases 1. and 2. would appear to have the same abnormality, as they do not mutually correct.

(2) The coagulation defect in Cases 1. and 2. is not the same as in the haemophiliac. Case 1. and the haemophiliac are mutually corrective as are Case 2. and the haemophiliac.

Figure (16)

Figure (18)

Thromboplastin generation test in Christmas disease - Case (1).

Ordinate - percentage blood thromboplastin.

Abscissa - incubation time in minutes after addition of calcium.

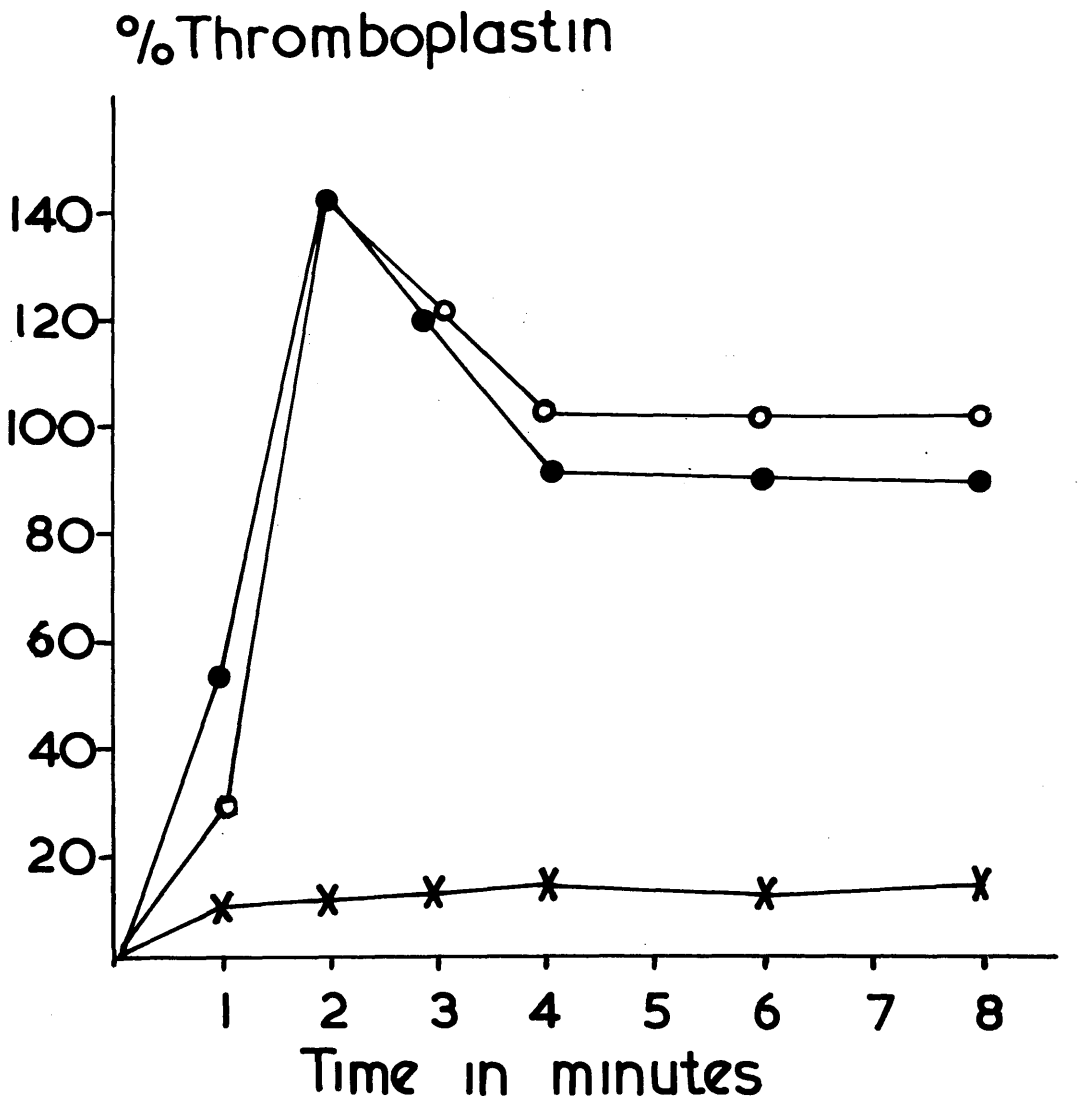
Normal serum and normal platelets constant.

Normal adsorbed plasma ●——●

Case (1) adsorbed plasma O——O

Haemophilic ads. plasma X——X

(Results of experiment similar to that described in the text).


%Thromboplastin
140
120
100
80
60
40
20
1
2
3
4
5
6
7
8
Time in minutes

(3) Alumina adsorbed normal plasma corrects the abnormality in the haemophiliac but not in Case 1.

(4) Normal plasma corrects the abnormality in haemophilia and in Case 1.

One-stage clotting times.

Normal	15"
Case 1.	15"
Case 2.	16"
Haemophiliac	16"

Prothrombin content by the globulin fraction technique.

Normal	100%
Case 1.	95%
Case 2.	95%
Haemophiliac	100%

Study of the defect by the thromboplastin generation technique.

Adsorbed plasma was prepared from the normal, from Case 1, Case 2 and the haemophiliac.

Serum was prepared from the normal, Case 1, Case 2 and the haemophiliac.

Platelets were prepared from the normal and from Case 2.

Normal reaction from the adsorbed plasma in these patients.

Case 1, normal and haemophiliac.

Normal serum Normal platelets Constant.	(1)	(2)	(3)	(4)	(5)	(6)
Normal ads. plasma	9	9	9	10	12	10
Case 1. ads. plasma	9	10	12	11	13	11
Haem. ads. plasma	35	32	31	27	28	26

See figure 16

Figure (17)

Figure (17)

Thromboplastin generation test in Christmas disease. (Case (1).

Ordinate - percentage blood thromboplastin.

Abscissa - incubation time in minutes after addition of calcium.

Normal adsorbed plasma and platelets constant.

Normal serum ●——●

Case (1) serum O——O

Haemophilic serum X——X

(Results of experiment similar to that described in the text).

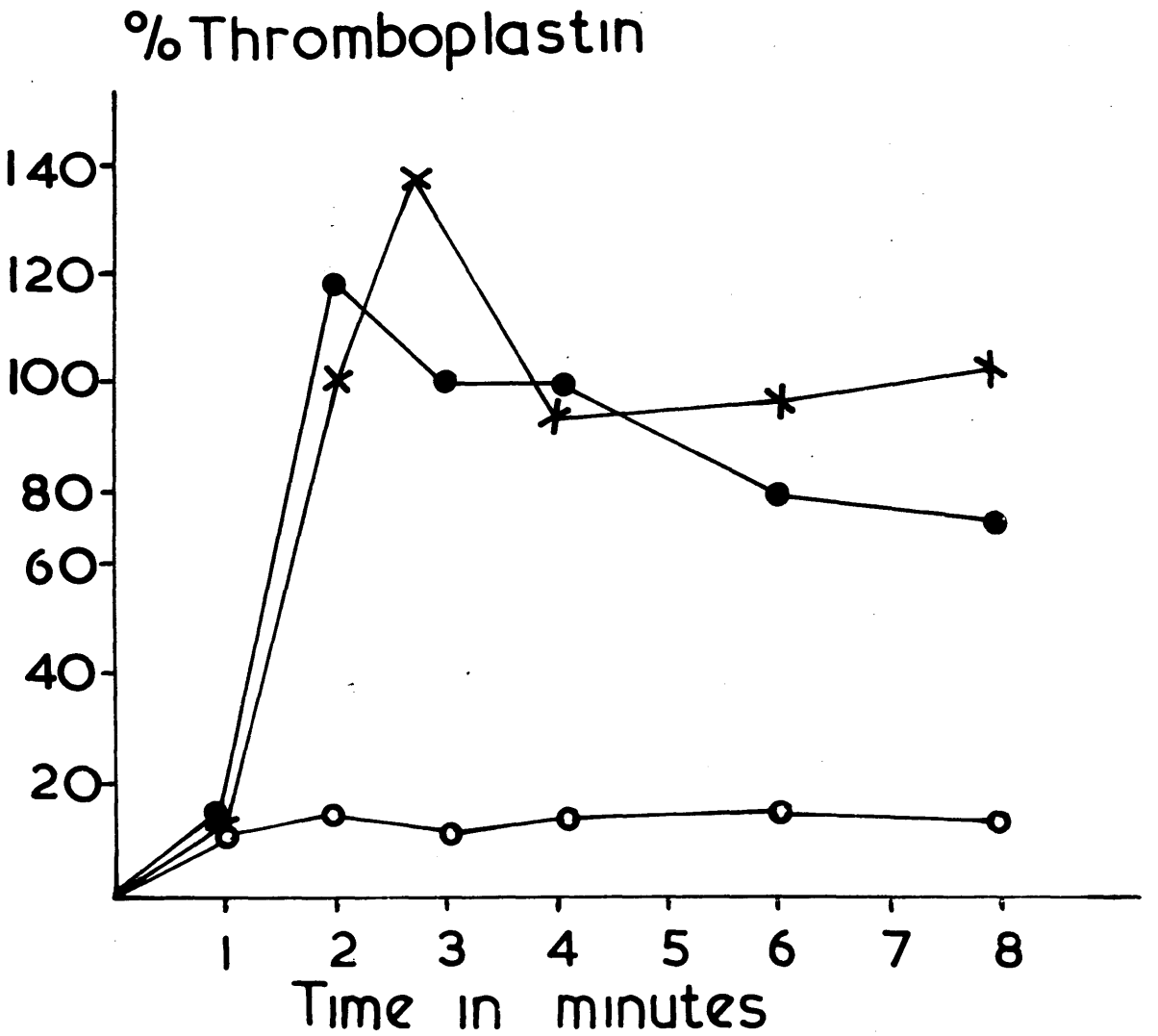

% Thromboplastin
140
120
100
80
60
40
20
1
2
3
4
5
6
7
8
Time in minutes

Figure (18)

Figure (18)

Thromboplastin generation test in Christmas disease. Case (2).

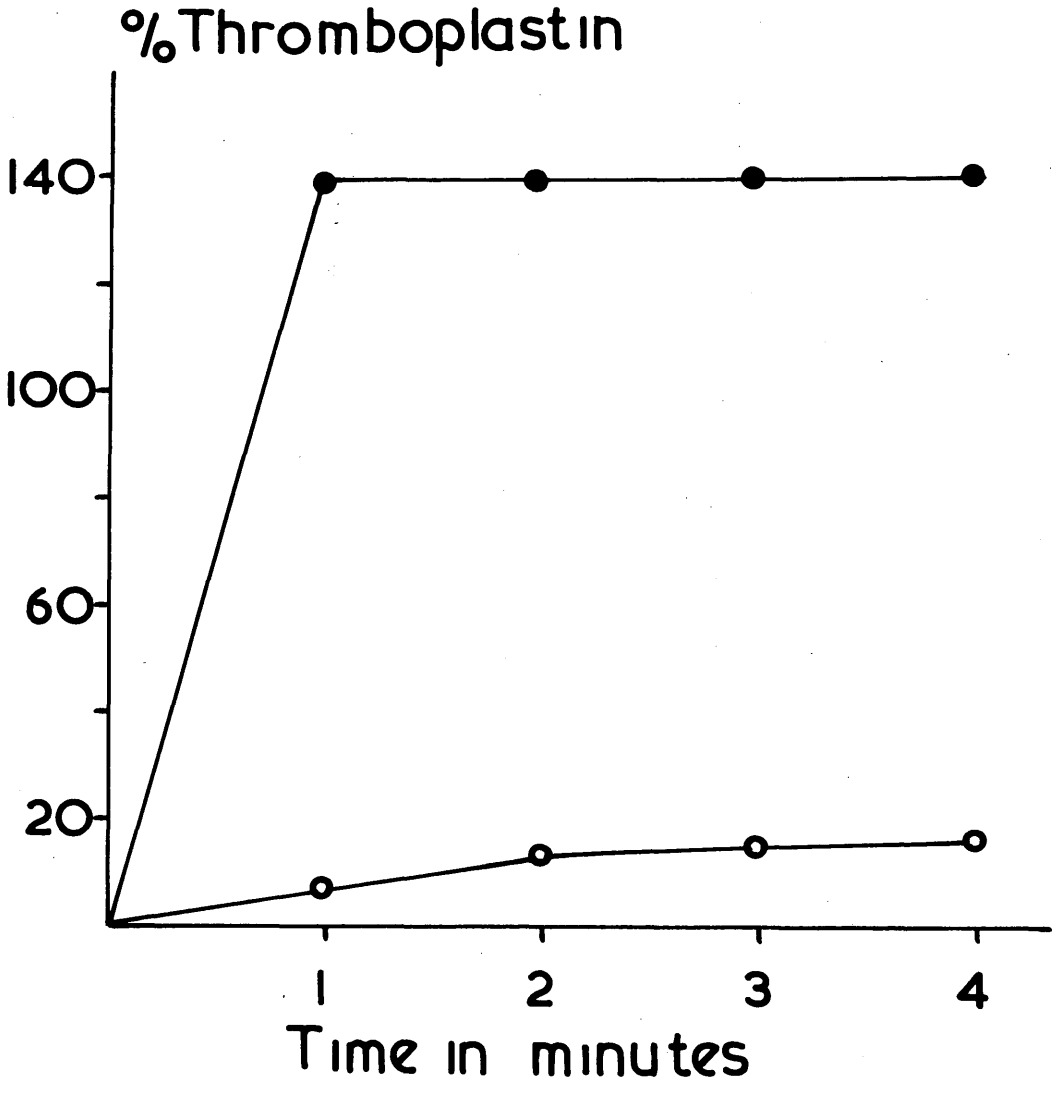
Ordinate - percentage blood thromboplastin.

Abscissa - incubation time in minutes after addition of calcium.

Normal adsorbed plasma and platelets constant.

Normal serum ●——●

Case(2) serum O——O


%Thromboplastin
140
100
60
20
1
2
3
4
Time in minutes

Defective serum reaction in these patients.

Normal ads plasma Normal platelets Constant	(1)	(2)	(3)	(4)	(5)	(6)
Normal serum	10	10	9	10	10	10
Case 1. serum	30	28	24	20	22	22
Haem. serum	10	9	9	9	10	10

See figure 17

Case 2. normal and haemophiliac.

Normal serum Normal platelets Constant	(1)	(2)	(3)	(4)	(5)	(6)
Normal ads plasma	8	8	9	9	10	9
Case 2. ads plasma	9	9	9	10	10	10
Haem. ads plasma	40	35	36	33	34	29

Normal ads plasma Normal platelets Constant	(1)	(2)	(3)	(4)	(5)	(6)
Normal serum	10	10	9	10	10	11
Case 2. serum	46	35	32	29	29	30
Haem. serum	11	9	9	9	9	10

See figure 18

Case 1.) Serum Case 2.) 50% of each	39	32	33	31	29	26

Platelet function - Comparison of platelets.

Normal ads plasma Normal serum Constant	(1)	(2)	(3)	(4)	(5)	(6)
Normal platelets	13	10	10	11	10	9
Case 2. platelets	14	11	11	10	10	10

Conclusions.

The site of the thromboplastin defect in these patients is in the serum. The adsorbed plasma from the patients behaves normally as a source of A.H.G. and factor V. The platelets are normal in their ability to form blood thromboplastin.

The ability of the adsorbed plasma from these patients to correct the thromboplastin defect in adsorbed haemophilic plasma.

1 cc. of plasma. 0.1 alumina. 2 mins.

Normal 60") one-stage clotting
Case 1. 82") times of adsorbed
Case 2. 95") plasmas.

	1	2	3	4	6	8	
Normal	37	10	12	12	13	13	
Haemophiliac	54	40	32	19	18	18	
Normal) 50% Haem.)	19	11	13	11	11	12	
Case 2) 50% Haem.)	23	12	13	13	13	13	
Case 1) 50% Haem.)	31	12	12	13	12	12	

Ads. plasma 1/5
Haemophilic platelets
Normal serum 1/10
m/40 $CaCl_2$

Figure (19)

Figure (13)

Thromboplastin generation test in Christmas disease. Case (1).

Ordinate - percentage blood thromboplastin.

Abscissa - incubation time in minutes after addition of calcium.

This figure shows the ability of adsorbed plasma from Case (1) to correct the thromboplastin defect in adsorbed haemophilic plasma.

Normal serum and platelets constant.

Adsorbed plasma variable.

●——● 50% Haem. 50% Nor.

O——O 50% Haem. 50% Case (1)

X——X Haem.

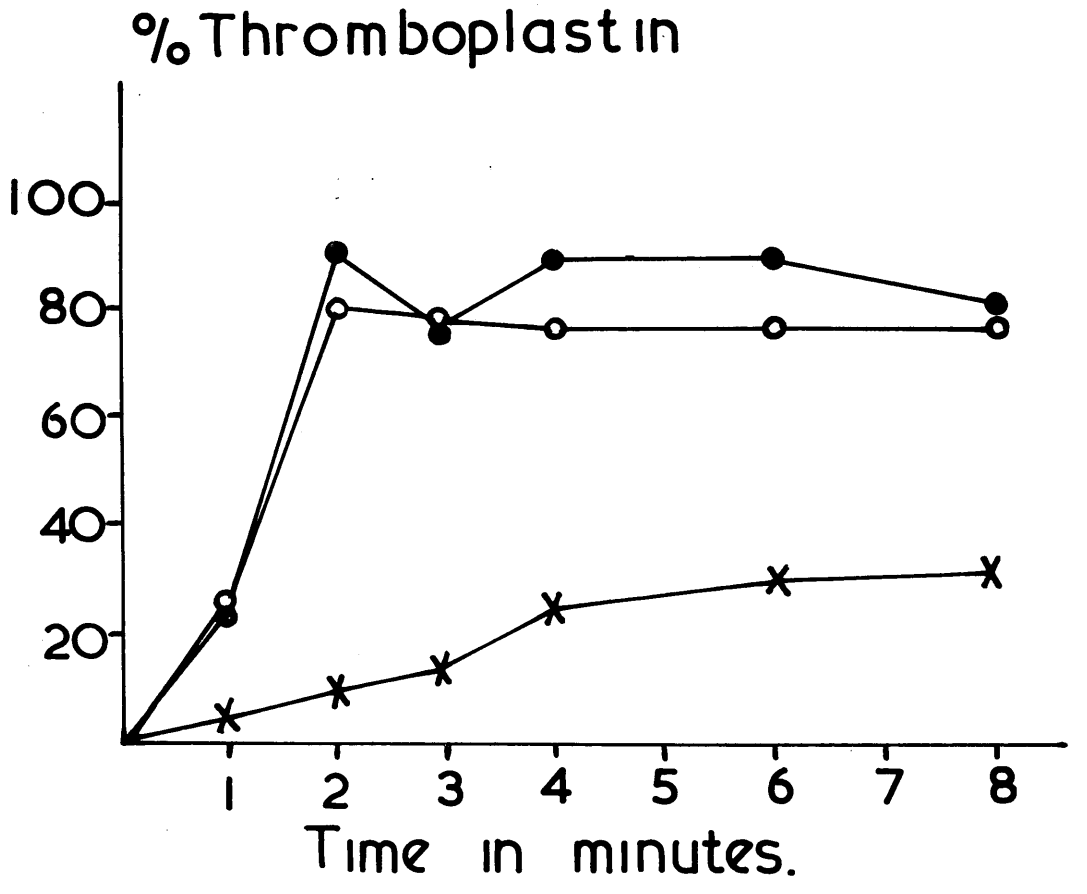

% Thromboplastin
100
80
60
40
20
1
2
3
4
5
6
7
8
Time in minutes.

Figure (20)

Figure (20)

Thromboplastin generation test in Christmas disease Case (2).

Ordinate - percentage blood thromboplastin.

Abscissa - incubation time in minutes.

This figure shows the ability of adsorbed plasma from Case (2) to correct the thromboplastin defect in adsorbed haemophilic plasma.

Normal serum and platelets constant.

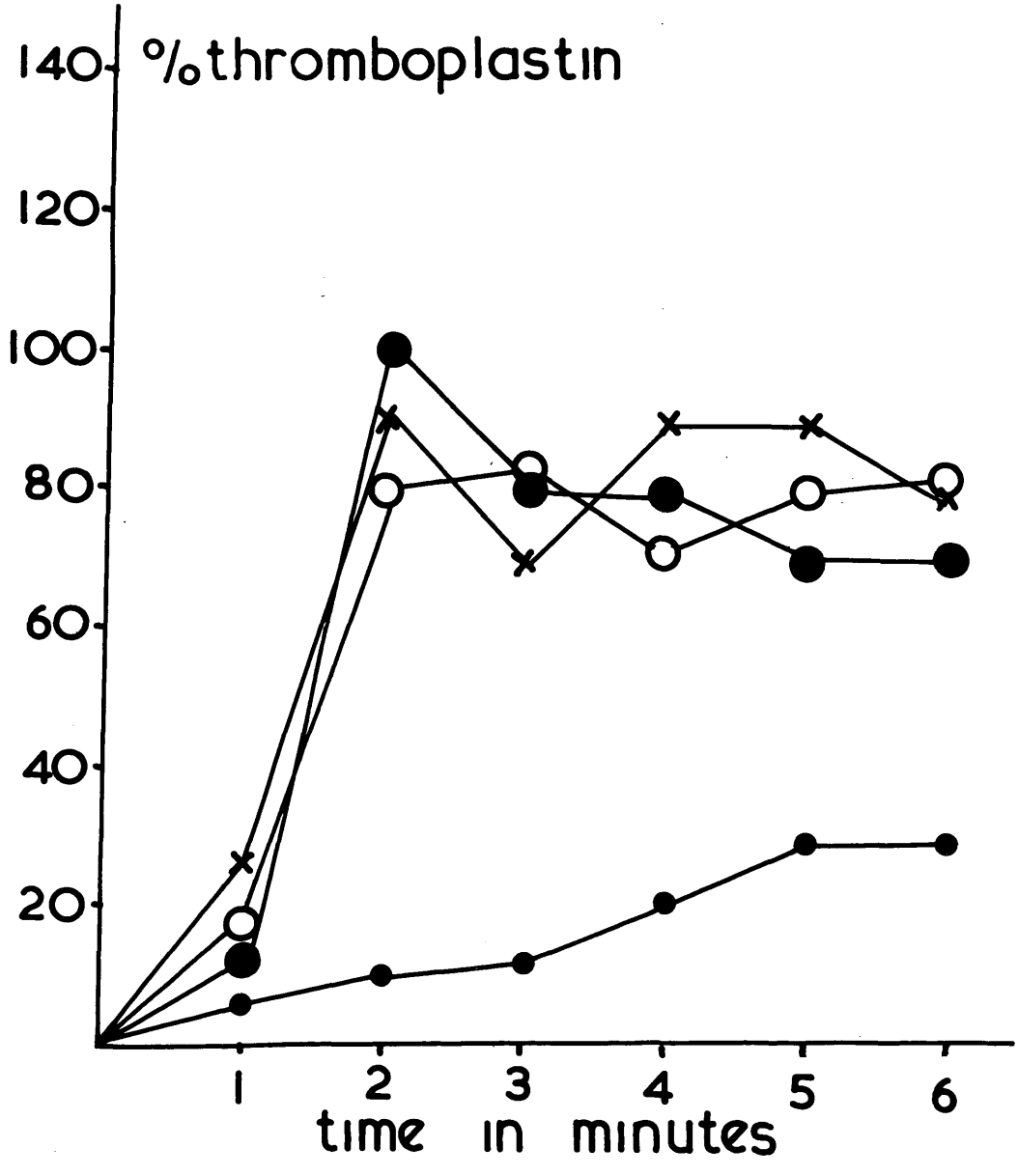
Adsorbed plasma variable.

●——● Normal.

X——X 50% Haem. 50% Nor.

O——O 50% Haem. 50% Case (2)

●——● Haem.


% thromboplastin
140
120
100
80
60
40
20
1
2
3
4
5
6
time in minutes

Conclusion: The adsorbed plasma from these patients is as good as the normal in correcting the haemophilic defect - see figures 19 and 20.

The action of serum from these patients as a source of serum factors in thromboplastin generation, in comparison with dilutions of normal serum.

	1	2	3	4	6	8
Normal serum	28	9	10	10	12	13
Case 1 serum	35	27	33	29	25	28
Case 2 serum	39	28	27	28	28	29
Haemophilic serum	34	10	8	11	11	10
Case 1)50% of Case 2) each	50	38	27	26	27	28
Normal serum 50% (from 1/10 =100%	30	12	9	10	12	12
Normal serum 25%	37	19	10	12	14	16
Normal serum 12.5%	44	30	19	17	21	25
Normal serum 6.25%	49	46	33	29	29	31

Normal alumina plasma 1/5)
Normal platelets) constant
m/40 $CaCl_2$)

Serum (variable) 1/10.

Comments: The serum from these patients has only a small percentage of the activity of normal serum in forming thromboplastin.

Figure (21)

Figure (3)

Thromboplastin generation technique in Christmas disease Case (2).

Ordinate - percentage blood thromboplastin.

Abscissa - incubation time in minutes.

This figure shows the property of normal serum to correct the abnormality. Since plasma is used from which prothrombin has not been removed the answer is complicated by the presence of thrombin.

Normal platelets constant.

●——● Normal plasma; normal serum.

O——O Case (2) plasma; normal serum.

●——● Case (2) plasma; Case (2) serum.

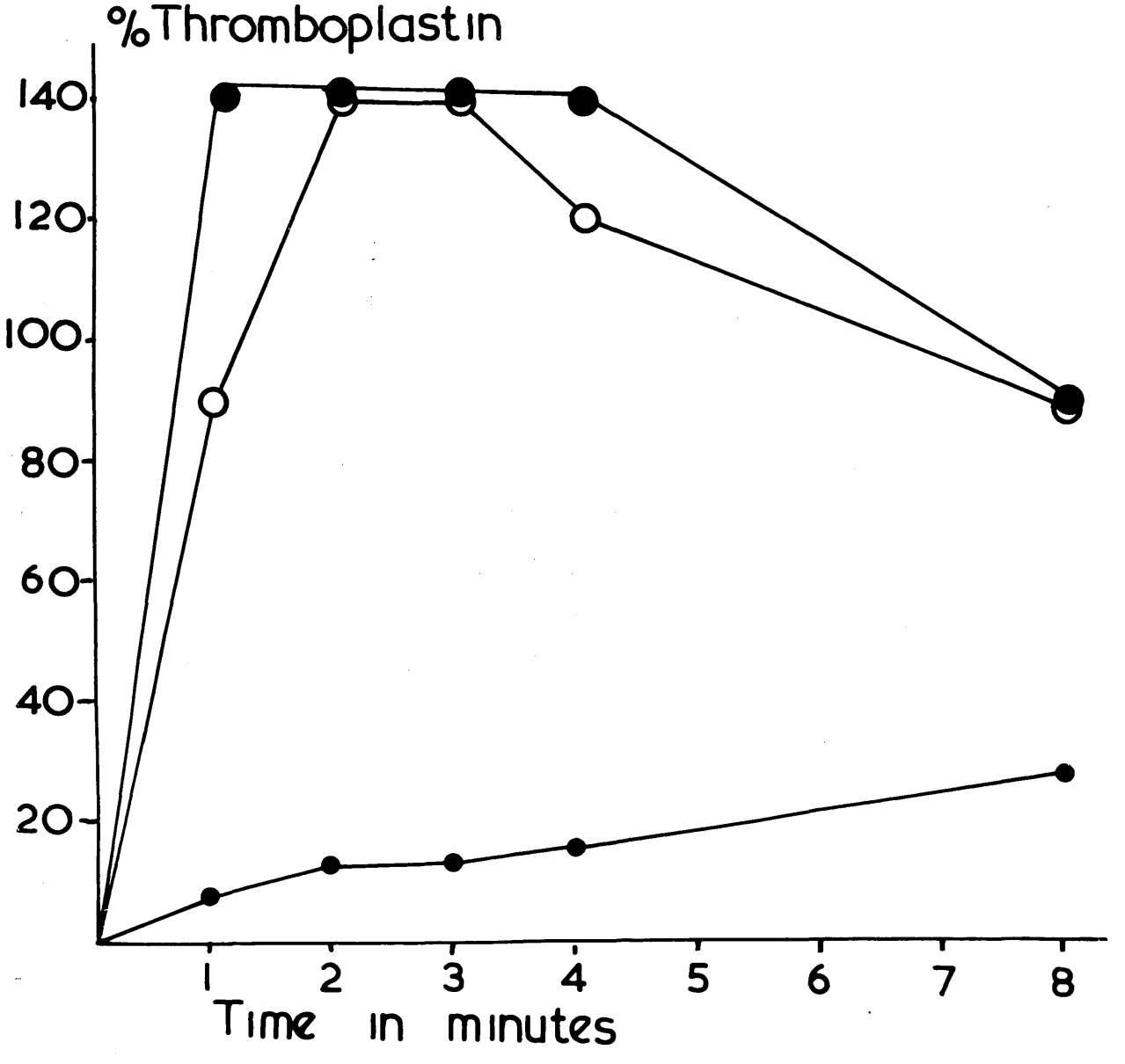

%Thromboplastin
140
120
100
80
60
40
20
1
2
3
4
5
6
7
8
Time in minutes

Figure (22)

Figure (22)

Correction of defective prothrombin consumption of Christmas disease by normal and haemophilic plasma.

Ordinate - clotting times of fibrinogen in seconds.

Abscissa - incubation time in minutes.

●——● Case (2)

X——X Case (2) + normal.

O——O Case (2) + haem.

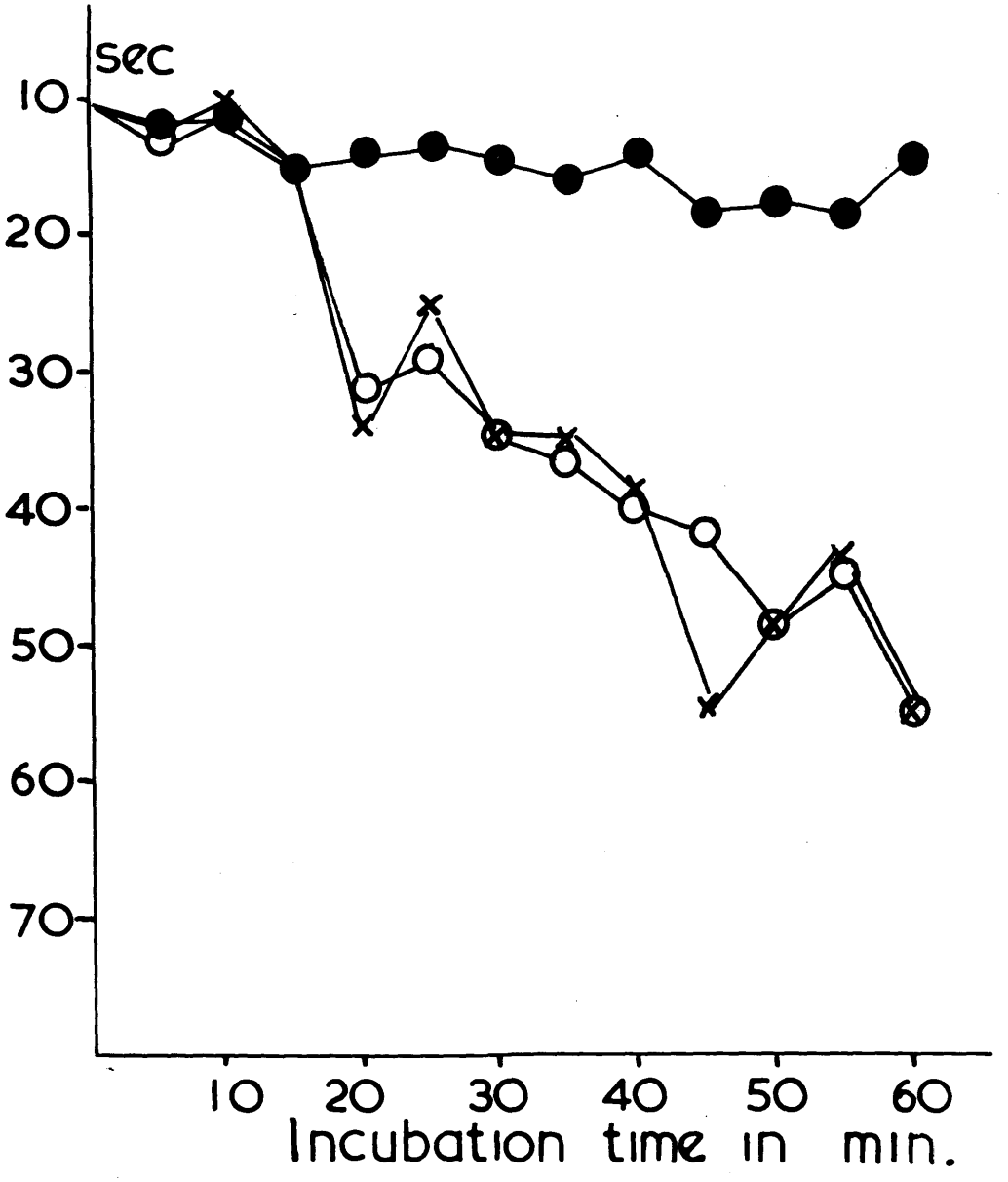

sec
10
20
30
40
50
60
70
10
20
30
40
50
60
Incubation time in min.

Figure (23)

Figure (23)

Correction of defective prothrombin consumption of haemophilia by normal and Christmas disease plasma.

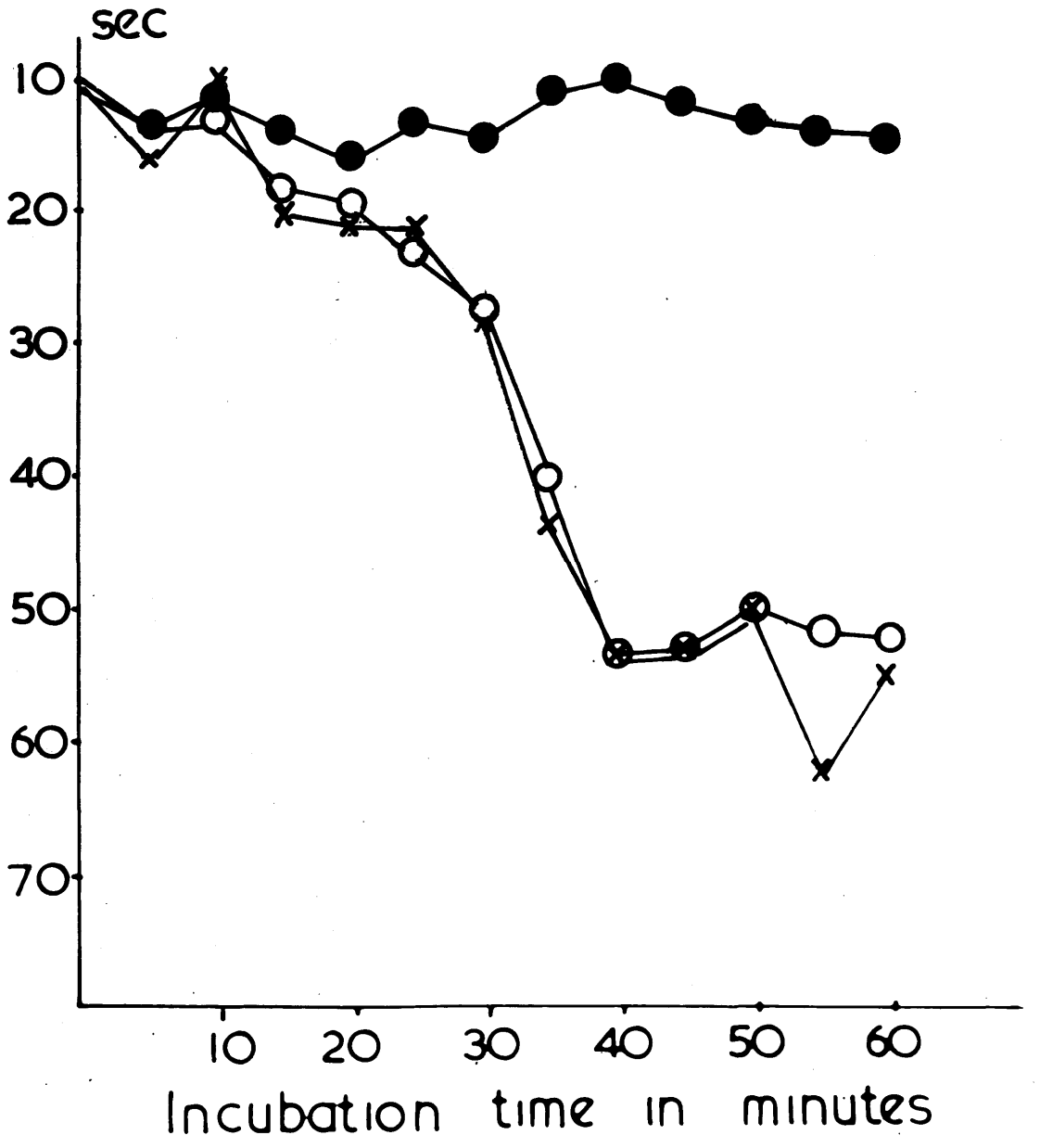
Ordinate - clotting times of fibrinogen in seconds.

Abscissa - incubation time in minutes.

●——● Haemophiliac.

O——O Haem.+ Case (2).

X——X Haem.+ Normal.


sec
10
20
30
40
50
60
70
10
20
30
40
50
60
Incubation time in minutes

The property of normal serum to correct the abnormality.

Since plasma is used from which the prothrombin has not been removed the answer is complicated by the presence of thrombin.

	(1)	(2)	(3)	(4)	(5)	(6)
Normal plasma 1/5) Normal platelets) Normal serum 1/10) $CaCl_2$)	8	8	8	8	8	9
Case 2. plasma 1/5) Normal platelets) Case 2. serum 1/10) $CaCl_2$)	46	35	32	29	25	18
Case 2. plasma 1/5) Normal platelets) Normal serum 1/10) $CaCl_2$)	11	8	8	9	10	11

See figure 21

Comment.

Normal serum corrects the defect.

Studies on prothrombin consumption.

(a) Correction of the prothrombin consumption of Case 2 by normal and haemophilic plasma.

(b) Correction of the prothrombin consumption of haemophilic plasma by normal and Case 2 plasma.

Figures 22 and 23

Comment.

In the preceding pages the differences between these two diseases has been demonstrated using the thromboplastin

Figure (24)

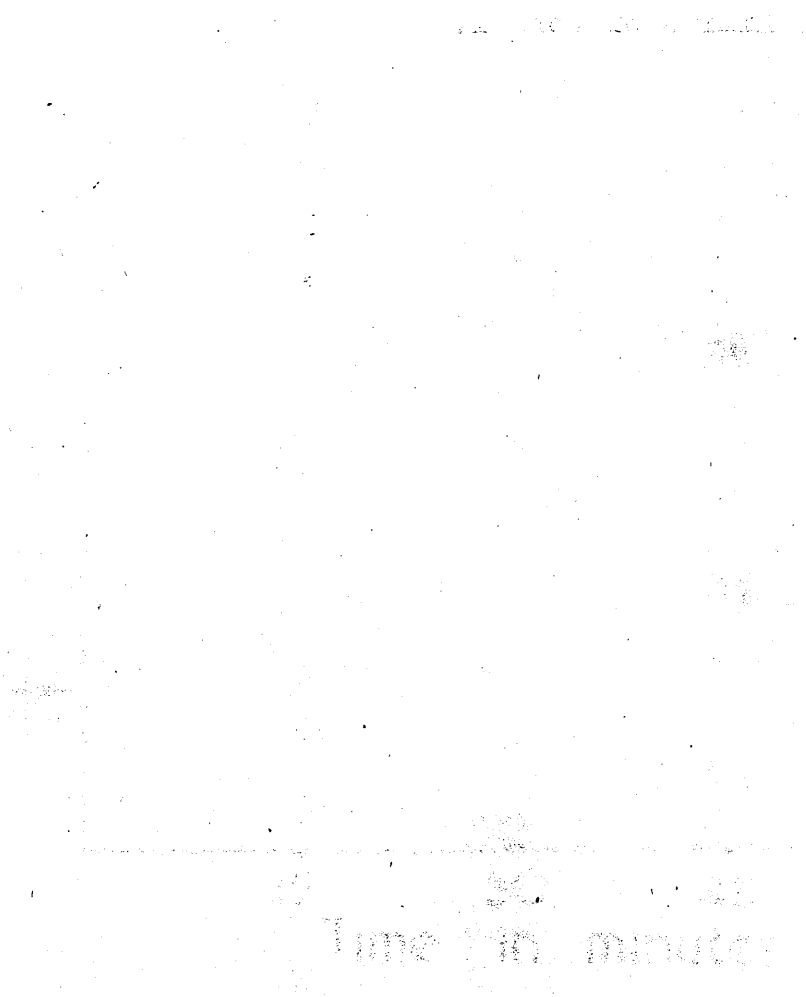


Figure (24)

Prothrombin consumption in Christmas Disease (Case 3).

Ordinate - percentage prothrombin.

Abscissa - incubation time in minutes.

●——● Case (3).

O----O Limits of normal.

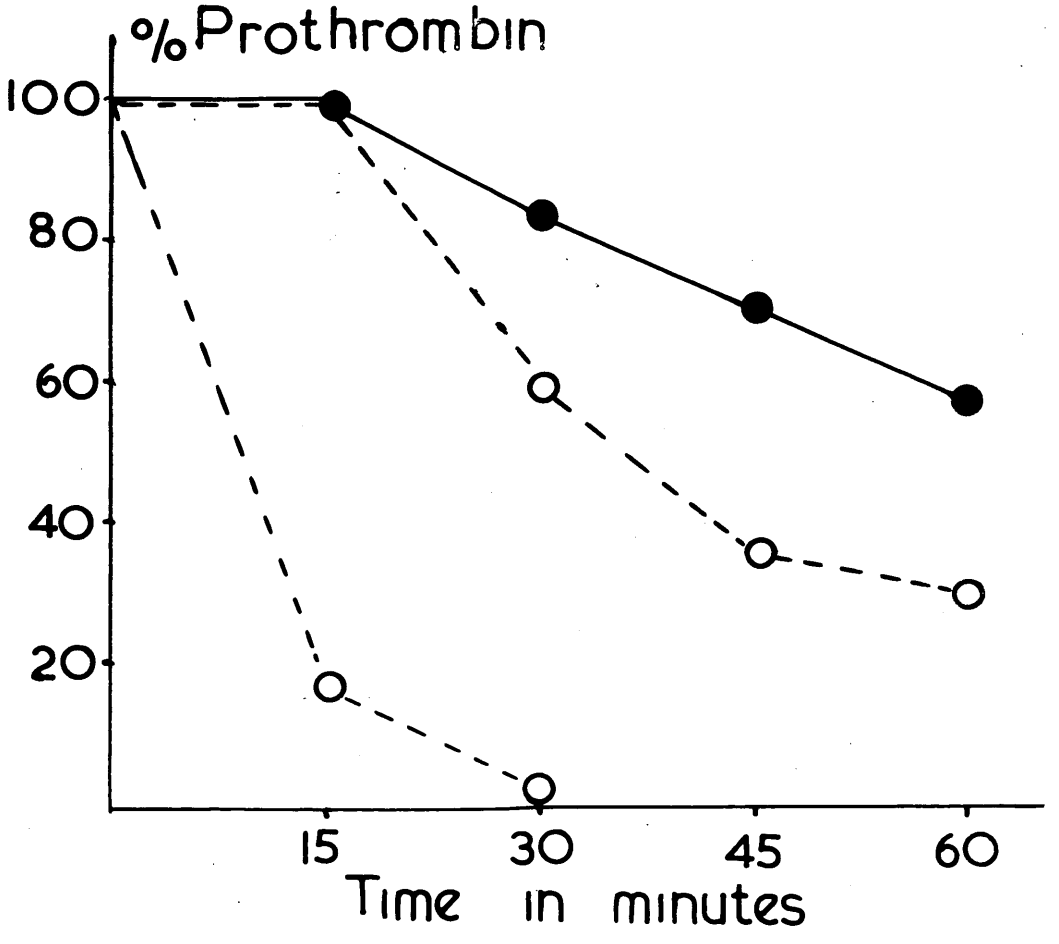

% Prothrombin
100
80
60
40
20
15
30
45
60
Time in minutes

Figure (25)

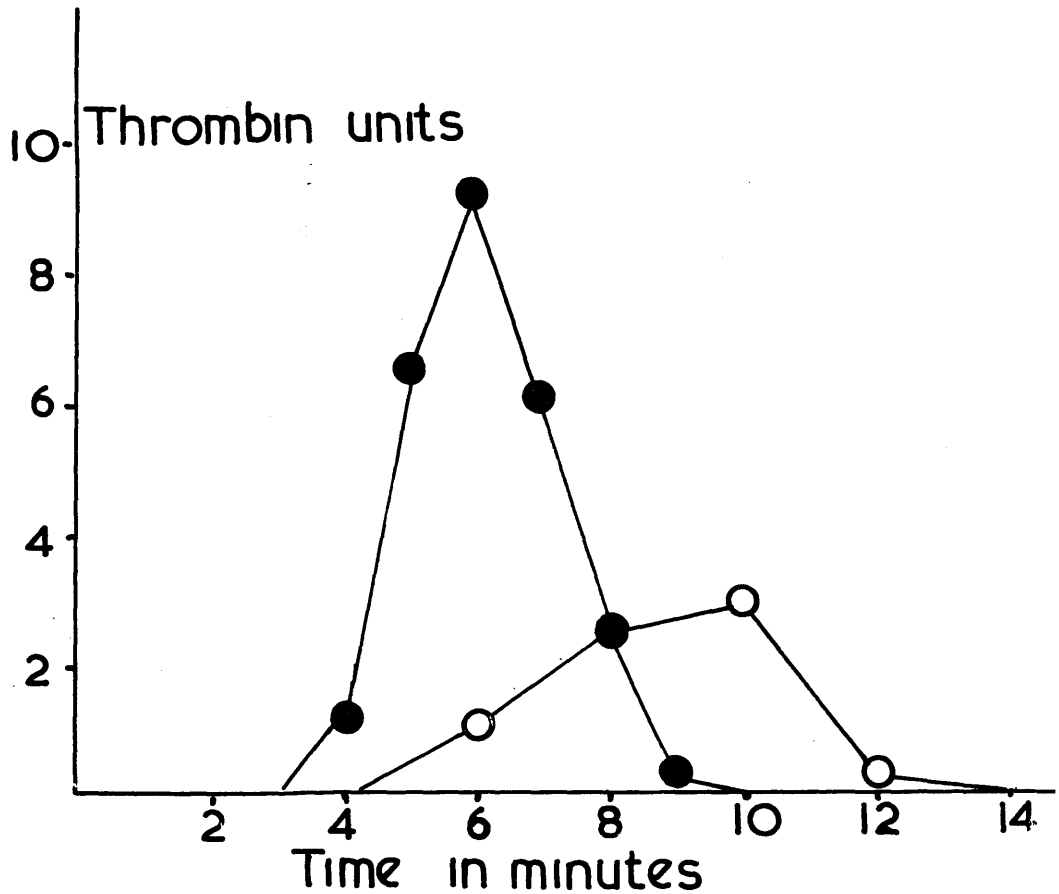
Thrombin generation from whole blood in Christmas disease. Case (3).

Ordinate - thrombin units.

Abscissa - time in minutes.

●——● thrombin generation from normal blood.

O——O thrombin generation from Christmas disease blood.


Thrombin units
10
8
6
4
2
2
4
6
8
10
12
14
Time in minutes

generation technique. Since this method of investigation is relatively new it was thought necessary to confirm the mutual correction of these defects by older and more accepted methods. By studying the prothrombin consumption of re-calcified plasma it was possible to show that the normal and haemophilic plasma corrected the prothrombin consumption of Case 2 plasma. Similarly small additions of plasma from Case 2 corrected the prothrombin consumption of haemophilic plasma as efficiently as did normal plasma.

Some of these observations on these patients were clearly of such fundamental importance that they required to be repeated. Some of these duplicate observations are recorded in the appendix (pages 619 - 636).

Case 3. L.S. (aged 14). Numerous bleeding episodes including haemarthroses dating from the age of 8 months. These haemorrhagic incidents were reported to respond well to transfusion.

Clotting time (Lee & White) (Method 1) 14-16 minutes.

Prothrombin Consumption (Merskey index) 100%; also by method of Douglas & Biggs (1953) was defective, Figure 24 .

One-stage "prothrombin" time - 15" (normal 15")

Thrombin generation - delayed formation of thrombin - see Figure 25 .

Figure (26)

Figure (30)

Family tree of Christmas disease - case (8). L.S.

Females represented O

Unaffected males represented □

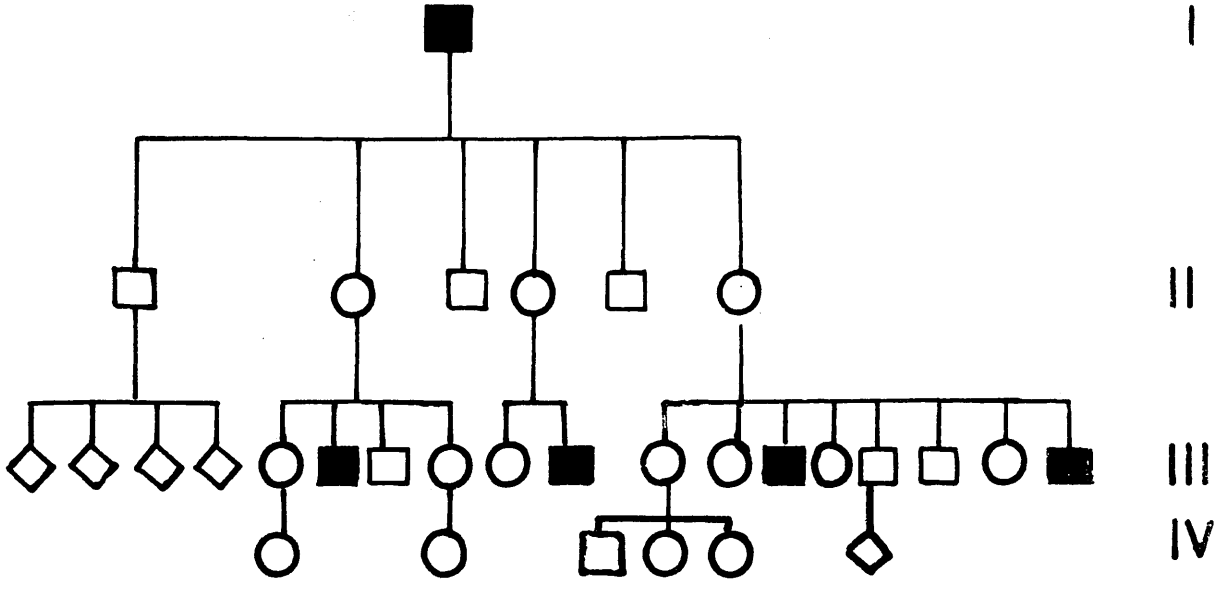
Affected males represented ■

Sex unknown represented◇ - these members of the family had not been traced and though it was known that there were the siblings as shown there was no information as to whether they were sons or daughters.

Case III_{10} is this patient.

Case III_{18} is a male aged 6 years with numerous haemorrhagic episodes, investigated by Dr. Merskey in Cape Town and found also to have Christmas disease.

Case 3 L.S.



The family history of this case is shown in Fig.26 . This is of considerable interest in that it illustrates quite clearly that the inheritance is that of a sex-linked recessive character as in haemophilia. Case III_{10} is this patient. Case III_{18} was a male aged 6 with numerous haemorrhagic episodes. This latter case is in South Africa and was investigated by Dr. Merskey in Cape Town and found to have the same disease. (Biggs et al 1952).

Case 4. A.W. This case was studied by Dr. J. O'Brien in Plymouth who observed that the blood of his case was able to correct the recalcification time of blood from other haemophiliacs. The patient was an adult male who gave a history of numerous haemorrhagic incidents. The family history was negative. On his most recent admission he had two teeth removed with associated severe haemorrhage. Plasma and serum from his patient were examined in Oxford. (See appendix page 634).

Clotting time (Lee & White) (Method 1) 28'-45'

Prothrombin Consumption (Merskey index) 160%

Case 5. J.E. This was a male aged 21. He gave a history of prolonged bleeding following tooth extractions on four occasions and on one required a transfusion of four pints of blood. At the age of 16 he had haematuria for

Figure (27)

Figure (27)

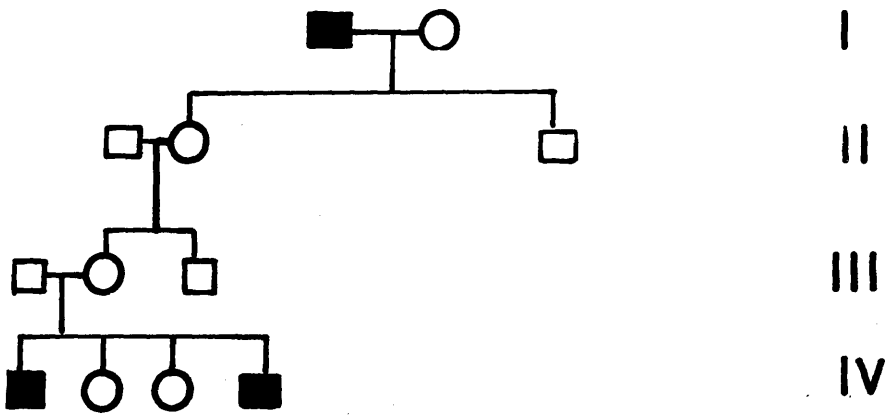
Family tree of Christmas disease - Case (5) J.E.

Females represented O

Unaffected males represented □

Affected males represented ■

Case 5 J.E.



three weeks and at 17 he had a haematoma of the buttock which was drained and took 4 weeks to heal. There was a younger brother aged $3\frac{1}{2}$, who bled for three weeks from a cut lip and the maternal great grandfather died of haemorrhage. (Fig 27) This patient had a relatively mild haemorrhagic diathesis and the laboratory findings were much less abnormal than in the other four cases. Attention was directed to this case by the ability of the plasma to correct the calcium clotting time of another haemophiliac.

Whole blood clotting time (Lee & White) 7-10 minutes.

Prothrombin Consumption Index (Merskey) 20%

One-stage prothrombin time 15"

Some of the results on these patients are summarised in the following Table.

TABLE I

	Case 1	Case 2	Case 3	Case 4	Case 5	Normal
Clotting Time (Lee & White method (1))	39-72	10-15	14-16	28-45	7-10	5-10
Prothrombin Consumption Index (Merskey %)	150	100	100	160	20	40
Quick's test (one-stage "prothrombin" time)	15	16	15	19	15	15
Bleeding time						
Tourniquet test			All Negative			
Platelet count						

Investigation of these cases by the Calcium clotting time.

In Table 2 is shown the ability of the plasma of these patients to correct the calcium clotting time of haemophilic plasma.

In Table 3 is given the calcium clotting time of these cases and its failure to be corrected by alumina-treated plasma, which is able to correct the defect in haemophilia.

In Table 4 the failure of all these cases to correct each other is demonstrated.

TABLE 2

Type of plasma added to haemophilic plasma.	Dilution of plasma added to haemophilic plasma. 1/2	1/10	1/50	1/100	0
Normal plasma	150	130	150	339	450
Case 1	240	-	-	-	555
Case 2	165	165	195	-	450
Case 3	-	177	-	329	895
Case 4	115	120	-	315	600
Case 5	140	175	220	240	780

(Recalcification times in seconds)

TABLE 3

	0	+ 1/2 Alumina plasma	+ 1/10 Haemophilic plasma	1/10 Normal plasma
Case 1	900	805	-	285
Case 2	455	-	-	150
Case 3	345	315	170	190
Case 4	390	340	150	175
Case 5	250			175
Haemophilic (1)	565	225		180
Haemophilic (2)	385	160		
Haemophilic (3)	250	159		159
Haemophilic (4)	895			188
Normal	145	265		

(Recalcification times in seconds)

TABLE 4

Cross over experiment using calcium clotting times to check that all five cases have the same disease.

Case 5 checked against Case 4.

Case 1 checked against Case 2.

Case 1, 3 and 4 checked against each other.

Case 5 with Case 4.

	+ 1/2 Case 5	+ 1/10 Case 5	+ 1/50 Case 5	+ 1/100 Case 5	0
Case 4	270	310	335	350	360

Case 1 with Case 2.

Case 1		15'
Case 2		7¼'
50% Case 1	50% Case 2	9'
10% Case 1	90% Case 2	9¾'
90% Case 1	10% Case 2	9¾'

Cases 1 with 3 and with 4.

	Saline	1/10 Case 3	1/10 Case 4	1/10 Case 1	1/10 Normal	1/100 Case 3	1/100 Case 4	1/100 Case 1	1/100 Normal
Case 3	310	-	313	345	205	-	335	380	325
Case 1	840	530	400	-	205	640	584	-	210
Case 4	400	300	-	440	175	350	-	400	240

(Recalcification times in seconds)

Other results on Cases 3, 4 and 5 are given in the appendix (pages 625-639)

Effect of various derivatives of plasma on the calcium clotting times of case 1.

These are shown in Table 5 .

Table 5.

Substance added to haemophilic or patient's plasma	Substance added to plasma of Case 1 in concentration:-			Substance added to the plasma of a haemophilic in concentration:-		
	10%	2%	0	10%	2%	0
Normal Plasma	105	130	210	120	150	450
Fibrinogen	230	240	237	150	195	450
Plasma heated to 56° C. 10 min.	180	190	215	200	240	450
Plasma stored 2 weeks	140	175	217	130	155	450
Seitz-filtered plasma	230	250	225	100	115	450
Haemophilic serum	120	150	210	430	475	450
Normal serum	90	100	230	190	265	450
Crude β globulin sample I	125	200	200	225	405	435
Crude β globulin sample II	120	135	200	420	375	435
Albumin	165	210	200	450	520	450
α globulin	200	210	200	455	4420	455
0-25% Sat. $(NH_4)_2SO_4$ from normal plasma	240	255	220	Corrects Haemophilic		
25-33% fraction	225	215	205	Does not correct Haemophilic.		
33-50% fraction	125	180	215	Does not correct Haemophilic.		

The missing coagulation component in Christmas disease has been called the Christmas factor. Some of the properties of this can be appreciated from the above table. (Biggs et al 1952).

The Christmas factor is adsorbed by aluminium hydroxide and by Seitz filtration. It is not present in the 0 to 33 per cent. saturated ammonium sulphate fraction of normal plasma, but is present in the 33 to 50 per cent fraction, and is present in the serum as well as in the plasma. This clotting factor was heat labile, relatively stable on storage in the refrigerator, and was contained in a crude ether fraction precipitated from plasma in association with alpha and beta globulins; it was not present in the ether fraction precipitated from the plasma in association with fibrinogen.

White, Aggeler and Emery (1953) studied the Christmas factor content of fractions of plasma obtained by Cohn's methods. They discovered that the Christmas factor content was concentrated in fractions III and IV_1. Little activity was found in fractions I and IV_4. None was found in fractions II or V. Since fraction III contained thrombin and fraction IV_1 was poorly soluble, neither was suitable for intravenous use in a highly concentrated form. Pronounced shortening of the whole blood coagulation time and a decided reduction in the residual serum prothrombin followed the intravenous administration of whole facton IV. (White et al 1953).

Figure (28)

Figure (2L)

Thromboplastin generation test in the mother of Christmas disease. Case (2).

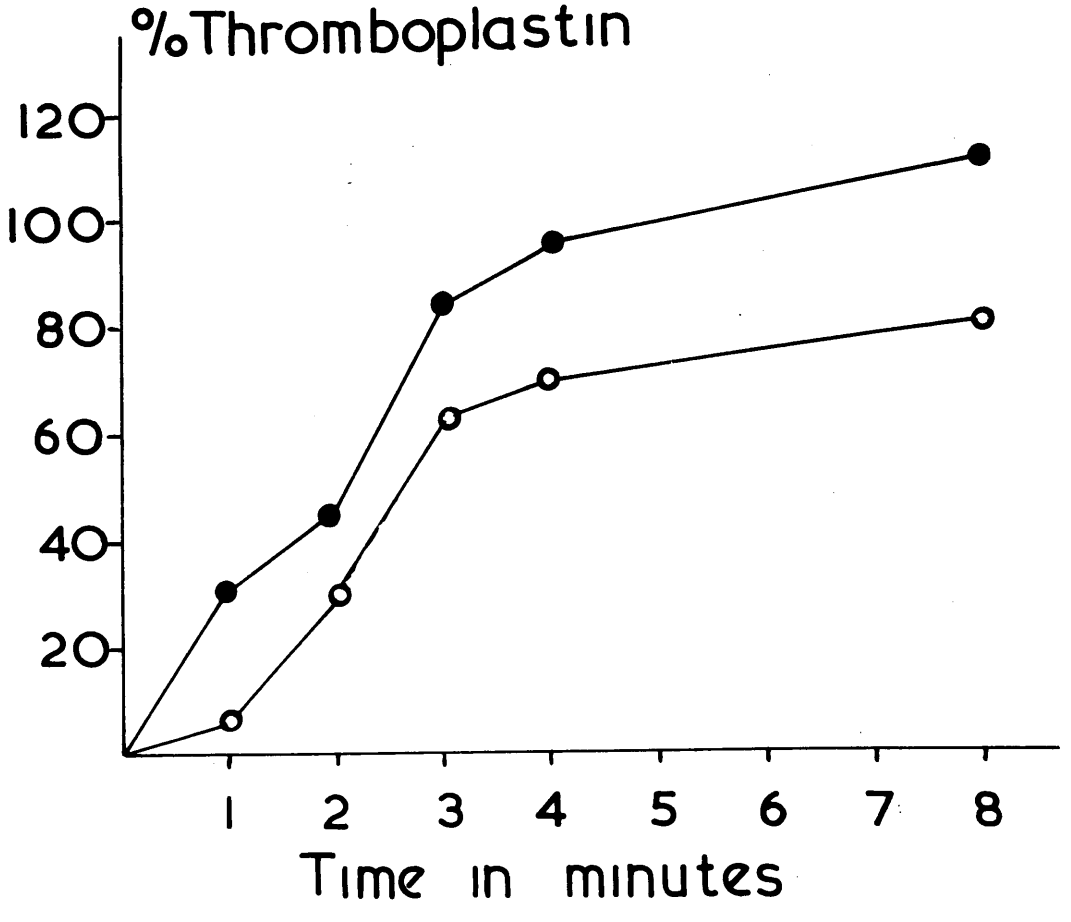
Ordinate - percentage blood thromboplastin.

Abscissa - incubation time in minutes after addition of calcium.

Normal adsorbed plasma and platelets constant.

●——● normal serum.

O——O serum from mother of Case (2).


%Thromboplastin
120
100
80
60
40
20
1
2
3
4
5
6
7
8
Time in minutes

Comparison of some of the features of antihaemophilic globulin and the Christmas factor.

In Table 6 some of the features of the Christmas factor are compared with those of antihaemophilic globulin.

The properties of Christmas Factor and Antihaemophilic Globulin

Table 6.

Method of Differentiation	Christmas Factor	Antihaemophilic Globulin
Ammonium sulphate fractionation.	Precipitated from normal plasma by 33-50% saturation.	Precipitated from normal plasma by 25% saturation.
Ether fractionation.	Precipitated from normal plasma in the crude β globulin fraction.	Precipitated from normal plasma in the fibrinogen fraction.
Test for presence in normal serum.	Present in large amounts.	Almost absent.
Test for presence in haemophilic serum.	Present in large amounts.	Absent.
Stability to heat.	Destroyed by heating to 56° C. for 10 min.	When isolated from plasma resists heating to 56° C. for 10 min.
Stability on storage.	Stable.	Often unstable.
Effect of Seitz filtration.	Adheres to the filter.	Is unaffected.
Effect of adsorbtion with $Al(OH)_3$	Very readily adsorbed	Not adsorbed.

Inheritance.

Cases 3 and 5 had positive family histories. The available family trees were not extensive, but so far as they

extended the inheritance fitted a sex linked recessive pattern. This has subsequently been confirmed by the investigations of other cases; the extensive family reported by MacMillan and Brown (1953) is a good example of the pattern of inheritance. The condition resembles haemophilia even in the matter of inheritance.

Assessment of the maternal clotting mechanism.

As in haemophilia it would be valuable to be able to detect the transmitters of this condition. In 1952 in Oxford, therefore, investigations were made on the mothers of Cases 1, 2 and 3. Only in the mother of Case 2 was any abnormality detected (figure 28) and this was accompanied by slightly abnormal prothrombin consumption. (46% Merskey Index) In the appendix (page 637) are described the results of later assessment of the mothers of other patients with Christmas disease and of their daughters. No conclusive difference from the normal was demonstrated in these.

Other cases reported in the literature.

The development of our understanding of blood thromboplastin formation enabled this study and the clear differentiation of these two disorders. Prior to this investigation it had been suspected that haemophilia was not simply one disease. Pavlovsky (1947) first observed that the clotting

time of mixtures of the blood of two patients, who were thought to suffer from haemophilia, was shorter than the clotting time of either specimen alone. He transfused 100 ml. of blood from one of these patients into the other and observed that this had the same corrective ability as the administration of the same quantity of normal blood. The antihaemophilic globulin activity of fractions of blood from some of his patients was almost equal to that from normal plasma.

Koller et al (1950) reported on a haemophilia-like disease which they called "Moena abnormality" (after the family name of their patients). The whole-blood clotting time was slightly prolonged and the prothrombin consumption markedly defective. Blood from these patients corrected the whole blood coagulation time and prothrombin consumption of other haemophilic patients.

Aggeler et al and White et al made an important and early contribution to the subject. Their studies were concurrent and independent to those reported by us in 1952. Their patient had a prolonged whole blood coagulation time and the prothrombin consumption was impaired, despite the presence of normal quantities of all previously described coagulation entities in the blood. The plasma had a normal one-stage clotting time and was corrected by platelet free

haemophilic plasma. They concluded that the plasma from their patient lacked a component necessary for blood thromboplastin formation, and they called this plasma thromboplastin component (P.T.C.). They demonstrated the following features of P.T.C.:-

(1) It can be adsorbed by barium sulphate and subsequently eluted with sodium citrate.

(2) It is present in the 40 to 50 per cent saturated $(NH_4)_2SO_4$ fraction of normal plasma and is not present in the 0 to 33 per cent saturated fraction which contains the A.H.G.

(3) P.T.C. is present in serum as well as in plasma.

(4) P.T.C. is present in normal quantities in the plasma in classical haemophilia.

(5) P.T.C. is not present in fraction I of Cohn, which contains the A.H.G. and fibrinogen, and is obtained by ether precipitation at low temperatures.

Further reports have appeared of cases having features similar to those reported by us and Aggeler and his colleagues in 1952. (Schulman and Smith (1952), Rosenthal, Dreskin and Rosenthal (1953), Lewis and Ferguson (1953), Van Creveld and Paulssen (1953), Soulier and Larrieu (1953), Cramer et al (1953), Beaumont et al (1953), MacMillan and Brown (1953).

Problems of terminology.

It is unfortunately true that differences of opinion concerning nomenclature have caused confusion. Until 1952

haemophilia was thought to be a single clinical and pathological entity. Aggeler and his co-workers (1952) in their original publication used the term P.T.C. deficiency. Subsequently they referred to the condition as deutero-haemophilia. We have used the term Christmas disease applying the name after one of the first two patients seen. The naming of clinical disorders after patients was introduced by Sir Jonathan Hutchison. It has been common practice recently in red cell serology and has also been followed by other workers in this field (e.g. Moena anomaly of Koller et al 1950, Hageman factor of Ratnoff and Calopy 1955). It has the advantage that no hypothetical implication is attached to such a name. Graham and Brinkhous (1953) have suggested that the term haemophilia should be retained for what is still the most common abnormality - deficiency of A.H.G. and that other allied conditions should be called haemophilioid states; Christmas disease was called haemophilioid-state C. Swiss and French workers have used the terms haemophilia A for A.H.G. deficiency and haemophilia B for Christmas disease (Soulier and Larrien 1953, Cramer et al 1953). Weiner (1953) suggested haemophilia I and II. Koller (1954) has extended his numerical numbering system and calls A.H.G. deficiency (factor VIII deficiency) and Christmas factor deficiency as factor IX deficiency. Seegers (1954)

has called Christmas disease platelet co-factor 2 deficiency and Fantl and Sawers (1954) refer to it as β -prothromboplastin deficiency.

The names for Christmas disease can therefore be enumerated as follows:-

Plasma Thromboplastin Component (P.T.C.) deficiency Aggeler et al (1952)

Deuterohaemophilia.

Plasma Thromboplastin Factor B (P.T.F.-B) Deficiency Aggeler et al (1954)

Haemophilia II (Weiner, 1953)

Haemophilia B. (Soulier and Larrieu, 1953, Cramer et al 1953)

Haemophilioid-state C (Graham and Brinkhous 1953)

Platelet Co-Factor-2. (Seegers 1954)

β -prothromboplastin Deficiency (Fantl & Sawers 1954)

Factor IX Deficiency (Koller 1954)

Haemophilia (lack of antihaemophilic globulin) may be called:

Haemophilia A (Soulier and Larrieu 1953)

Haemophilia I (Weiner, 1953)

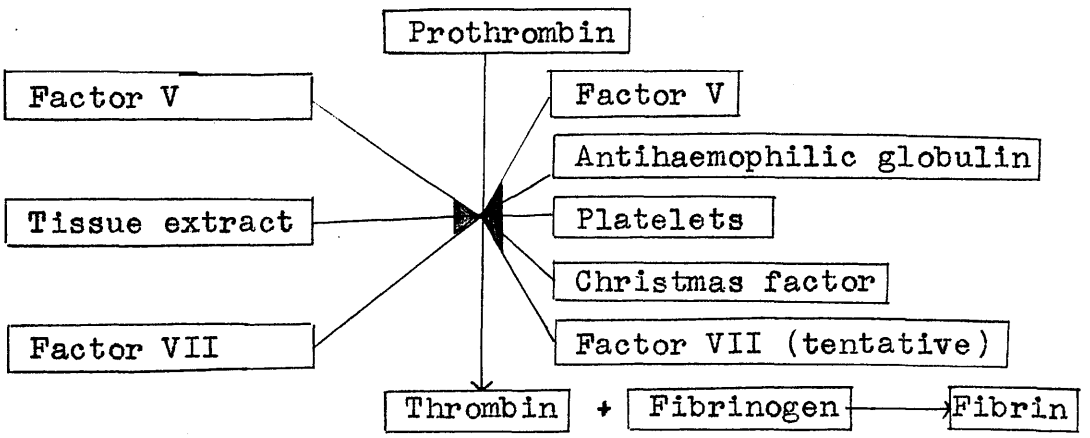
Factor VIII Deficiency (Koller 1954).

Christmas factor as a component of blood thromboplastin.

It was shown in Chapter 4 that the constituents required for the preparation of a powerful blood thromboplastin are

platelets, factor V, antihaemophilic globulin and the fraction of serum adsorbed on alumina. Christmas disease has the features of a thromboplastin deficiency; in particular there is defective prothrombin consumption. The missing component in Christmas disease - the Christmas factor - is present in normal serum not being consumed during clotting and is present in that fraction of serum adsorbed on alumina. This fraction of serum also contains the factor VII of Koller. Whether the factor VII participates in blood thromboplastin formation has not been finally decided. This problem will be discussed in the next chapter. For the present it is advisable to label factor VII as a possible component of blood thromboplastin.

The scheme of blood coagulation thus becomes:



The relationship of the coagulation defect in Christmas disease and that produced by the coumarin drugs will be dealt with in the next chapter.

The discovery of Christmas disease is of very great academic interest and of some practical importance. It is clear that cases of haemophilia in the past may have been either deficiencies of antihaemophilic globulin (haemophilia) or Christmas factor (Christmas disease). A differentiation is important in the problem of practical therapeutics of the conditions. There is for example, no value in treating Christmas disease with a preparation of antihaemophilic globulin which does not contain the Christmas factor.

The discovery of Christmas disease was reported in the British Medical Journal in December 1952 (Brit. med. J. ii, 1378 Christmas disease; A condition previously mistaken for haemophilia).

Though I shared in the investigations of Cases 3, 4 and 5, the assessment of Cases 1 and 2 by the thromboplastin generation technique was my own work; the other investigations described were done jointly.

SUMMARY

(1) The problem of occasional mutual correction of "haemophilic" blood was investigated by the application of the thromboplastin generation technique. This led to the clear differentiation of "haemophilia" into two conditions - haemophilia - lack of antihaemophilic globulin and Christmas disease - lack of the Christmas factor.

(2) Using the thromboplastin generation technique the abnormality in haemophilia is in the adsorbed plasma whereas in Christmas disease it is in the serum. The antihaemophilic globulin (A.H.G.) is used up during clotting and is therefore not present in normal serum. The Christmas factor (C.F.) is not consumed during clotting and is therefore present in serum. C.F. is adsorbed on aluminium hydroxide whereas A.H.G. is not.

(3) Female transmitters of Christmas disease have been investigated. In one mother there was defective prothrombin consumption and a serum abnormality on thromboplastin generation.

(4) The literature of the condition is briefly reviewed and the problems of terminology discussed.

REFERENCES

Aggeler, P.M., White, S.G., Glendening, M.B., Page, E.W., Leake, T.B. and Bates, G. (1952). Plasma thromboplastin component (P.T.C.) deficiency. A new disease resembling haemophilia. Proc. Soc. exp.Biol. and Med. 79, 692.

_____(1952). P.T.C. factor deficiency: A previously undescribed haemophilia-like disease due to deficiency of a heretofore unknown thromboplastin component. Amer. J. Med. 13, 90.

Aggeler, P.M., White, S.G. and Spaet, T.H. (1954). Deuterohemophilia: Plasma thromboplastic factor B deficiency. Christmas disease. Haemophilia B. Blood, 9, 246.

Beaumont, J.L., Cayla, A., Dupin, H. and Bernard, J.(1953). Syndrome hemorragique congenital du au defaut d'un facteur plasmatique prothromboplastique different du facteur antihemaphilique (Hemophilic B). Le Sang, 488.

Biggs, R., Douglas, A.S., Macfarlane, R.G., Dacie, J.V., Pitney, W.R., Merskey, C., and O'Brien, J.R.(1952). Christmas disease. A condition previously mistaken for haemophilia. Brit. med. J. 2, 1378.

Cramer, R., Flückinger, P., Gasser, C., Koller, F.,Loeliger,A. and Matter, M. (1953). Haemophilia B. Two cases of haemophilia B. due to deficiency of a new clotting factor (Christmas factor). Acta Haemat. 10, 65.

Cramer, R., Matter, M., and Loeliger, A. (1953). Die haemophilie B. Zwei familiäre Falle der Bluterkrankheit bedingt durch den Mangel eines neuen Gerrinungsfaktors (Christmas factor). Helv. Ped. Acta. 8, 185.

Fantl, P. and Sawers, R.J. (1953). Haemophilia; a syndrome under review. Med. J. Aust. 1,925.

Graham,J.B. and Brinkhous, K.M. (1953). Letter to the editor. Brit. med. J. 2, 97.

Koller, F., Krusi, G. and Luchsinger, G. (1950). Ueber eine besondere form hemorrhagischer diasthese. Schweiz med. Wchnschr. 80,1101.

Koller, F. (1954). Is haemophilia a nosologic entity? Blood, 9, 286.

Lewis, J.H. and Ferguson, J.H. (1953). Haemorrhagic diathesis due to P.T.C. (plasma thromboplastin component) deficiency. Proc. Soc. exper. Biol.and Med. 82, 445.

MacMillan, R.L. and Brown, K.W.G. (1953). Christmas disease - a variant of haemophilia. Canad. med. Ass. J. 69, 623.

Pavlovsky, A. (1947). Contribution to the pathogenesis of haemophilia. Blood, 2, 185.

Poole, J.C.F. (1953). A haemorrhagic state resembling haemophilia. Lancet. 1, 122.

Ratnoff, O.D. and Colopy, J.E. (1955). A familiar haemorrhagic trait associated with a deficiency of a clot promoting fractionof plasma. J. clin. Invest. 34, 602.

Rosenthal, R.L., Dreskin, O.H. and Rosenthal, N.(1953). New haemophilia-like disease caused by deficiency of a third thromboplastin factor. Proc. Soc. exper. Biol. and Med. 82, 171.

Schulman, I. and Smith, C.H. (1952). Haemorrhagic disease in an infant due to deficiency of a previously undescribed clotting factor. Blood, 7, 794.

Seegers, W. (1954). A theoretical consideration of the blood clotting mechanisms in haemophilia. Schweiz. med. Wchnschr. 84, 781.

Soulier, J.P. and Larrieu, M.J.(1953). Nouvelle methode de diagnostic de l'hemophilic dosages des facteurs antihemophiliques A et B. Le Sang, 24, 205.

Van Creveld, S. and Paulssen, M.M.P. (1953). Haemorrhagic diathesis due to absence of Christmas factor. Lancet 264,823.

Weiner, A.S. (1953). Letter to the editor. Brit. med.J. 1,559.

White, S.G., Aggeler, P.M., Glendening, M.B., Page, E.W., Leake, T.B. and Bates, G. 1952. P.T.C.factor: a new plasma thromboplastin component distinct from the antihaemophilic factor. J. clin. Invest. 31,673.

White, S.G., Aggeler, P.M. and Emery, B.E. (1953). The plasma thromboplastin component (P.T.C.) potency of plasma fractions. Proc. Soc. exp. Biol. & Med. 83,69.

CHAPTER 6

OTHER THROMBOPLASTIN-PRECURSOR DEFICIENCIES

CONTENTS.

Failure of blood thromboplastin formation ascribed to deficiencies of plasma thromboplastin antecedent (P.T.A.), the fourth thromboplastin component, and the Hageman factor are discussed.

A family is described where the features correspond to those described as P.T.A. deficiency.

CHAPTER 6

OTHER THROMBOPLASTIN-PRECURSOR DEFICIENCIES

In addition to haemophilia and Christmas disease there have been described other constitutional deficiencies of factors needed for blood thromboplastin formation. This is in addition to deficiencies of factor V or factor VII which have been mentioned in Chapter 3 .

PLASMA THROMBOPLASTIN ANTECEDENT (P.T.A.) DEFICIENCY.

This condition was described by Rosenthal, Dreskin and Rosenthal (1953 and 1955). The family studied by these authors consisted of thirteen members comprising four generations. Six of the members of the family had, to a variable degree, a haemorrhagic tendency. The inheritance was of interest in that both males and females were affected and the transmission was as an autosomal dominant trait with a probable high degree of penetrance and variable expression of the gene. The disease can be transmitted by either sex to male or female offspring.

The clinical severity of the haemorrhagic tendency was much less than in most cases of haemophilia and Christmas disease. The patients rarely developed spontaneous bleeding;

the haemorrhage usually followed trauma or a surgical procedure. Haemarthrosis was rare.

The laboratory investigation revealed that this was a blood thromboplastin deficiency. The whole blood clotting time showed a slight prolongation and there was some impairment of prothrombin utilisation. Some of the patients had a normal whole blood clotting time. The one-stage clotting time, tourniquet test, bleeding time and platelet count were all normal.

The properties of P.T.A. are compared with those of antihaemophilic globulin (A.H.G.) and the Christmas factor (C.F.). The blood from these patients corrects the deficiency in haemophilia or Christmas disease. A.H.G. is used up during the clotting of normal blood and therefore there is none remaining in normal serum. P.T.A. (like C.F.) is not consumed during clotting and therefore is present in serum. P.T.A. (like A.H.G.) is not adsorbed on barium sulphate. C.F. is removed by barium sulphate adsorption. P.T.A. (like C.F.) does not disappear on storage as does A.H.G.

POSSIBLE FOURTH THROMBOPLASTIN COMPONENT.

(Spaet, Aggeler and Kinsell 1954).

The characters of this coagulation defect are similar to those found in P.T.A. deficiency except that the plasma

and serum of the patient suffering from this disorder and those of a patient with P.T.A. deficiency were mutually corrective. Furthermore it was found that when either alumina-adsorbed plasma or serum from this patient was substituted for its normal counterpart in the thromboplastin generation test, markedly abnormal results were obtained.

HAGEMAN FACTOR.

Ratnoff and Colopy (1955) describe a further component of the coagulation system which they believe is required in the conversion of prothrombin to thrombin. They have called this the Hageman factor after the first patient studied and they present evidence that this coagulation component is different from those previously discussed.

Three patients (one male and two females) were studied; the two females belonged to the same family. It is of particular interest that none of these patients had any evidence of a haemorrhagic tendency. The disturbance of coagulation was discovered in the first two patients because of a prolonged whole blood clotting time done as a routine prior to operation. The operations were carried out without abnormal haemorrhage occurring.

Plasma from the patients had a normal one-stage clotting time and therefore there was no deficiency of factors V or VII. High spun platelet-free patients' plasma was incoagulable and

the defect was therefore believed to be due to a plasma factor rather than to a platelet abnormality. This high-spun platelet-free plasma remained incoagulable without the addition of an anticoagulant; it clotted in normal time, however, on the addition of plasma from patients with haemophilia, Christmas disease, P.T.A. deficiency and fourth thromboplastin component deficiency. There was deficient prothrombin consumption in these patients.

The Hageman factor can be prepared from normal serum. The Christmas factor and factor VII are removed by adsorption on barium sulphate and the fourth thromboplastin component by heating to 60° C. Such preparations are likely to contain P.T.A.

No patients of this type have been seen.

PATIENTS WITH A COAGULATION ABNORMALITY HAVING THE CHARACTERS OF "P.T.A. DEFICIENCY".

The family now to be described did not have typical deficiencies of either A.H.G. or C.F. and may have belonged to the same type of disorder as that described by Rosenthal et al or Spaet et al. A possible explanation is that they were partial deficiencies of both A.H.G. and C.F.

A father and daughter suffered from a mild haemorrhagic disorder.

One-stage clotting time normal. Ability of the plasma

to correct the one-stage clotting time of tromexan plasma was normal. Prothrombin consumption by Merskey technique was normal in both father and daughter.

The results of the thromboplastin generation technique were as follows:

(1) Platelets from father and daughter were as good as normal platelets.

Normal ads. plasma and normal serum constant.

	(1)	(2)	(3)	(4)	(5)	(6)
Normal platelets	20	10	9	8	10	9
Father platelets	31	11	10	9	10	12
Daughter platelets	21	8	8	9	10	10

(2) Adsorbed plasma-serum prepared from normal, father, daughter and haemophiliac.

Ads. plasma 1/5. Serum 1/20.

Platelets constant throughout experiment.

Nor. ads. plasma	(Normal serum	39	19	12	10	11	12
	(Father serum	53	33	21	14	13	13
	(Daughter serum	48	30	16	13	14	13
	(Haem. serum	11	10	11	11	12	10
Daughter ads. plasma	(Normal serum	39	22	12	11	11	10
	(Father serum	49	34	28	18	15	14
	(Daughter serum	48	37	24	15	14	12
	(Haem. serum	21	12	11	10	11	12
Father ads. plasma	(Normal serum	45	27	15	12	11	11
	(Father serum	60	44	32	24	16	14
	(Daughter serum	59	50	35	21	16	13
	(Haem. serum	31	12	11	11	10	11

Figure (29)

Figure (VI)

Thromboplastin generation test in a patient of P.T.A. type.

Ordinate - percentage blood thromboplastin.

Abscissa - incubation time in minutes after addition of calcium.

Platelets constant.

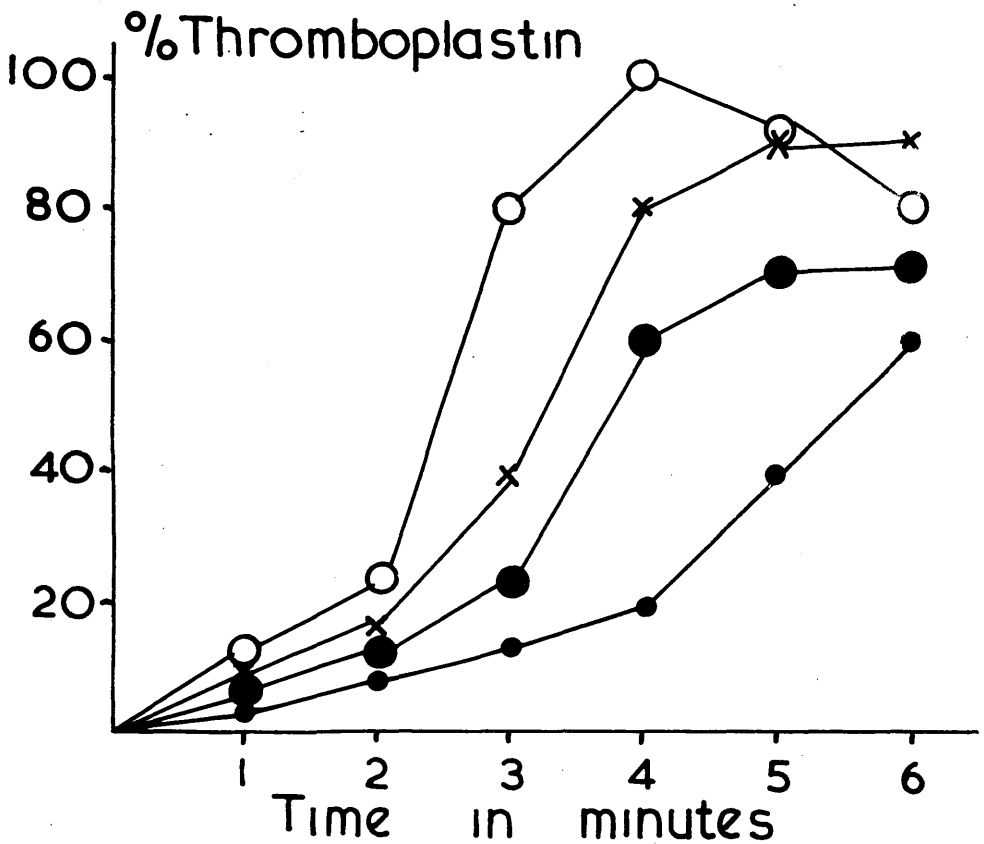
O——O Normal ads. plasma; normal serum.

X——X Patient ads. plasma; normal serum.

●——● Normal ads. plasma; patient serum.

●——● Patient ads. plasma; patient serum.

Patient = father of family described.


%Thromboplastin
100
80
60
40
20
1
2
3
4
5
6
Time in minutes

Haem.	(Normal serum	68	57	53	48	44	42
ads.	(Father serum	57	50	50	48	49	46
plasma	(Daughter serum	65	66	64	60	60	59
	(Haem. serum	59	35	29	26	28	26

Comment.

The defect only shows up clearly when the patients' ads. plasma and patient serum are used together in the test (see figure 29). It is tempting to assume that these patients represent partial deficiencies both of antihaemophilic globulin and the Christmas factor rather than the deficiency of an additional coagulation factor - the so called plasma thromboplastin antecedent.

SUMMARY

(1) In addition to deficiencies of antihaemophilic globulin (A.H.G.) and Christmas factor (C.F.), other plasma components have been said to be required for blood thromboplastin formation. These include plasma thromboplastin antecedent (P.T.A.) of Rosenthal et al, the fourth thromboplastin component of Spaet et al and the Hageman factor (Ratnoff and Colopy).

(2) A family with a mild haemorrhagic diathesis is described; the features may be the same as the cases described by Rosenthal et al or Spaet et al. The characteristic feature was the failure to obtain clear evidence of an abnormality in thromboplastin formation in the generation technique unless the adsorbed plasma and the serum were both derived from the patient.

REFERENCES

Ratnoff, O.D. and Colopy, J.E. (1955). A familial haemorrhagic trait associated with a deficiency of a clot promoting fraction of plasma. J. clin. Invest. 34, 602.

Rosenthal, R.L., Dreskin, O. H. and Rosenthal, N. (1953). New haemophilia-like disease caused by deficiency of a third thromboplastin factor. Proc. Soc. exp. Biol. & Med. 82, 171.

Rosenthal, R.L.,Dreskin, O.H. and Rosenthal, N. (1955). Plasma thromboplastin antecedent (P.T.A.) deficiency. Clinical, coagulation, therapeutic and hereditary aspects of a new haemophilia-like disease. Blood. 10,120.

Spaet, T.H., Aggeler, P.M. and Kinsell, B.G. (1954). A possible fourth thromboplastin component. J. clin. Invest. 33, 1095.

Part III. The Action of Anticoagulant Drugs

CHAPTER 7

ACTION OF THE COUMARIN DRUGS

CONTENTS

Chemical Structure.
Prothrombin.
(1) Comparison with patient with idiopathic prothrombin deficiency.

(2) Assay by area method and the globulin fraction technique.

Comparison by these two methods.

Antithrombin assay.

Factor VII.
(1) Effect of normal serum and normal plasma and of preparations of prothrombin and factor VII in correcting the one-stage clotting time of tromexan plasma.

(2) The effect of additions on the shape of the two stage curve of tromexan plasma. These additions include normal serum, normal plasma, prothrombin deficient plasma, tromexan serum.

The progress of levels of prothrombin and factor VII during tromexan therapy and following cessation of therapy with and without vitamin K_1.

Factor V.

Effect of storage of plasma on the one-stage clotting time.

Calcium concentration and the one-stage clotting time.

Other one-stage techniques and comparison with prothrombin content on two stage techniques:-

(a) with an excess of factor VII.
(b) with preincubation of brain and serum.
(c) with blood thromboplastin.
(d) with Russell's viper venon.

Blood thromboplastin in coumarin therapy.

(1) whole blood clotting time.
(2) Calcium clotting time.
(3) Prothrombin consumption.
(4) Thrombin Generation Test.
(5) Thromboplastin generation technique.
    (i) failure of serum to form blood thromboplastin.
    (ii) antihaemophilic globulin content.

Comparison of coumarin defect with that in Christmas disease.

(a) Comparison of serum thromboplastin defect in coumarin therapy and that in Christmas disease.

(b) The action of dindevan plasma on the prothrombin consumption of Christmas disease plasma.

(c) Comparison of normal and Christmas plasma and of normal and Christmas serum to correct the one-stage clotting time of tromexan plasma.

(d) The thrombin-thromboplastin generation from Christmas disease and tromexan plasma with additions of normal, tromexan and Christmas disease serum.

CHAPTER 7

ACTION OF THE COUMARIN DRUGS

If advance is to be made in the treatment of thrombo-embolic disease it is of importance to have a better understanding of the nature of the interference with blood coagulation produced by the anticoagulant drugs.

Some of the historical aspects of this problem with reference to "sweet clover disease" in cattle and the discovery of dicumarol as the aetiological factor have been described in Chapter 3 . From a scrutiny of the experimental evidence also outlined previously it is suggested that the administration of the coumarin drugs leads to a deficiency of prothrombin and factor VII. It is the object of this present chapter to produce new evidence in support of this concept and to examine the extent of these deficiencies using improved techniques. It is also intended to examine the nature and extent of any interference with the blood's intrinsic thromboplastin mechanism produced by these drugs.

CHEMICAL STRUCTURE.

The coumarin anticoagulant drugs are relatively simple chemical compounds but the chemical processes involved in the production of their effect are very poorly understood.

Figure (30)

Figure (xx)

Formulae of coumarin drugs.

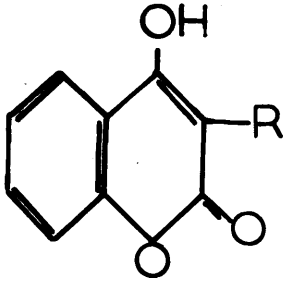
(1) 3 substituted - 4 hydroxy coumarin derivatives.

(2) Cyclic acetals.

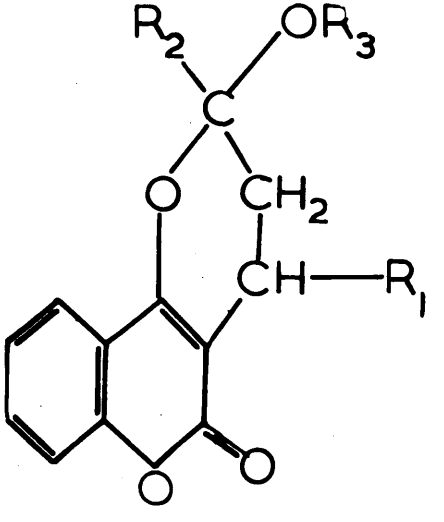
(3) Two coumarin ring systems.

(4) Derivatives of 1:3 - indanedione.

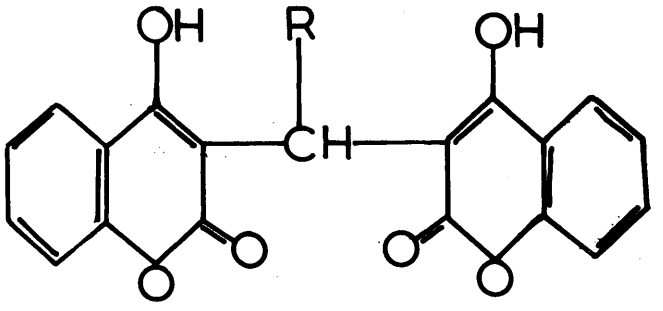
1.



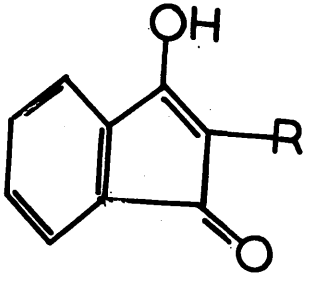
2.



3.



4.



They may be divided into four main groups according to their chemical structure (see figure 30). (Hunter and Shepherd,1955).

(1) 3 - substituted - 4 hydroxy coumarin derivatives represented by the general formula I in which R is an aryl or aralkyl group. Available examples of this group are Marcoumar made by Roche and the recently marketed Sinthrome made by Geigy.

(2) Cyclic acetals (see formula II) in which R_1 is an aryl group and R_2 and R_3 are alkyl groups. Cyclocumarol available as cumopyran made by Abbot Laboratories Ltd. is a compound of this type.

(3) Compounds having two coumarin ring systems and having the general formula III - Dicumarol itself and Tromexan (Geigy) belong to this group.

(4) Derivatives of 1:3 - indanedione - see formula IV. These compounds though not coumarin derivatives, are so closely related to the latter both in chemical structure and mode of action, that they are usually classified along with coumarin anticoagulants. Dindevan (phenyl indanedione and the newer dipaxin belong to this group.)

While several interesting relationships between chemical structure, physical properties and anticoagulant activity have been pointed out, we are still unable to define the minimum structural characteristics which are required to confer anticoagulant powers on a molecule.

The drug of the coumarin group in routine use in the wards of the Radcliffe Infirmary, Oxford, at the time of the greater part of this study was tromexan.

Figure (31)

Figure (21)

Two-stage prothrombin test in normal plasma, tromexan plasma and prothrombin deficient plasma (Area method).

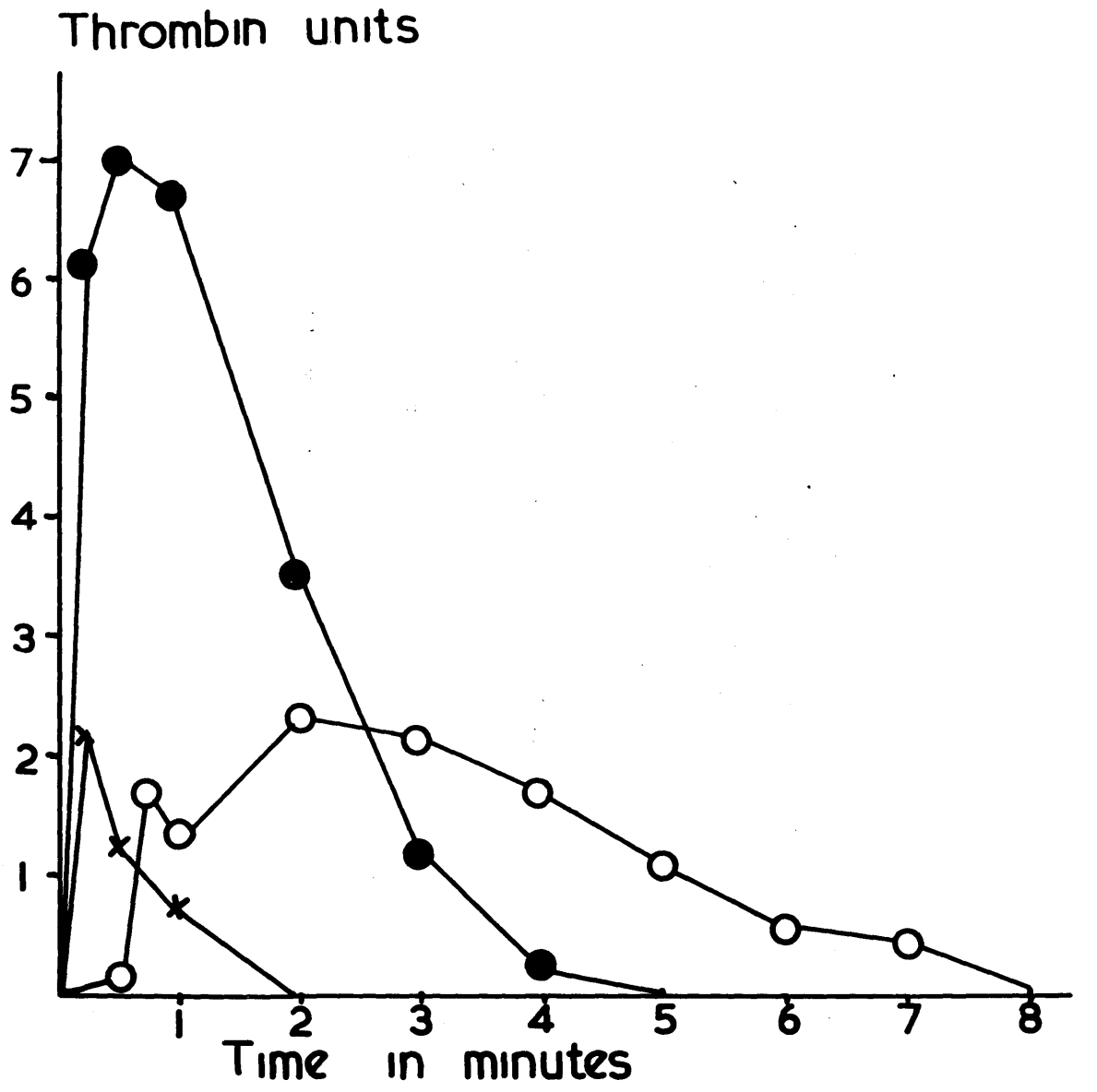
Ordinate - Thrombin units.

Abscissa - Incubation time in minutes after addition of calcium.

●——● Normal plasma.

O——O Tromexan plasma.

X——X Prothrombin deficient plasma.


Thrombin units
7
6
5
4
3
2
1
1
2
3
4
5
6
7
8
Time in minutes

Clinical trials with tromexan have been reported by Reinis and Kubik (1948), von Kaulla and Pulver (1948); Burt et al (1949), Burke and Wright 1950) and Stirling and Hunter (1951). A smaller number of observations have been made on dicumarol and dindevan.

Prothrombin.

Biggs and Douglas (1953) have reported a case of idiopathic prothrombin deficiency. This patient is described in Chapter 12 . This case is unique in that it is the only case so far described in which isolated prothrombin deficiency has been demonstrated conclusively. A comparison of the defect in this patient with that in tromexan plasma assisted the elucidation of the present problem.

When the area two-stage test was performed on a normal plasma, this patient's plasma and a tromexan plasma, the results obtained are shown in Figure 31 . It will be observed that the patient with idiopathic prothrombin deficiency develops his small amount of thrombin very rapidly, but the tromexan plasma produces much thrombin over several minutes. The one-stage clotting time of these plasma samples are compared with the corresponding prothrombin contents estimated by the area method (Table 7).

TABLE 7

Comparison of one-stage clotting times with prothrombin content in normal, tromexan and prothrombin deficient plasma.

	One-stage clotting time.	Prothrombin content by area method.
Normal plasma	15"	100%
Tromexan plasma	35"	68%
Prothrombin deficient plasma	18"	10%

The importance of these results lies in the observation that the patient with only 10 per cent of prothrombin has a one-stage clotting time little altered from normal, whereas the tromexan plasma with 68 per cent of prothrombin has a grossly prolonged one-stage clotting time. It seems a reasonable assumption that the one-stage clotting time is insensitive to change in prothrombin, but is sensitive to the main defect in tromexan plasma.

When the plasma of the patient with idiopathic prothrombin deficiency or a normal plasma was mixed with tromexan plasma in the same proportions, the patient's plasma (containing only 10 per cent prothrombin) was as effective as the normal plasma in shortening the one-stage clotting time of the tromexan plasma.

Figure (32)

Figure (32)

Antithrombin assay in tromexan plasma.

Ordinate - Thrombin units.

Abscissa - Incubation time in minutes after addition of thrombin to plasma.

——— tromexan plasma.

----- normal plasma.

(Mean of 25 observations.)

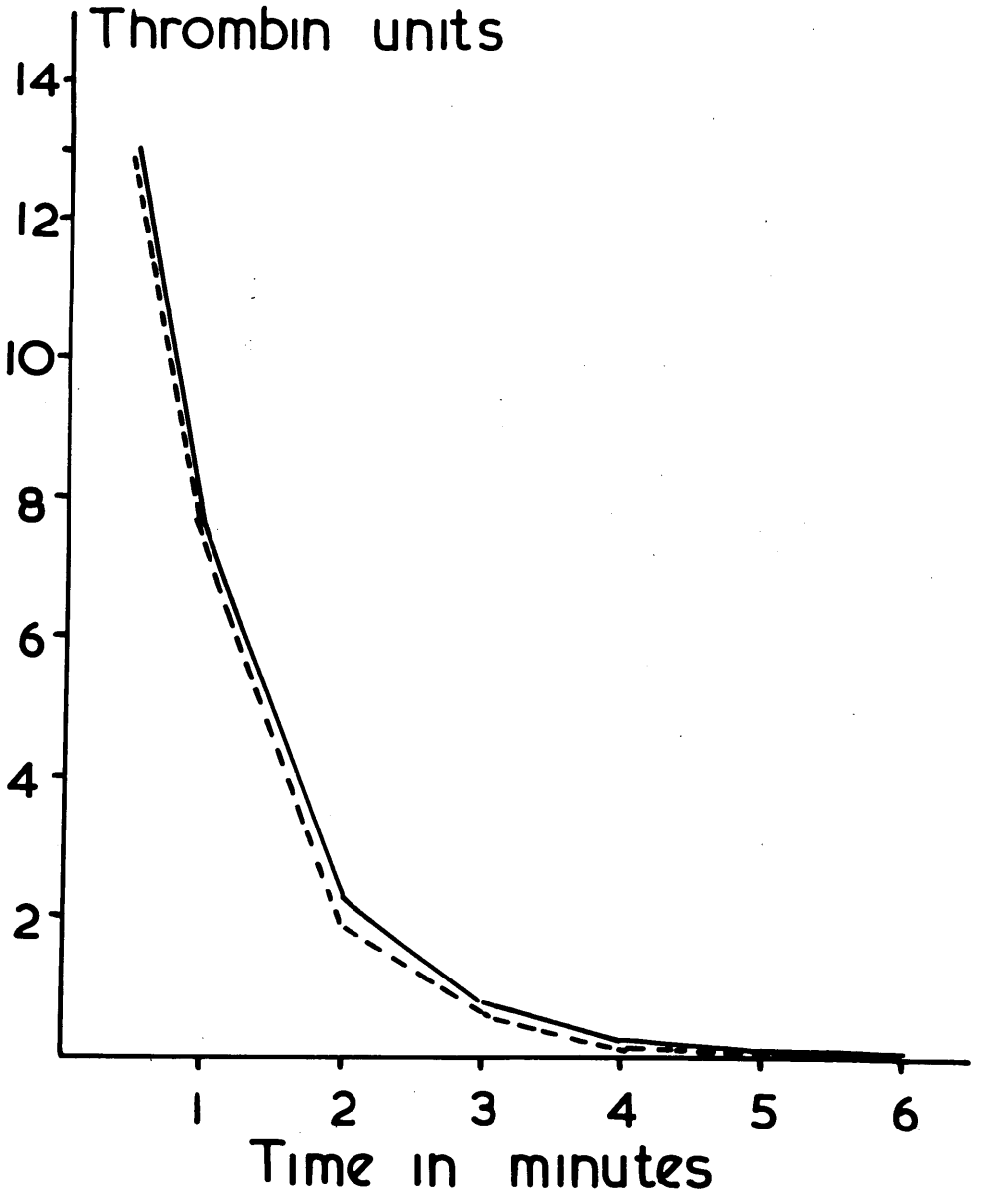

Thrombin units
14
12
10
8
6
4
2
1
2
3
4
5
6
Time in minutes

TABLE 8

The effect of dilutions of normal and prothrombin-deficient plasma in correcting the one-stage clotting time of tromexan plasma.

Dilutions	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
Normal plasma	24	27	30	32	36	41	40	39
Prothrombin deficient plasma	22	26	28	32	33	37	37	38

Clotting time of tromexan plasma = 43"

" " " normal " -15"

This correction cannot reasonably be attributed to the addition of prothrombin.

The prothrombin content of tromexan plasma was assayed by the two methods mentioned above. 324 specimens chosen at random from the daily routine specimens were examined, 140 were tested by the area method and 184 by the globulin fraction method. To apply the area two-stage method of prothrombin assay to tromexan plasma the antithrombin content must be similar to the normal. This has been found to be so. The results of antithrombin assay on normal and tromexan plasma are given in the appendix and the results are shown in figure 32 .

In Figures 33 & 34 the results of the prothrombin assay are shown by histograms. It will be seen that using both methods the prothrombin content of the specimens lay generally from 35-100 per cent while by Quick's one-stage method the figures ranged from less than 5 per cent to 30 per cent. The mean prothrombin content by the globulin fraction method was 64 per cent and by the area method 69 per cent. From a comparison of the two histograms it will be seen that there is reasonable similarity in the prothrombin content of the two series. The mean of all of the observations on prothrombin content was 65 per cent and the corresponding mean by Quick's one-stage percentage was 11 per cent. Figure 35 shows by histogram the combined results obtained using both methods (area method and the globulin fraction technique).

A small number of observations have been made on the prothrombin content during dicumarol and dindevan therapy. (See pages 683, 1246-7 of the appendix). The results are similar to those obtained in tromexan therapy.

METHODS OF PROTHROMBIN ASSAY.

Area method (Biggs and Douglas 1953). In figures 36 & 37 are illustrated further examples of this technique on normal and tromexan plasma. In figure 37 the areas which are used to make the assay are shown by shading.

Figure (33)

Figure (33)

Prothrombin assay in tromexan plasma by the area method. (140 observations).

Ordinate - percentage of observations.

Abscissa - "prothrombin" content of tromexan plasma.

This histogram shows in the shaded representation the percentages as read from Quick's saline dilution curve and in the solid the percentages of prothrombin as measured by the two-stage area method.

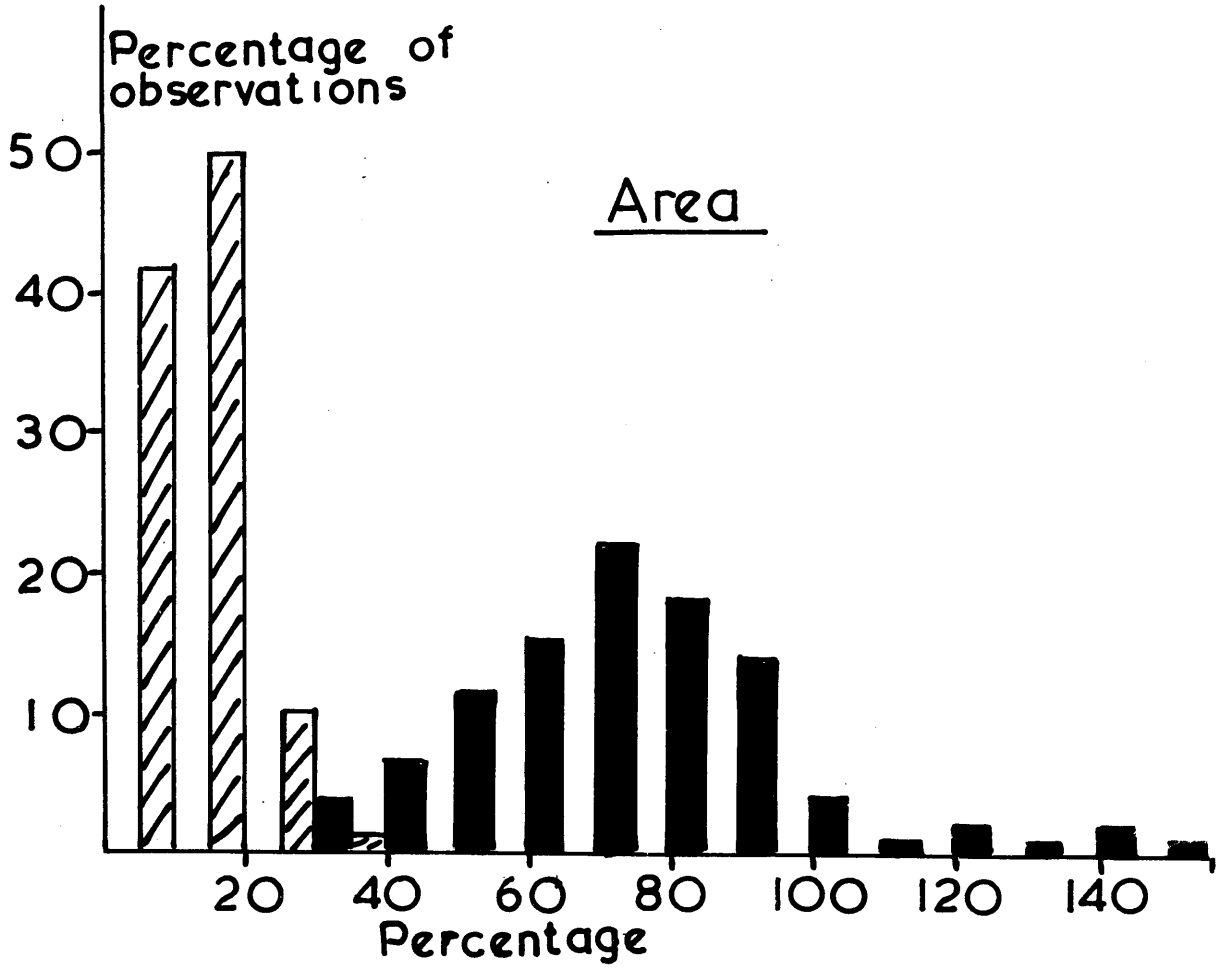

Percentage of observations
50
40
30
20
10
Area
20
40
60
80
100
120
140
Percentage

Figure (34)

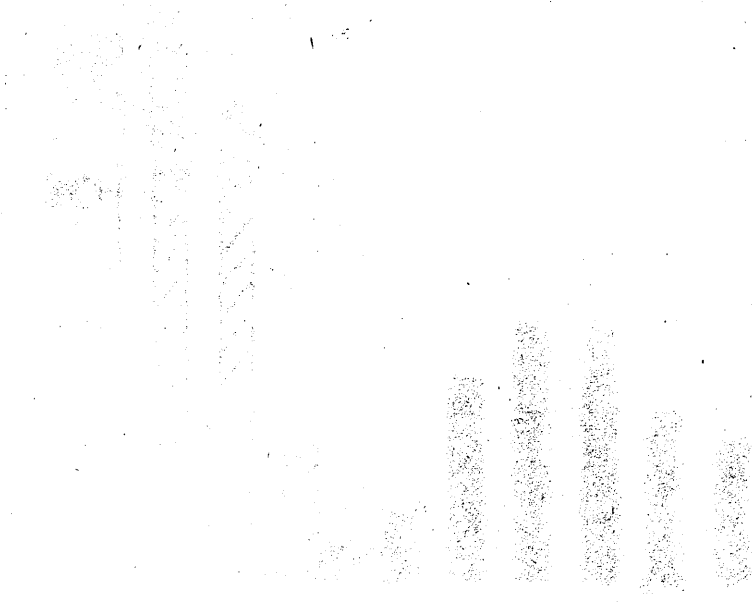


Figure (14)

Prothrombin assay in tromexan plasma by the globulin fraction method (184 observations).

Ordinate - percentage of observations.

Abscissa - "prothrombin" content of tromexan plasma.

This histogram shows in the shaded representation the percentages as read from Quick's saline dilution curve and in the solid the percentages of prothrombin as measured by the globulin fraction technique.

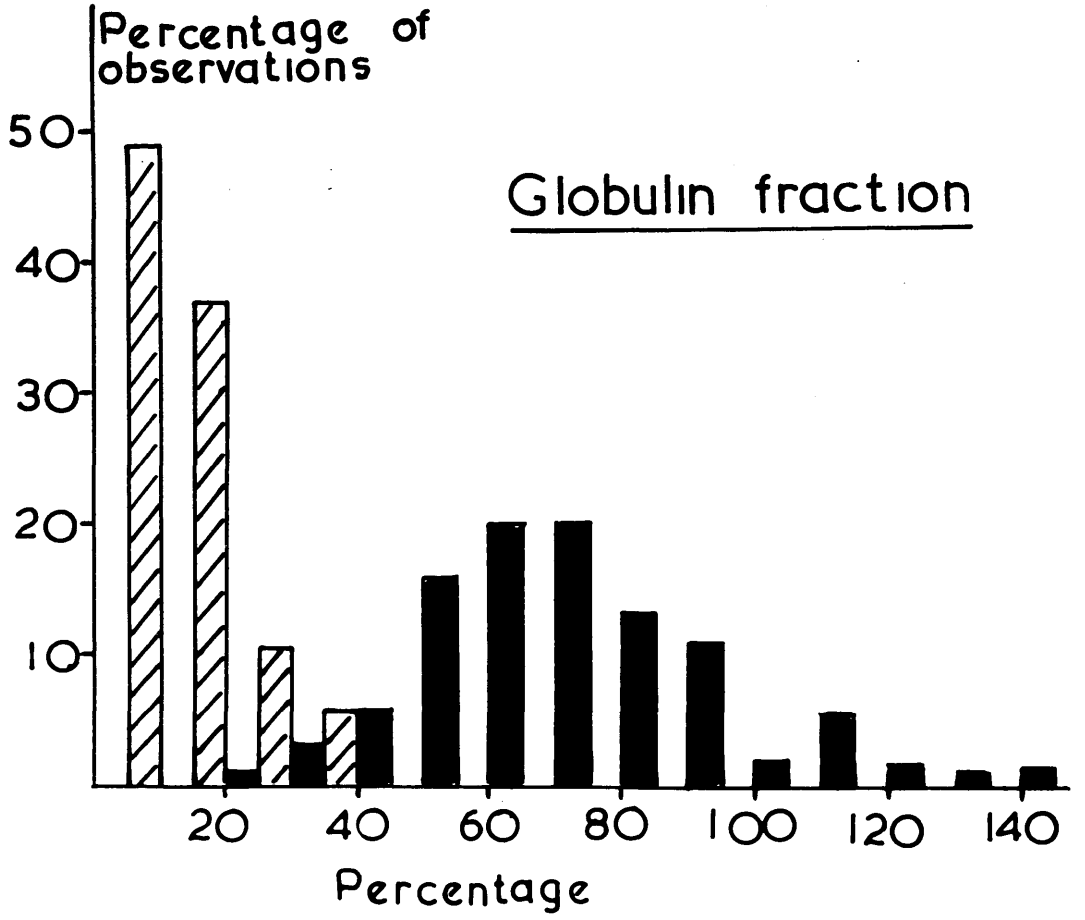

Percentage of observations
50
40
30
20
10
Globulin fraction
20
40
60
80
100
120
140
Percentage

Figure (35)

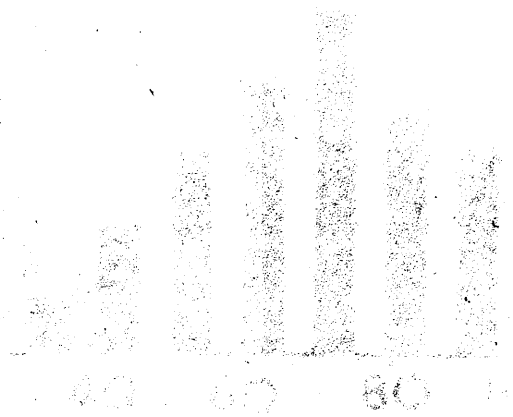


Figure (35)

Prothrombin assay in tromexan plasma by the area method and the globulin fraction technique.

Ordinate - number of observations.

Abscissa - "prothrombin" content of tromexan plasma.

This histogram combines the results shown in figures 33 and 34 - 140 observations by the area method and 184 by the globulin fraction technique. The shaded representation shows the prothrombin content as read from Quick's saline dilution curve and the solid the content as assayed by the two-stage techniques.

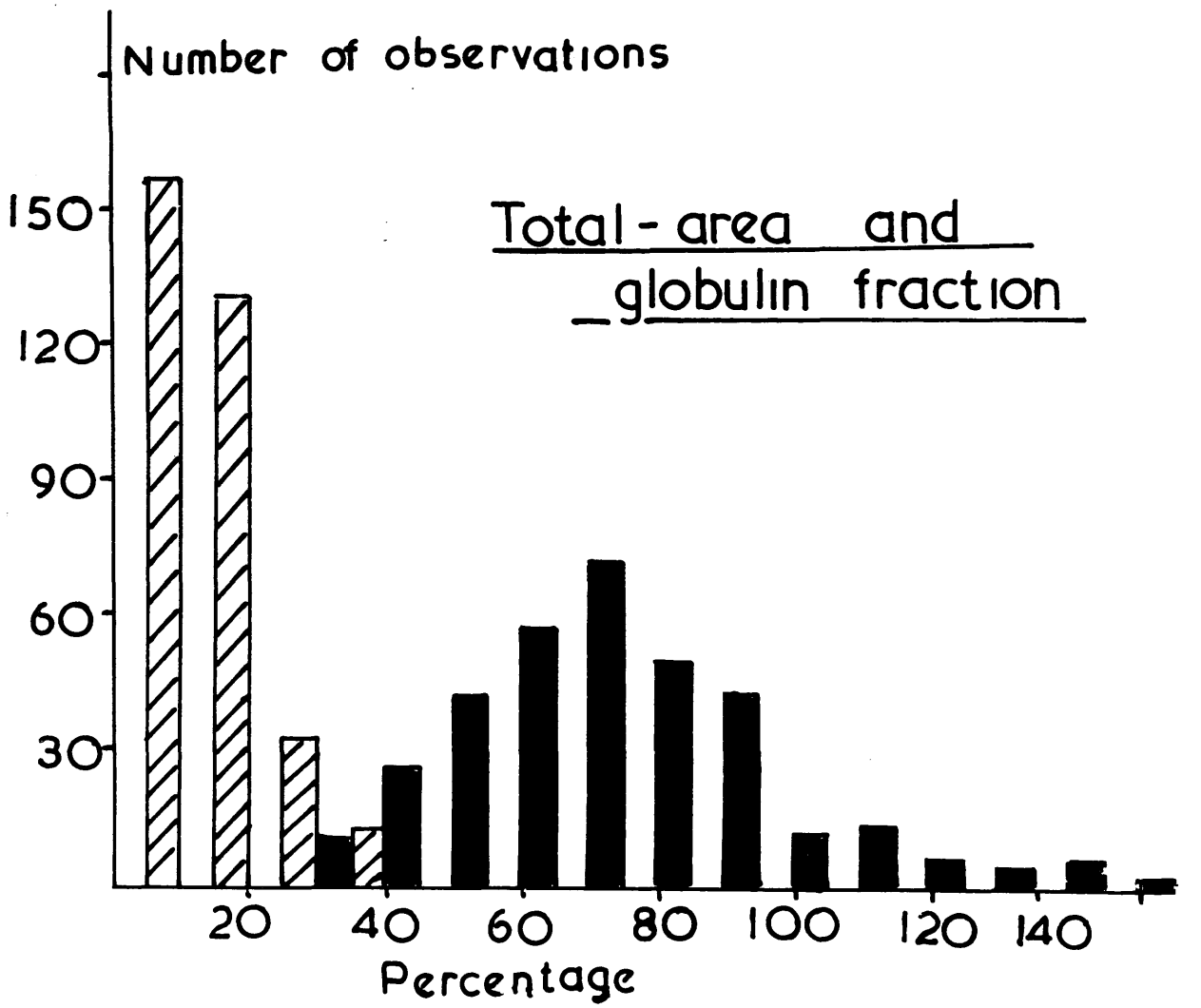

Number of observations
150
120
90
60
30
Total - area and
globulin fraction
20
40
60
80
100
120
140
Percentage

Figure (36)

Figure (36)

Two-stage prothrombin test in a normal plasma and a tromexan plasma. (Area method).

Ordinate - Thrombin units.

Abscissa - Incubation time in minutes after addition of calcium.

O——O Normal plasma.

●——● Tromexan plasma.

This figure shows a further example of the two-stage test in tromexan plasma.

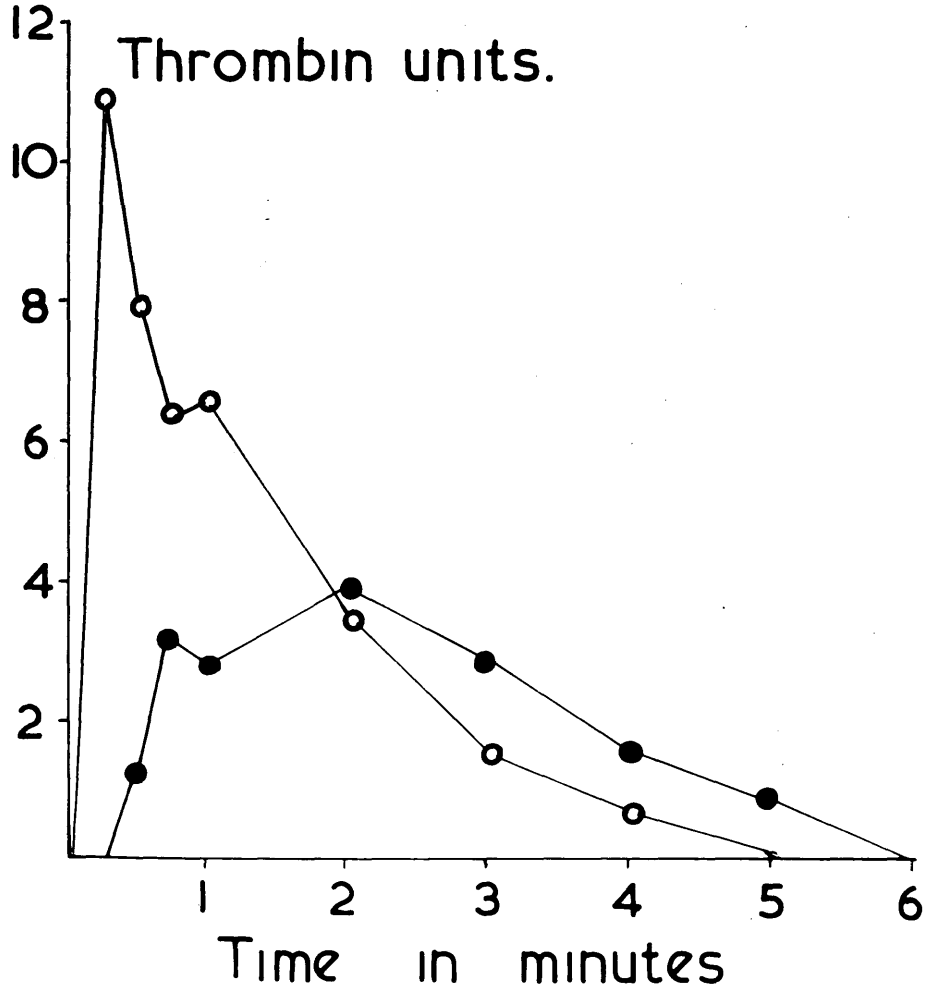

Thrombin units.
12
10
8
6
4
2
1
2
3
4
5
6
Time in minutes

Figure (37)

Figure (37)

Two-stage prothrombin test in a normal plasma and a tromexan plasma. (Area method).

Ordinate - Thrombin units.

Abscissa - Incubation time in minutes after addition of calcium.

The shading in this figure indicates the areas which require to be computed in order to make the assay.

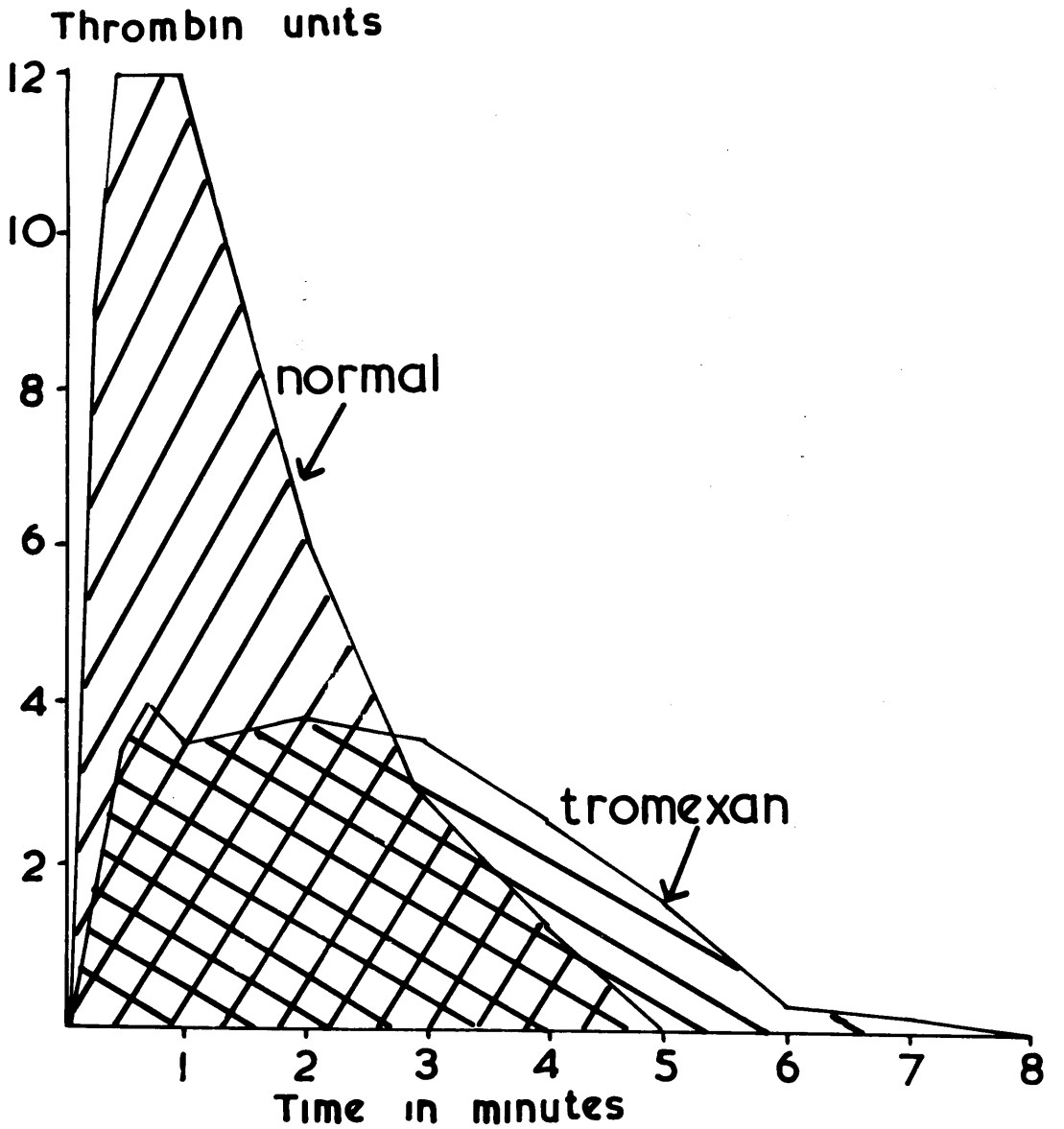

Thrombin units
12
10
8
6
4
2
normal
tromexan
1
2
3
4
5
6
7
8
Time in minutes

Figure (38)

Figure (38)

Prothrombin assay by the globulin fraction technique.

Ordinate - Thrombin units.

Abscissa - Time in minutes after addition of calcium.

●——● - Thrombin development in normal.

O——O - Thrombin development in tromexan.

This figure shows that the globulin fraction from the normal develops its thrombin rapidly. The fraction from the tromexan plasma develops thrombin more slowly but ultimately attains the same thrombin level. This tromexan plasma therefore contained a normal amount of prothrombin.

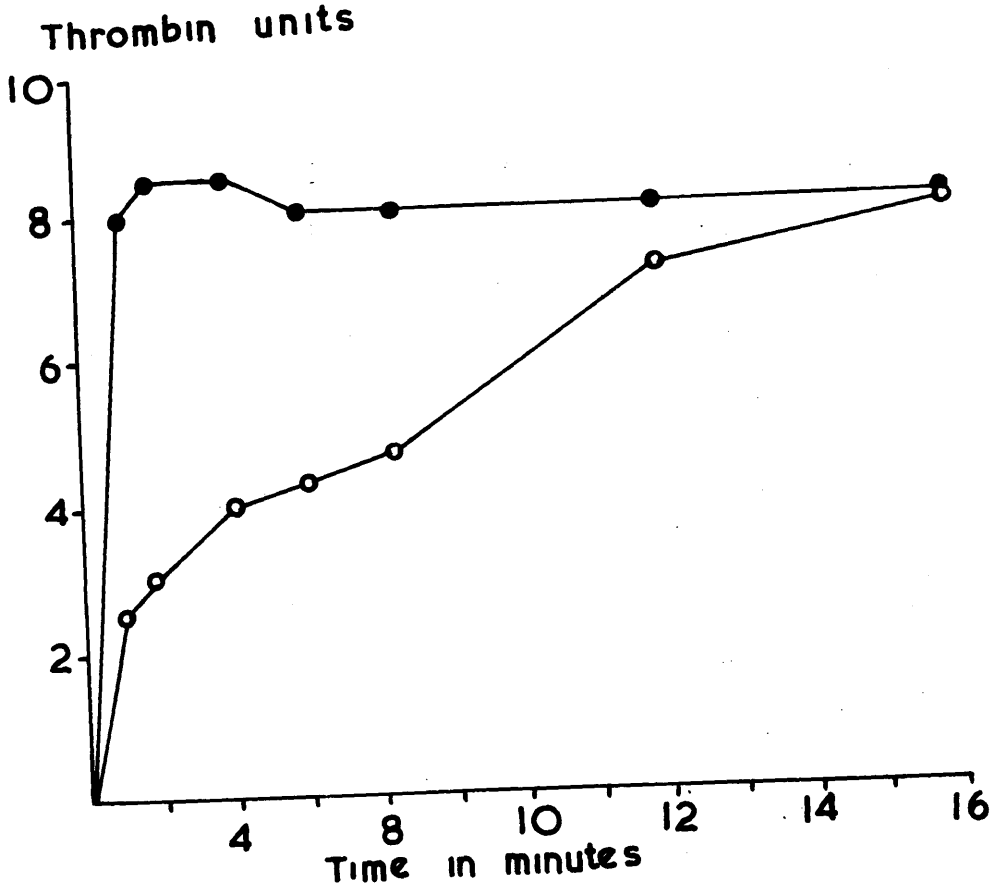

Thrombin units
10
8
6
4
2
4
8
10
12
14
16
Time in minutes

Figure (39)

Figure (89)

Prothrombin assay by the globulin fraction technique.

Ordinate - Thrombin units.

Abscissa - Time in minutes after addition of calcium.

●——● Thrombin development in normal.

O——O Thrombin development in tromexan.

This figure shows that the globulin fraction from the normal develops its thrombin rapidly whereas that from the tromexan develops more slowly. The tromexan specimen does not attain the same level of thrombin as the normal and was therefore deficient in prothrombin having only 75% of the normal.

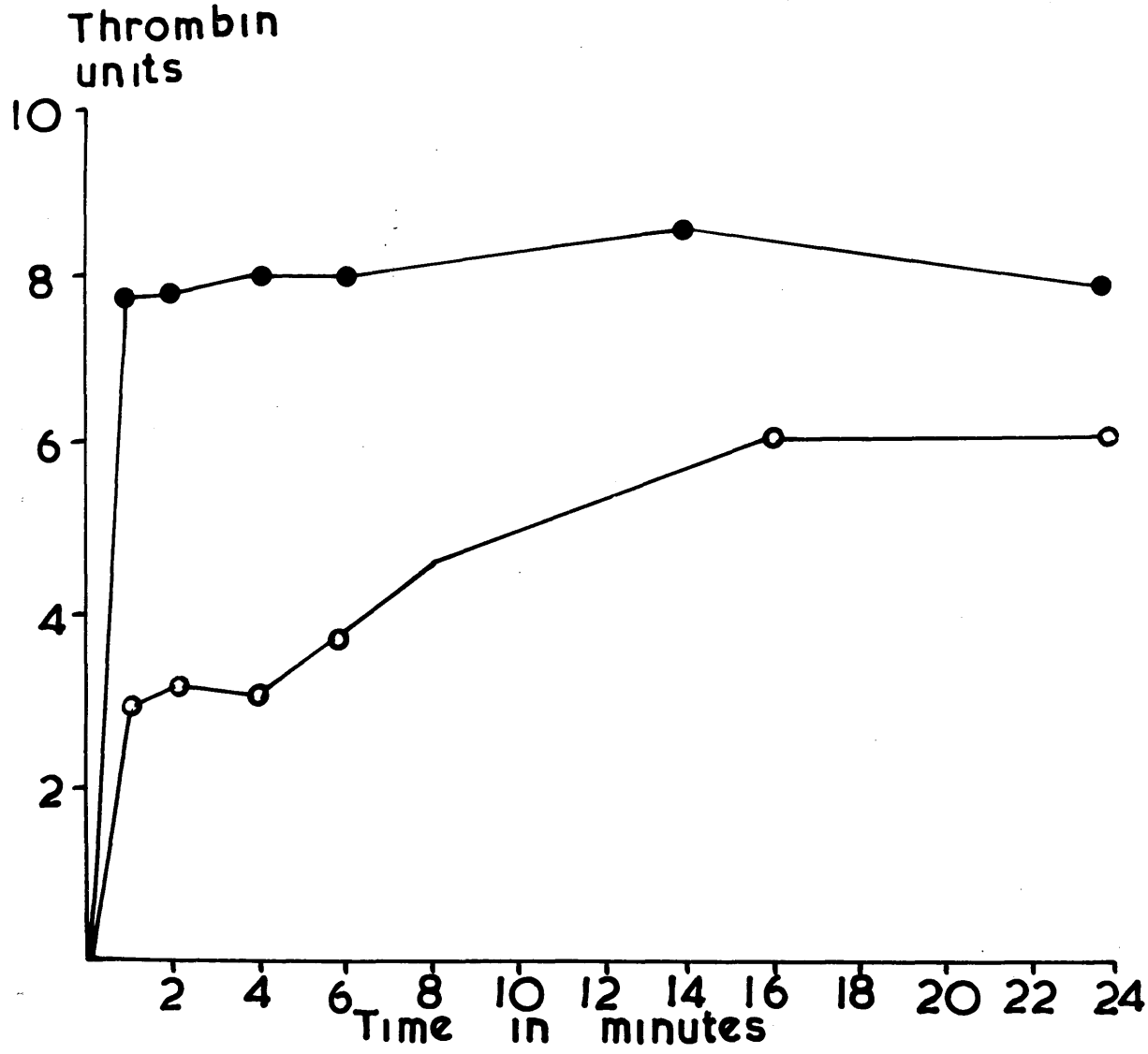

Thrombin
units
10
8
6
4
2
2
4
6
8
10
12
14
16
18
20
22
24
Time in minutes

Figure (40)

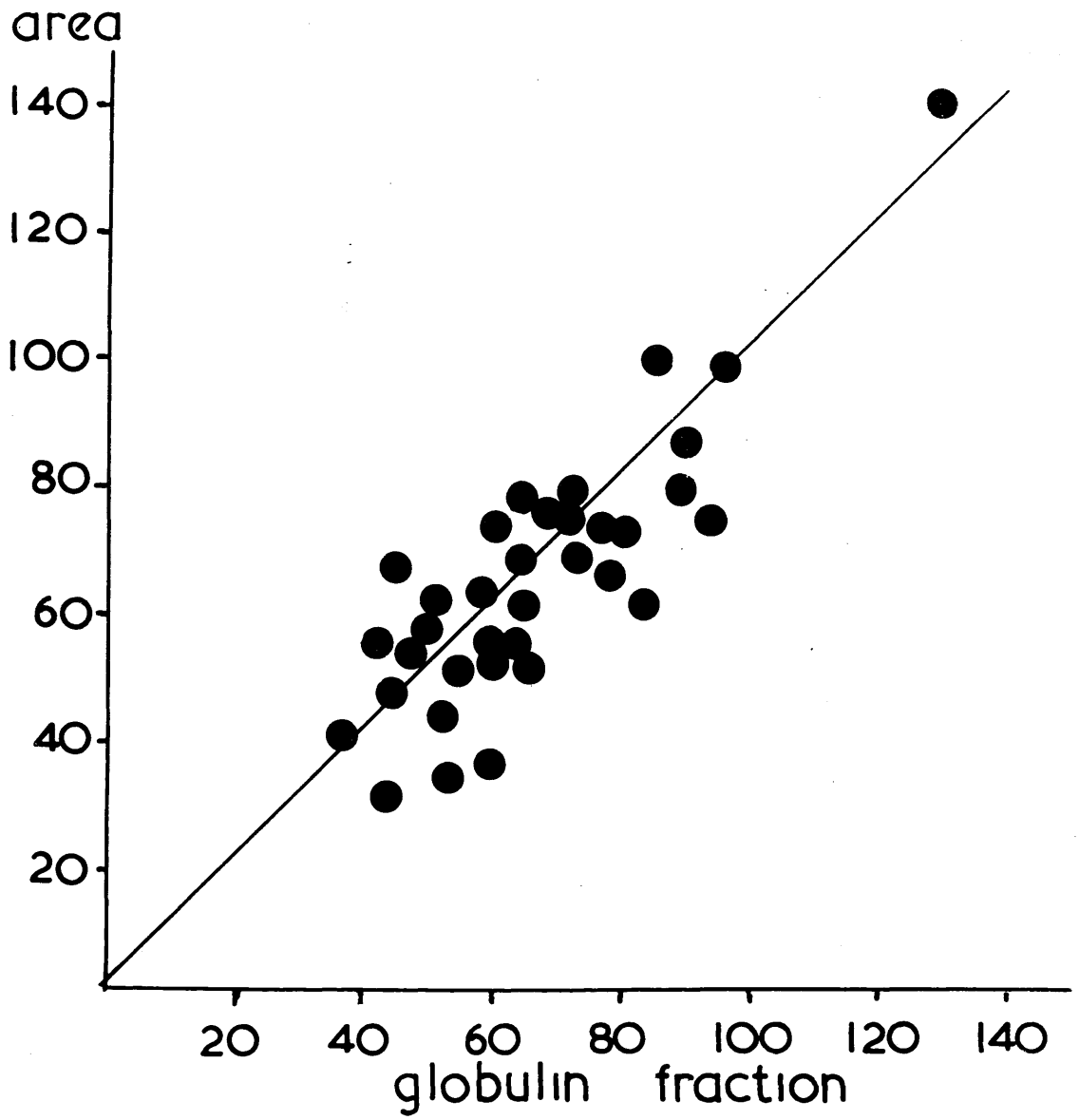
Figure (40)

Correlation of results of prothrombin assay by area method and the globulin fraction technique.

Ordinate - prothrombin percentage as assayed by area method.

Abscissa - prothrombin percentage as assayed by globulin fraction technique.

Thirty five specimens of tromexan plasma were examined by both assay procedures and the correlation of results is indicated in this figure.


area
140
120
100
80
60
40
20
20
40
60
80
100
120
140
globulin fraction

Globulin fraction method (Douglas and Biggs 1953). Figures 38 & 39 illustrate the progress of thrombin production from globulin fractions prepared from normal and tromexan plasmas. The amount of thrombin eventually produced from one of these is normal (figure 38) whereas in the other (figure 39) it is low. The impaired rate of thrombin formation can also be appreciated.

To test the validity of these two methods in their property of assaying prothrombin, 35 specimens were examined using both techniques. The correlation of results is shown in Fig. 40 It will be observed that although some of the points are at some distance from the 45° line there is reasonable correlation of the results.

Factor VII.

Reduction of one-stage clotting time by the addition of serum.

It has been shown that after normal coagulation when the blood is incubated in glass tubes for one hour at 37° C., prothrombin and factor V are reduced to very low levels in the resulting serum (Alexander, Goldstein and Landwehr 1951, Douglas and Biggs 1953, Merskey 1950). It will be seen (Table 9) that the one-stage clotting time of tromexan plasma was reduced almost to normal by the addition of normal serum. This observation confirms previous reports.

TABLE 9

The action of normal serum and normal plasma in correcting the one-stage clotting time of tromexan plasma.

Number of observations	Clotting time (seconds) of Tromexan Plasma	Clotting time (seconds) of Normal Plasma	Clotting time (seconds) after addition to Tromexan Plasma of 1/10 proportion of	
			Normal Plasma	Normal Serum
51	36-39	15	-	16
24	39-54	15	20-22	-

The details of these results are given in the appendix pages 736-741. Tromexan serum was able to produce some shortening but was not so effective as normal serum in this property. The tromexan plasma used in these observations was collected from patients on the third or fourth day of therapy. These results support the view that deficiency of prothrombin is a minor feature of the clotting defect caused by tromexan. Prothrombin deficient plasma can correct the defect as efficiently as normal plasma; normal serum is more effective than normal plasma. The lack of factor VII appears to be the main defect and it is the deficiency which probably controls the results of the one-stage "prothrombin" time in these

patients. If it is remembered that a level of 10 per cent of prothrombin caused only slight lengthening of the one-stage "prothrombin" time, then the level of 65 per cent found as an average in patients treated with tromexan is unlikely to alter the one-stage "prothrombin" time significantly.

It seems unlikely that the extent of the deficiency of prothrombin is contributory to the prolonged one-stage clotting time. This is of course an assumption which may be unjustified. In the prothrombin deficient patient there was a normal concentration of factor VII. When there is a deficiency of factor VII as in tromexan therapy the moderate deficiency of prothrombin may, under these rather different conditions, be contributing to the lengthening of the one-stage clotting time.

Reduction of one-stage clotting time by the addition of preparations of prothrombin and factor VII.

Preparations of the alumina-adsorbed fraction of plasma and serum are able to shorten the one-stage clotting time tromexan plasma (see page 643 of the appendix).

The comparison in the effect of normal serum and plasma to correct the one-stage clotting time of tromexan plasma.

When nine parts of tromexan plasma are mixed with one part of normal serum or one part of the corresponding plasma then the serum produces a greater correction than the plasma.

Figure (41)

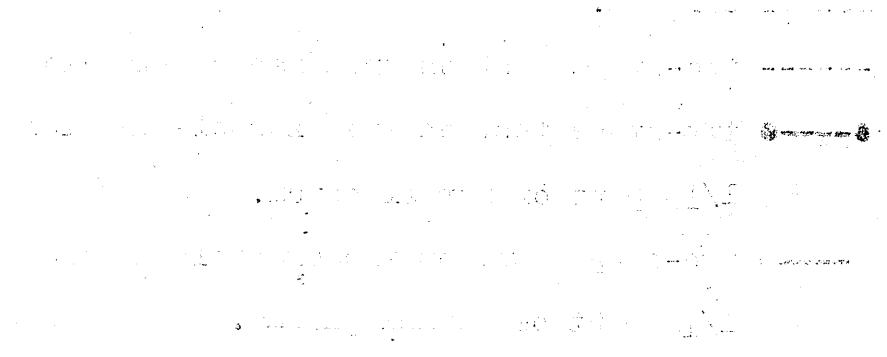


Figure (41)

The effect of normal serum and plasma on the two-stage test on tromexan plasma.

Ordinate - Thrombin units.

Abscissa - Incubation time in minutes after addition of calcium.

------ two-stage test on tromexan plasma alone.

●——● two-stage test on tromexan plasma plus 1/10 part of normal serum.

O——O two-stage test on tromexan plasma plus 1/10 part of normal plasma.

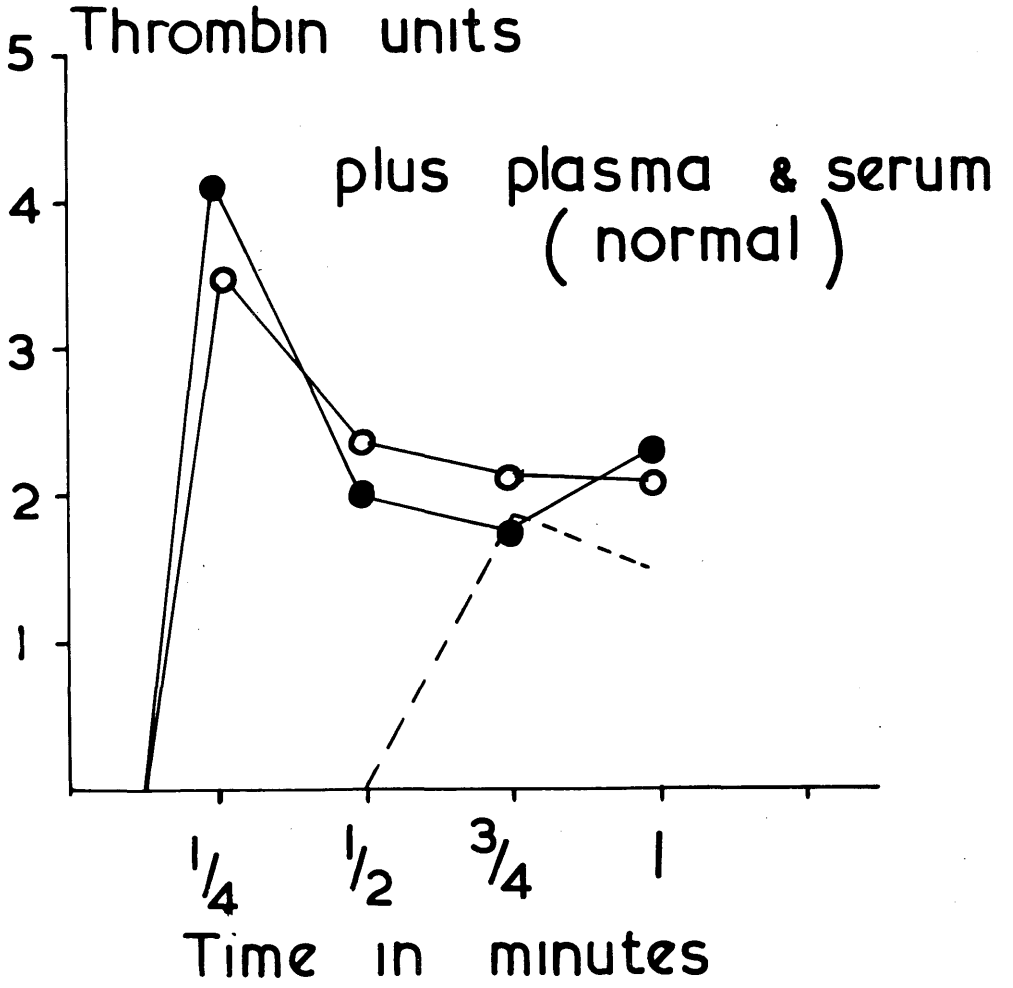

Thrombin units
5
4
3
2
1
plus plasma & serum
(normal)
1/4
1/2
3/4
1
Time in minutes

Figure (42)

Figure (42)

The effect of normal plasma on the two-stage test on tromexan plasma.

Ordinate - Thrombin units.

Abscissa - Incubation time in minutes after addition of calcium.

●——● Tromexan plasma alone.

O——O Tromexan plasma plus $1/_{10}$ part of normal plasma.

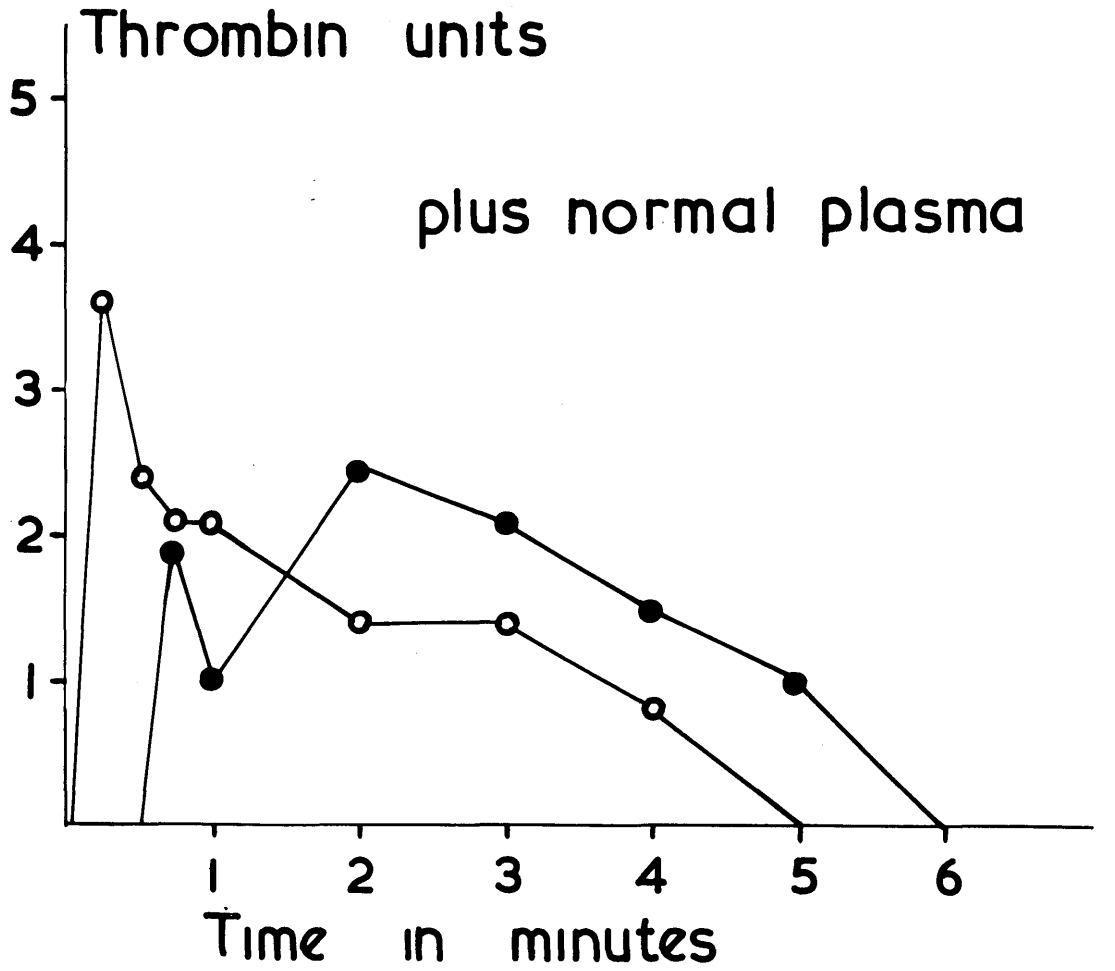

Thrombin units
plus normal plasma
5
4
3
2
1
1
2
3
4
5
6
Time in minutes

Figure (43)

Figure (43)

The effect of normal serum on the two-stage test on tromexan plasma.

Ordinate - Thrombin units.

Abscissa - Incubation time in minutes after addition of calcium.

●——● Tromexan plasma alone.

O——O Tromexan plasma plus $1/_{10}$ part of normal serum.

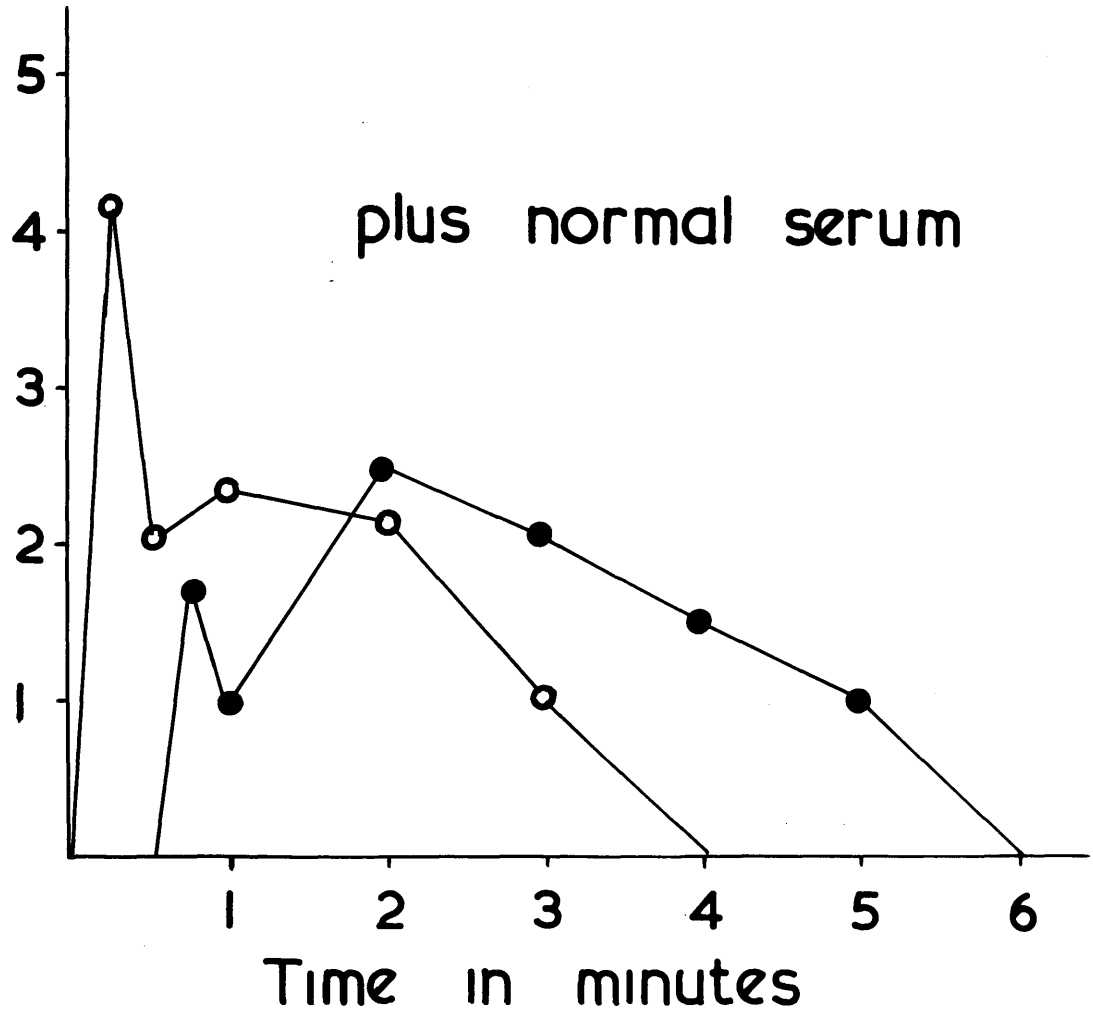

5
4
3
2
1
plus normal serum
1
2
3
4
5
6
Time in minutes

Figure (44)

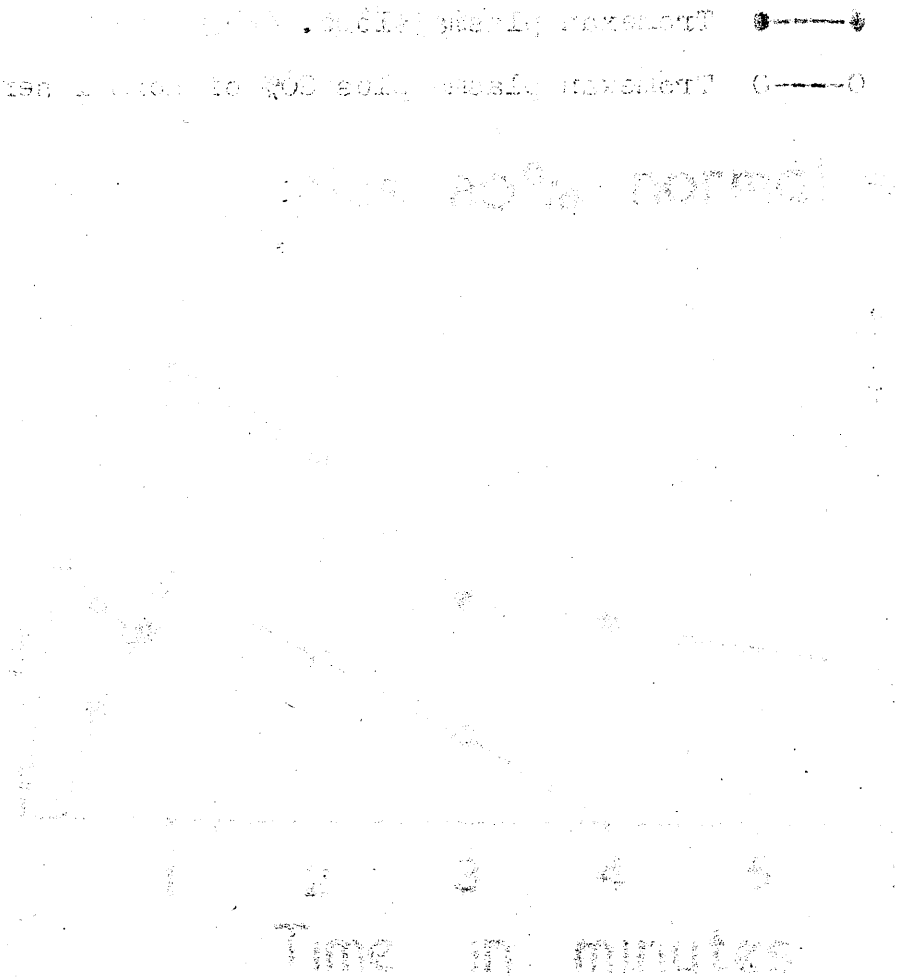


Figure (44)

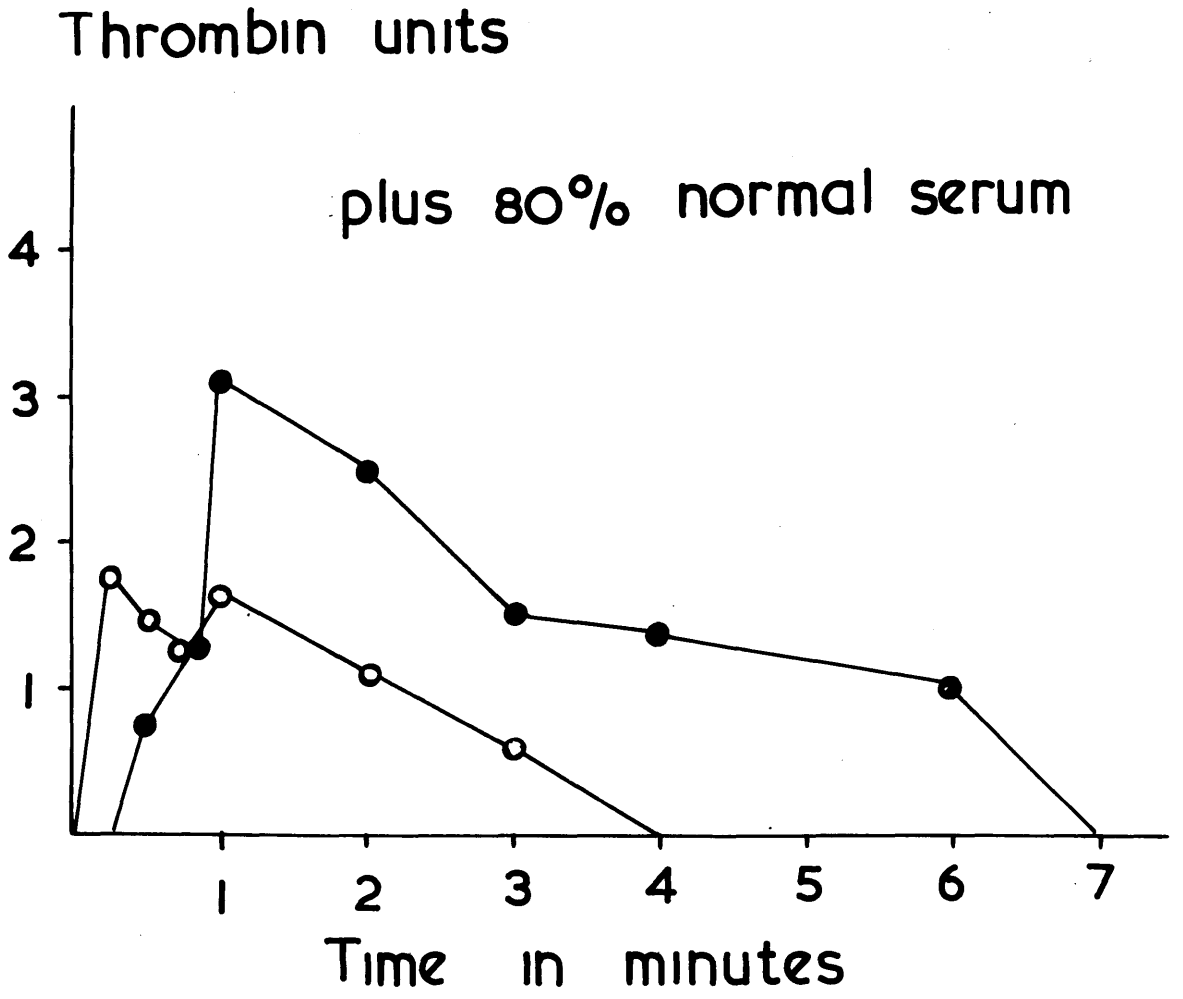
The effect of the addition of an excess of serum on the shape of the two-stage test on tromexan plasma.

Ordinate - Thrombin units.

Abscissa - Incubation time in minutes after addition of calcium.

●——● Tromexan plasma alone.

O——O Tromexan plasma plus 80% of normal serum.


Thrombin units
plus 80% normal serum
4
3
2
1
1
2
3
4
5
6
7
Time in minutes

(see Table 9). This is in spite of the fact that the plasma contains prothrombin whereas there is none in the serum. This greater power of the serum was not due to the citrate in the plasma. (appendix page 740). Some change must arise during clotting so that the serum is more active than the corresponding plasma. Even tromexan serum is able to produce some shortening on its own plasma. The addition of normal serum to normal plasma results in some reduction in the normal one-stage clotting time (appendix page 741).

The effect of normal serum and plasma on the two-stage test on tromexan plasma

These additions to tromexan plasma reduce the delay in thrombin generation demonstrable by the two-stage technique. This explains the correction in the one-stage test. Figures 41, 42, and 43 illustrate this and the data are given in the appendix. Even the addition of considerable amounts of serum to tromexan plasma fails to completely correct the shape of the curve on the two-stage technique, though the one-stage test may be restored to normal. (see figure 44).

The effect of the addition of prothrombin deficient plasma on the shape of the two-stage curve of thrombin production from coumarin plasma

In figures 45 and 46 are shown the effect of the additions to the tromexan plasma of plasma from the patient with

Figure (45)

Figure (25)

The effect of prothrombin deficient plasma on the two-stage test on tromexan plasma.

Ordinate - Thrombin units.

Abscissa - Incubation time in minutes after addition of calcium.

●——● Tromexan plasma alone.

O——O Tromexan plasma plus 1/10 part of plasma from the prothrombin deficient patient.

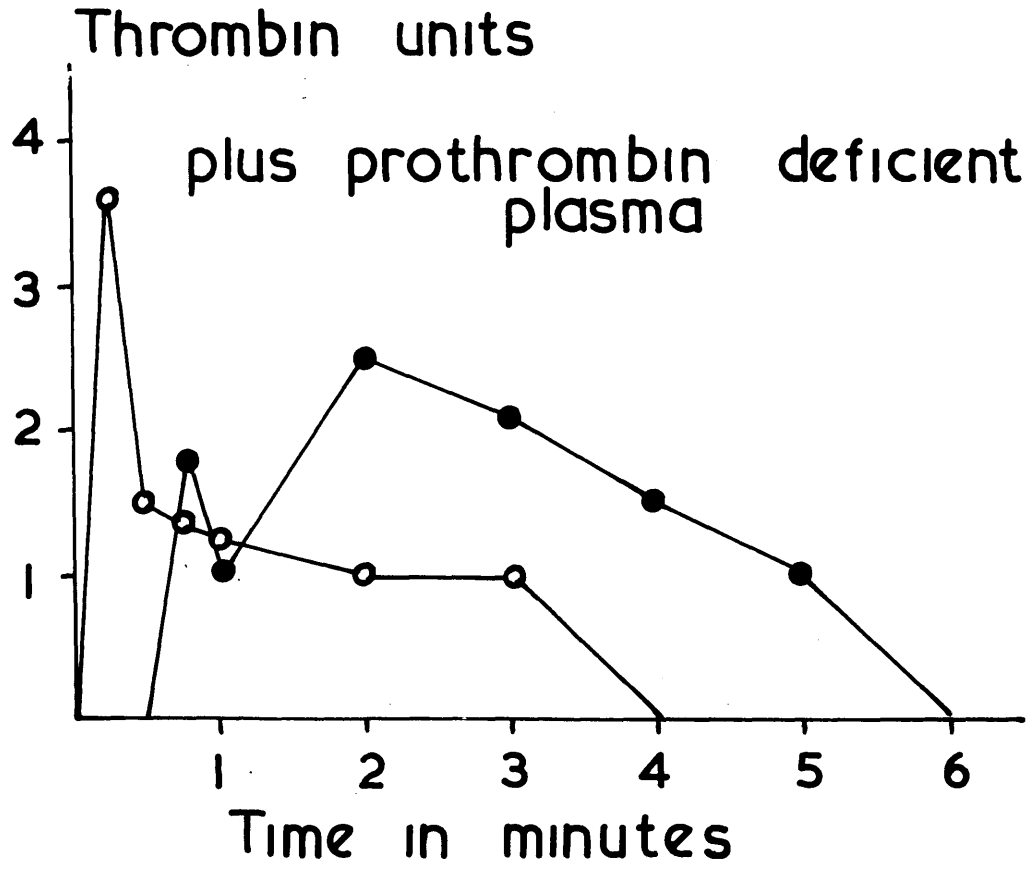

Thrombin units
plus prothrombin deficient plasma
4
3
2
1
1
2
3
4
5
6
Time in minutes

Figure (46)

Figure (46)

The two-stage test on plasma from a patient on tromexan, on plasma from the prothrombin deficient patient and on a mixture of the two.

Ordinate - Thrombin units.

Abscissa - Incubation time in minutes after addition of calcium.

●——● Tromexan plasma.

------ Prothrombin deficient plasma.

O——O 50% mixture of these two plasmas.

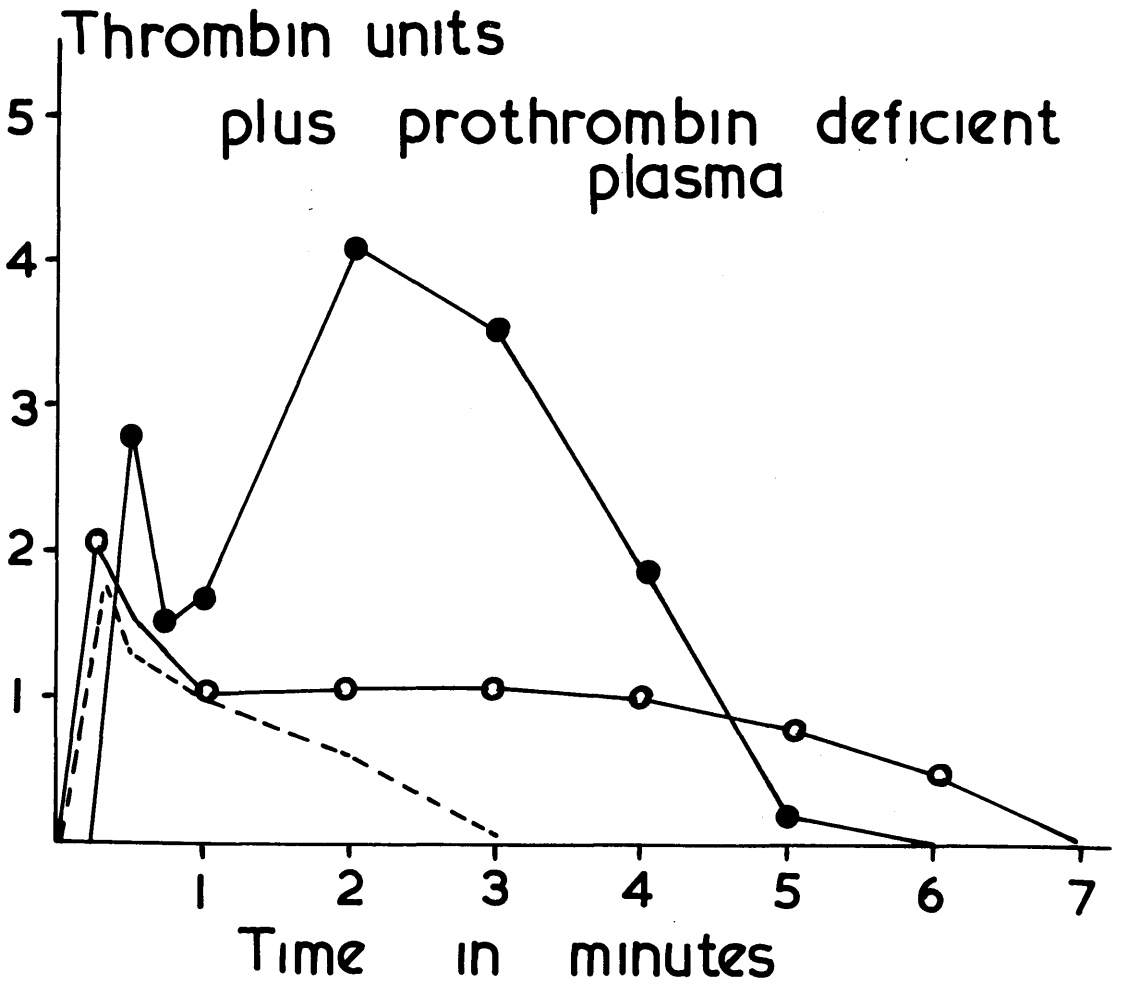

Thrombin units
plus prothrombin deficient plasma
5
4
3
2
1
1
2
3
4
5
6
7
Time in minutes

Figure (46)

The two-stage test on plasma from a patient on tromexan, on plasma from the prothrombin deficient patient and on a mixture of the two.

Ordinate - Thrombin units.

Abscissa - Incubation time in minutes after addition of calcium.

●——● Tromexan plasma.

------ Prothrombin deficient plasma.

O——O 50% mixture of these two plasmas.

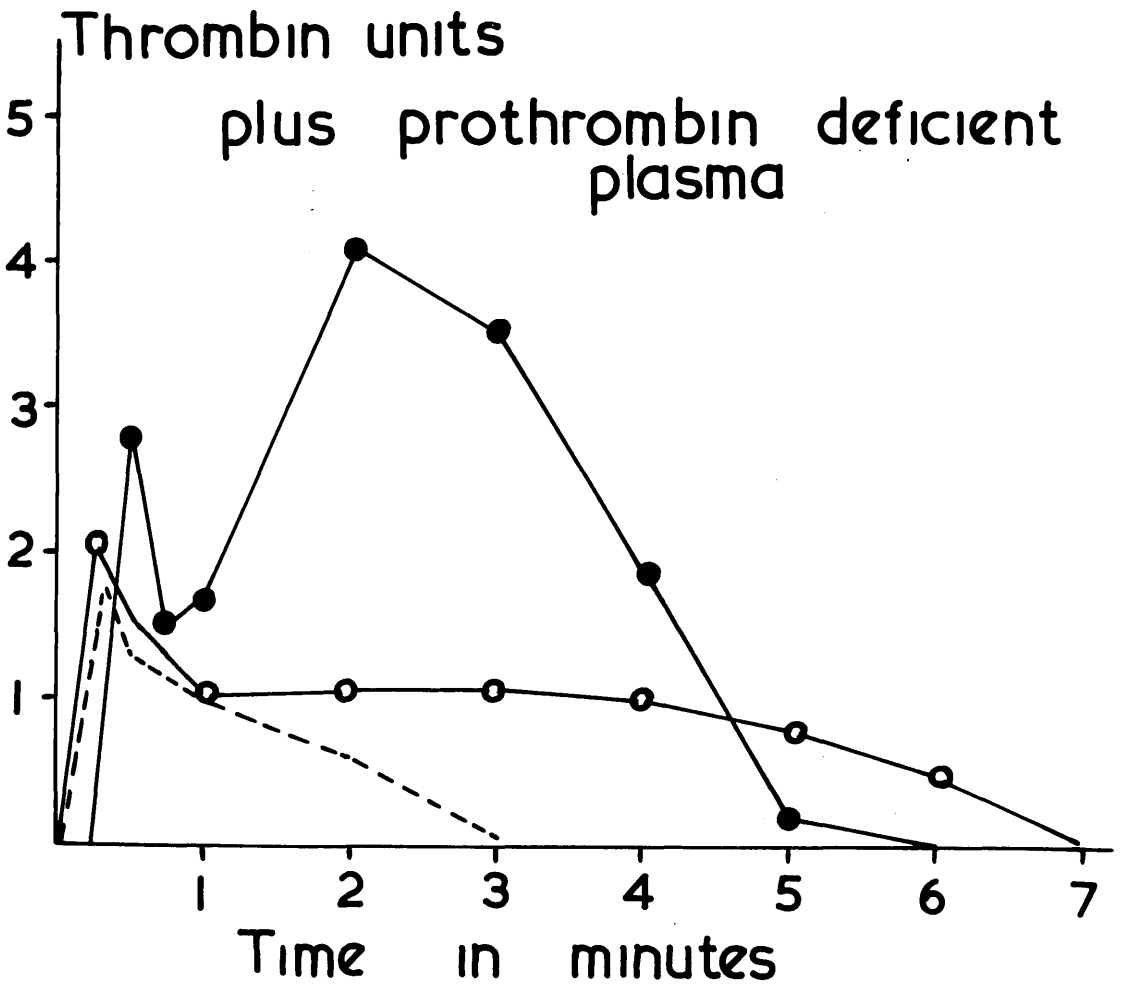

Thrombin units
plus prothrombin deficient plasma
5
4
3
2
1
1
2
3
4
5
6
7
Time in minutes

Figure (47)

Figure (47)

The effect on the two-stage test on tromexan plasma of the addition of its own serum.

Ordinate - Thrombin units.

Abscissa - Incubation time in minutes after addition of calcium.

○——○ Tromexan plasma.

●——● Tromexan plasma + $1/10$ part of its own serum.

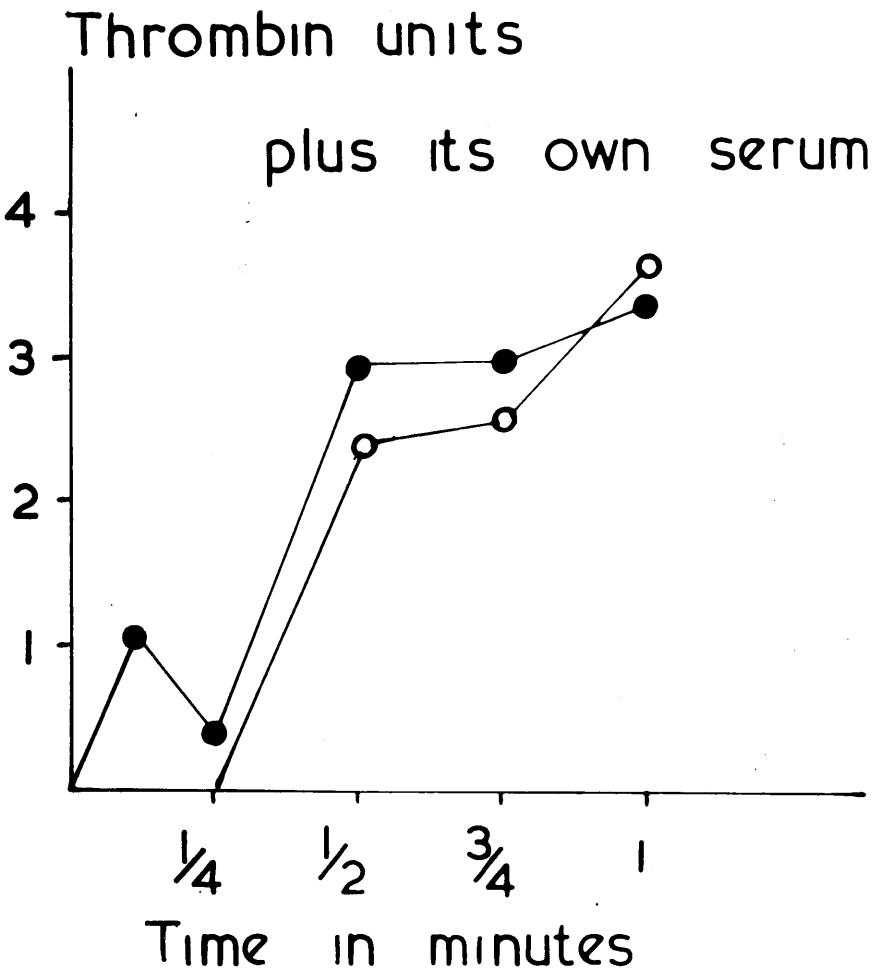
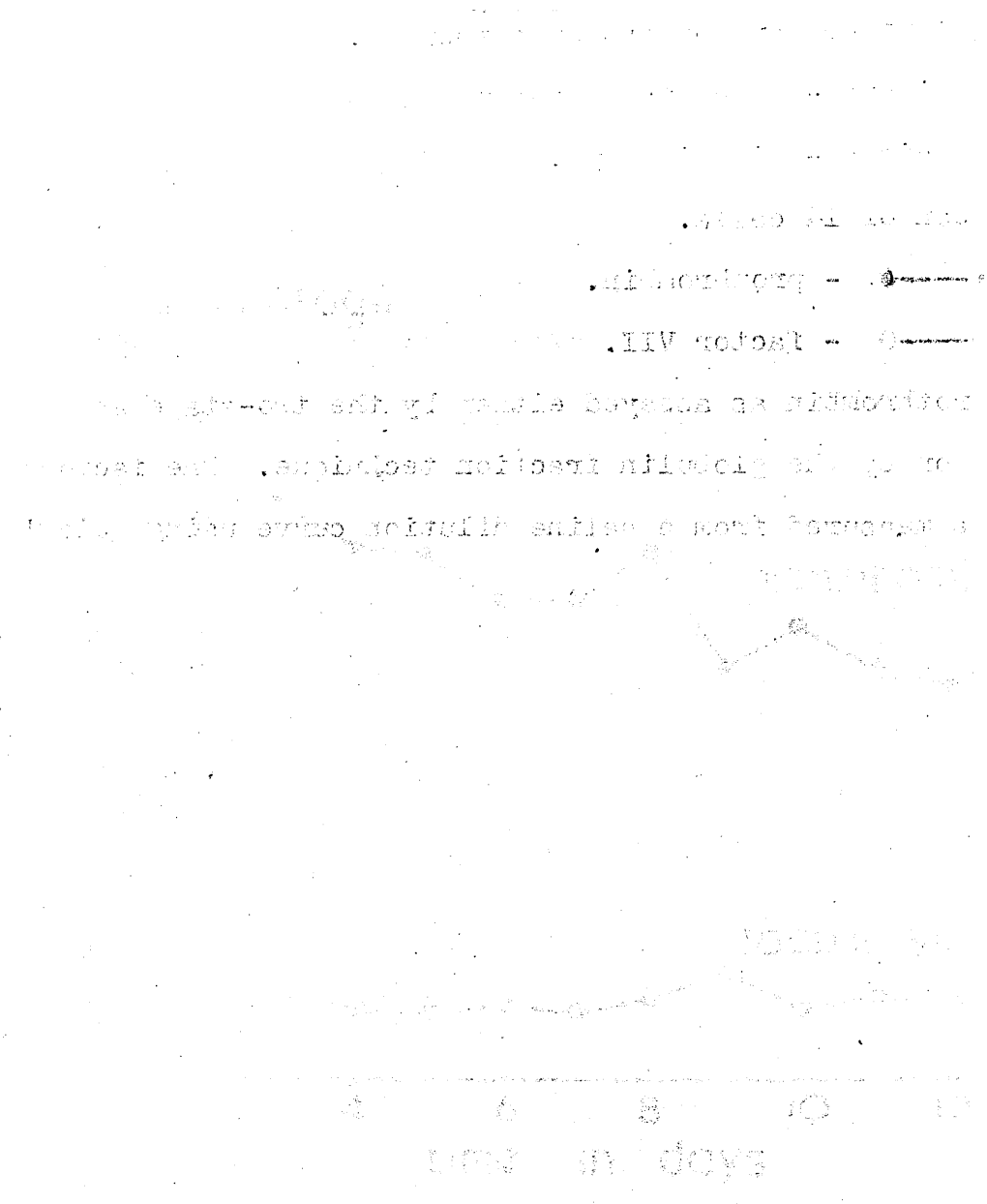

Thrombin units
plus its own serum
4
3
2
1
1/4
1/2
3/4
1
Time in minutes

Figure (48)



prothrombin deficiency. The much more rapid production of thrombin is illustrated.

The effect of the addition of tromexan serum on the shape of the two-stage curve of thrombin production from its own plasma.

The more rapid production of thrombin from tromexan plasma following the addition of its own serum is illustrated in figure 47 .

The levels of prothrombin and factor VII during tromexan therapy.

Since reduction of factor VII is the main factor which controls the one-stage prothrombin time in the blood of patients treated with tromexan the one-stage test can be used to give an approximate measure of factor VII. Using the one-stage test and the two methods of prothrombin assay, the progress of therapy in fourteen cases was followed daily for 14 days.

In figure 48 is shown the mean of 14 cases. The factor VII falls rapidly at the onset of therapy, the fall in prothrombin is more gradual and much less in extent. During the early days of therapy (e.g. 3-4 days) the prothrombin content is little altered from normal, whereas the factor VII is already at a low level. Such plasma is particularly useful as a substrate for the study of factor VII deficiency.

Figure (49)

Figure (49)

Progress of prothrombin and factor VII subsequent to stopping tromexan therapy.

Ordinate - Percentage prothrombin or factor VII.

Abscissa - Time in days after starting therapy.

Mean of 2 cases.

O——O - prothrombin.

●——● - factor VII.

Prothrombin was assayed by the globulin fraction technique and the factor VII from a saline dilution curve using Quick's test.

The solid black area represents the tromexan therapy and the cessation of therapy is shown accordingly.

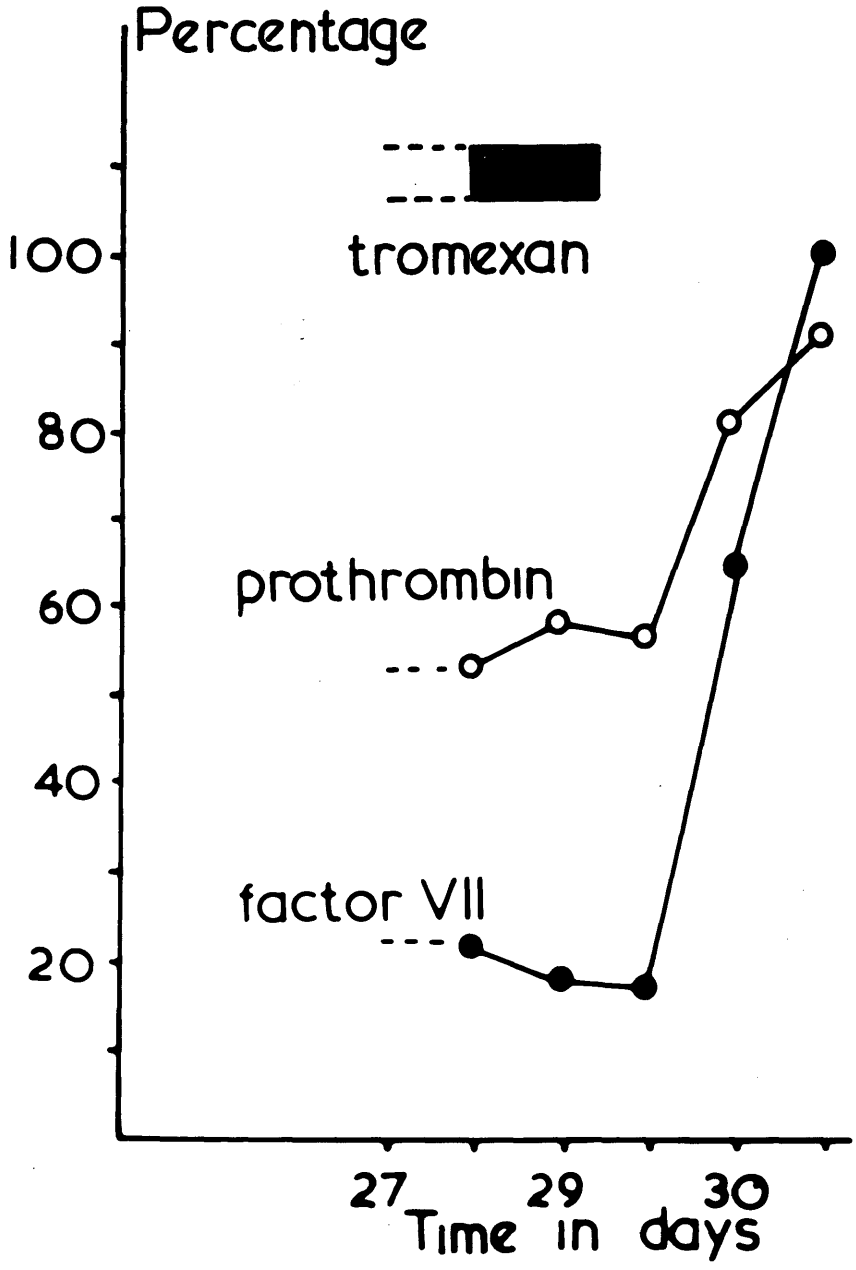

Percentage
tromexan
100
80
60
40
20
prothrombin
factor VII
27
29
30
Time in days

Figure (50)

Figure (50)

Progress of prothrombin and factor VII following oral administration of 1000 mg. of oral vitamin K_1

Ordinate - clotting time of fibrinogen in seconds.

Abscissa - time in hours.

O——O prothrombin.

●——● factor VII.

The administration of the vitamin K_1 is shown.

Prothrombin was assayed by the globulin fraction technique and the factor VII from a saline dilution curve using Quick's test.

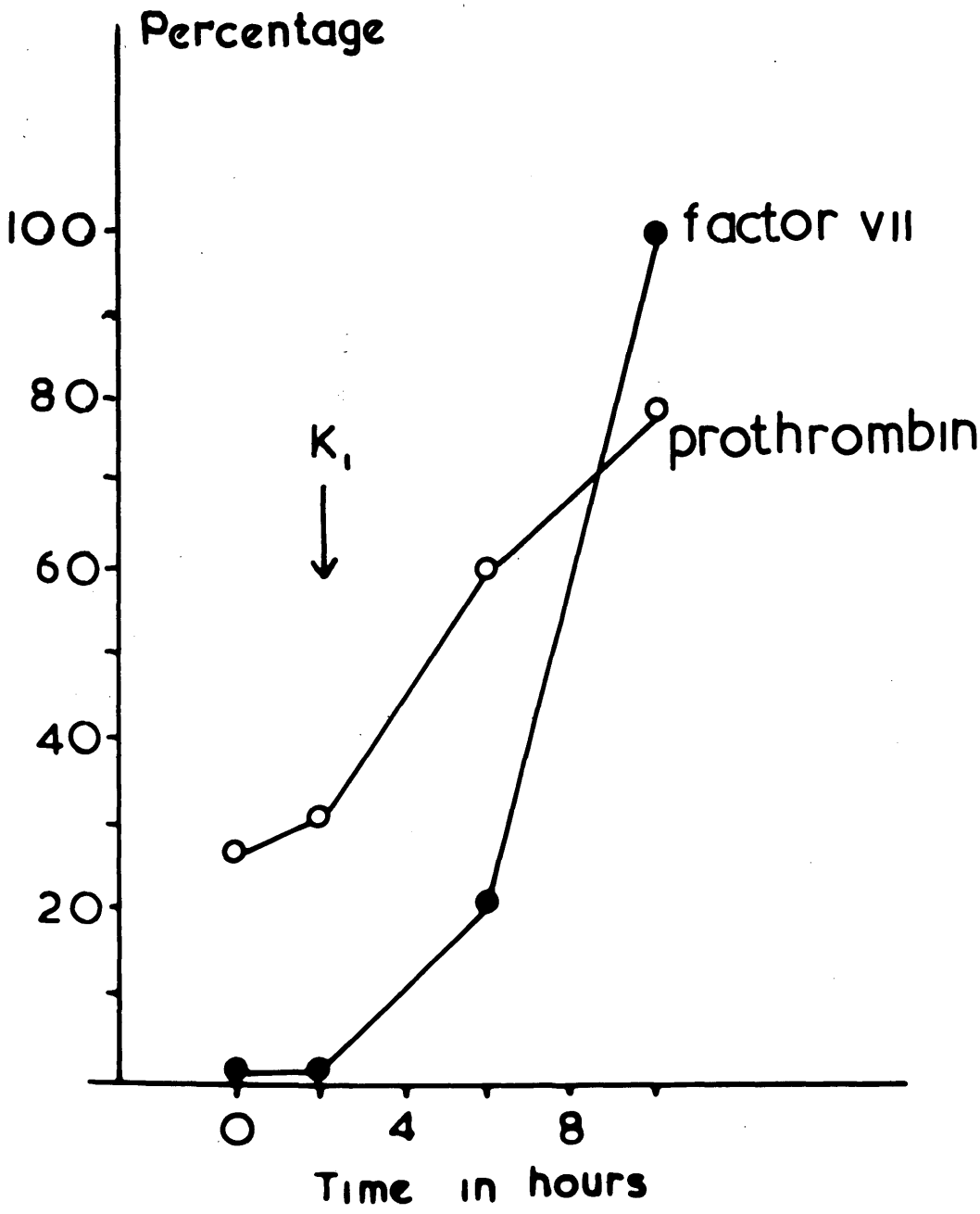

Percentage
100
80
60
40
20
factor VII
prothrombin
K1
0
4
8
Time in hours

Figure (51)

Figure (31)

Factor V content of tromexan plasma.

Ordinate - Thrombin units.

Abscissa - incubation time in minutes after addition of calcium.

10 normal plasmas and 10 tromexan plasmas were examined in the one experiment. The factor V was prepared from each of these plasmas and the activation of prothrombin studied. The continuous line is the result from the factor V of the tromexan plasmas expressed as a mean. The discontinuous lines represent the activation by the factor V from the normal plasmas diluted also to 50%. The activation of the prothrombin with no added factor V is shown as 0%.

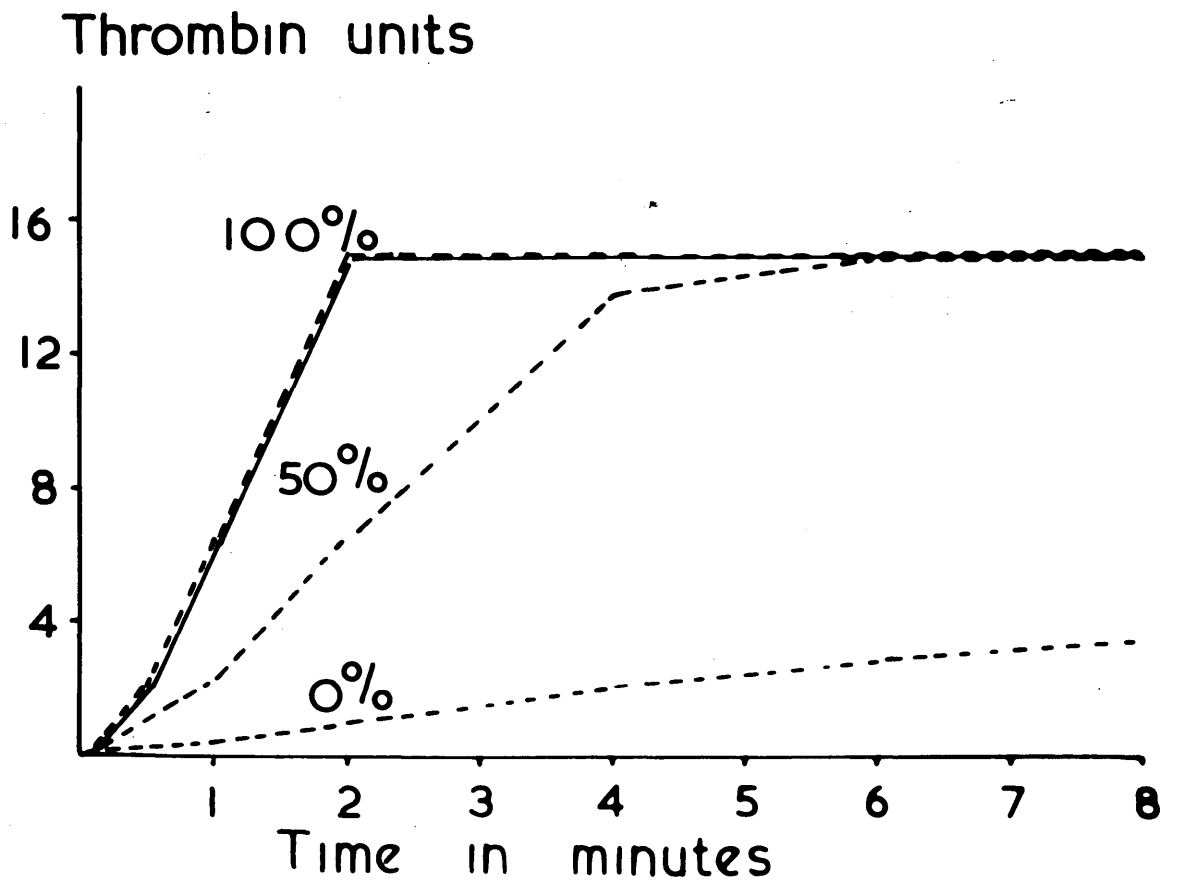

Thrombin units
16
12
8
4
100%
50%
0%
1
2
3
4
5
6
7
8
Time in minutes

Figure (52)

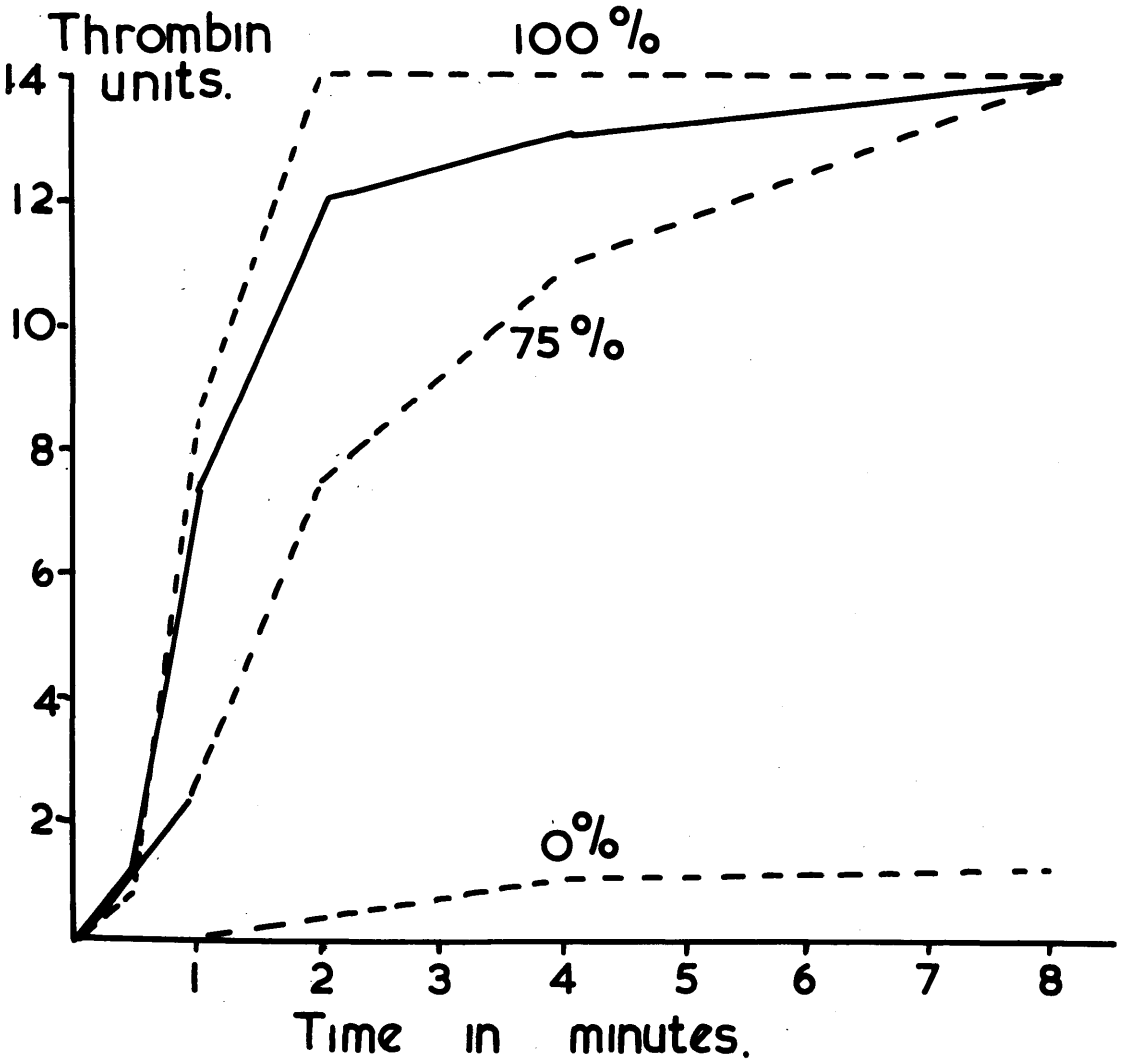
Figure (32)

Factor V content of tromexan plasma.

Ordinate - Thrombin units.

Abscissa - incubation time in minutes after addition of calcium.

One normal plasma and ten tromexan plasmas were examined in the one experiment. The factor V was prepared from each of these plasmas and the activation of prothrombin studied. The continuous line is the mean of the results from the factor V of the tromexan plasmas. The discontinuous lines represent the activation by the factor V from the normal.


Thrombin units.
14
12
10
8
6
4
2
100 %
75 %
0%
1
2
3
4
5
6
7
8
Time in minutes.

The levels of prothrombin and factor VII on the cessation of therapy.

In two patients the return of prothrombin and factor VII after the termination of therapy is shown in figure 49 . This represents the mean of two observations.

In one patient, under the effect of tromexan, who had a level on Quick's one-stage test of less than 5% for some days and in whom bleeding had occurred, the effect of a massive dose of vitamin K_1 was studied (1000 mg.). The rapid return of both these factors towards normal can be observed in Figure 50 .

The action of vitamin K_1 is studied in greater detail in Chapter 24 .

Factor V in Tromexan Therapy.

There have been reports that part of the defect in coumarin plasma is attributable to a deficiency of factor V (Olwin 1949). In two experiments described in detail in the Appendix no evidence was found of any deficiency of factor V. The results are shown in Figures 51-52.

Effect of storage of tromexan plasma on the clotting time by Quick's test.

When Quick's test is performed on tromexan plasma which has been stored in the deep freeze the clotting time shortens and the percentage as read off a dilution curve becomes greater (See appendix - page 648).

Figure (53)

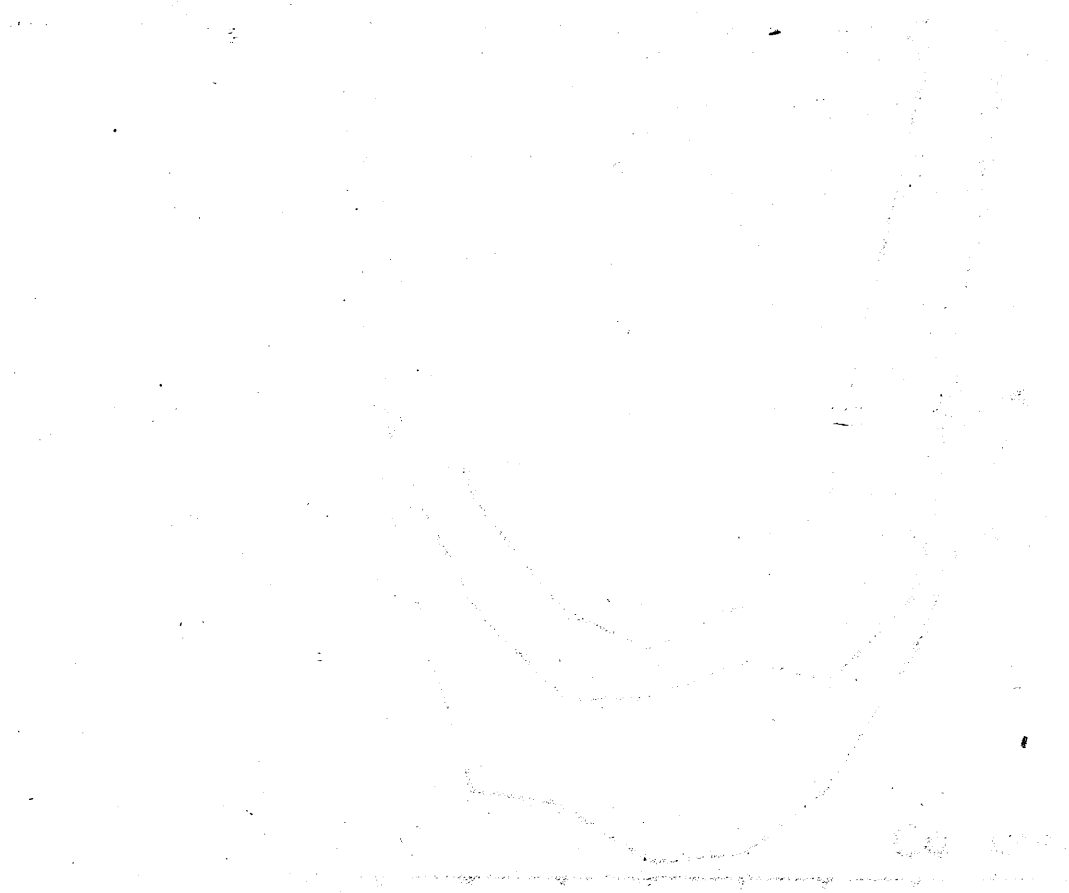


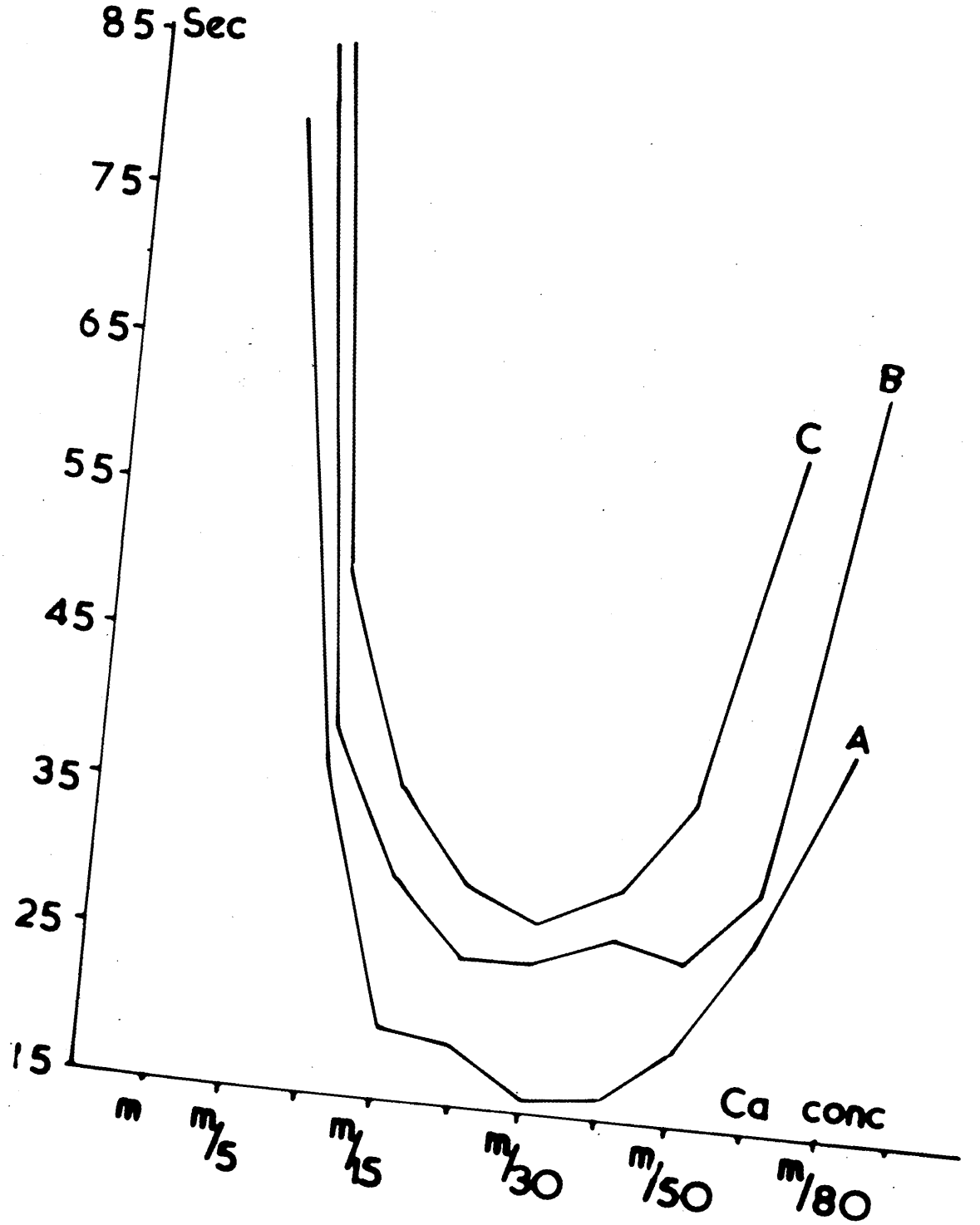
Figure (52)

Effect of calcium concentration on the one-stage clotting time of tromexan plasma.

Ordinate - Clotting time in seconds.

Abscissa - Concentration of calcium.

B and C were the clotting times of the tromexan plasmas with the varying concentrations of calcium. A represents the clotting times of a normal plasma, with the different concentrations of calcium.


85 Sec
75
65
55
45
35
25
15
B
C
A
m
m/5
m/15
m/30
m/50
m/80
Ca conc

Calcium concentration and the one-stage clotting time of tromexan plasma.

It will be seen from figure 53 that the response in the clotting time of the tromexan plasma to varying concentrations of calcium was similar to the normal.

One-stage techniques and the coagulation defect caused by tromexan.

The clear cut pattern of gross Factor VII deficiency associated with slight and often insignificant reduction in prothrombin in these patients, may come as a surprise to those familiar with the use of other methods which purport to measure prothrombin. By other methods the level of prothrombin in the blood of patients treated with coumarin drugs is apparently much lower than that recorded in this investigation.

(a) One-stage methods with an excess of factor VII.

Even using one-stage methods in which an excess of factor VII is added to the clotting mixture the recorded level of prothrombin is lower than that given by the two methods used here. See appendix page 705 et seq. Such methods have been described by Koller et al 1951 and Owren and Aas 1951. A modification of their technique was used involving the substitution of adsorbed normal plasma as the source of factor V and fibrinogen in place of Seitz-filtered ox plasma.

The reason for the discrepancy is not clear, but it must be realised that although one-stage methods are technically simple their interpretation using dilution curves is complex. The two methods of prothrombin assay used in this investigation give close agreement and have a sound theoretical basis. (Biggs and Douglas 1953, Douglas and Biggs 1953); it is the author's belief that for reasons unknown the one-stage techniques, even those modified by the addition of serum may give falsely low values when used for the measurement of prothrombin.

(b) Preincubation of brain and serum.

Third day tromexan plasma with a one-stage clotting time of 75" (normal 19") and normal prothrombin content was used as substrate in comparison with normal plasma.

Incubation mixture 0.3 Brain 0.3 normal serum

0.3 $CaCl_2$

At stated intervals 0.1 ml. of this incubation mixture and a further 0.1 ml. of m/40 $CaCl_2$ were added simultaneously to the substrate.

Substrate	30"	1	2	3	4	6
Normal plasma	10	9	10	9	9	11
Tromexan plasma	10	8	10	10	10	13

Comment - the clotting time of the tromexan substrate was essentially the same as the normal.

When this technique was applied as a possible measure of prothrombin, reading the results from a dilution curve, the quantity recorded was too low when compared with the globulin fraction technique. The speed of clotting of the tromexan plasma was normal, when the serum and brain have been preincubated, provided the tromexan plasma contained a normal amount of prothrombin.

(c) Using blood thromboplastin.

The same tromexan plasma, with normal prothrombin content was used again as substrate (75" - one-stage clotting time as compared with 19" for the control).

Incubation mixture-0.3 platelets, 0.3 ads. normal plasma 1/5, 0.3 normal serum 1/10, 0.3 m/40 $CaCl_2$. At intervals as shown 0.1 added to substrate together with simultaneous addition of 0.1 of m/40 $CaCl_2$.

Substrate	1	2	3	4	5	6
Normal plasma	9	9	8	9	9	9
Tromexan plasma	8	9	9	8	9	9

Provided the prothrombin content is normal, the reaction of the tromexan plasma to the blood thromboplastin is normal. When this technique was applied to the measurement of prothrombin, the result being read from a dilution curve the answer again was too low when compared with the globulin

Figure (54)

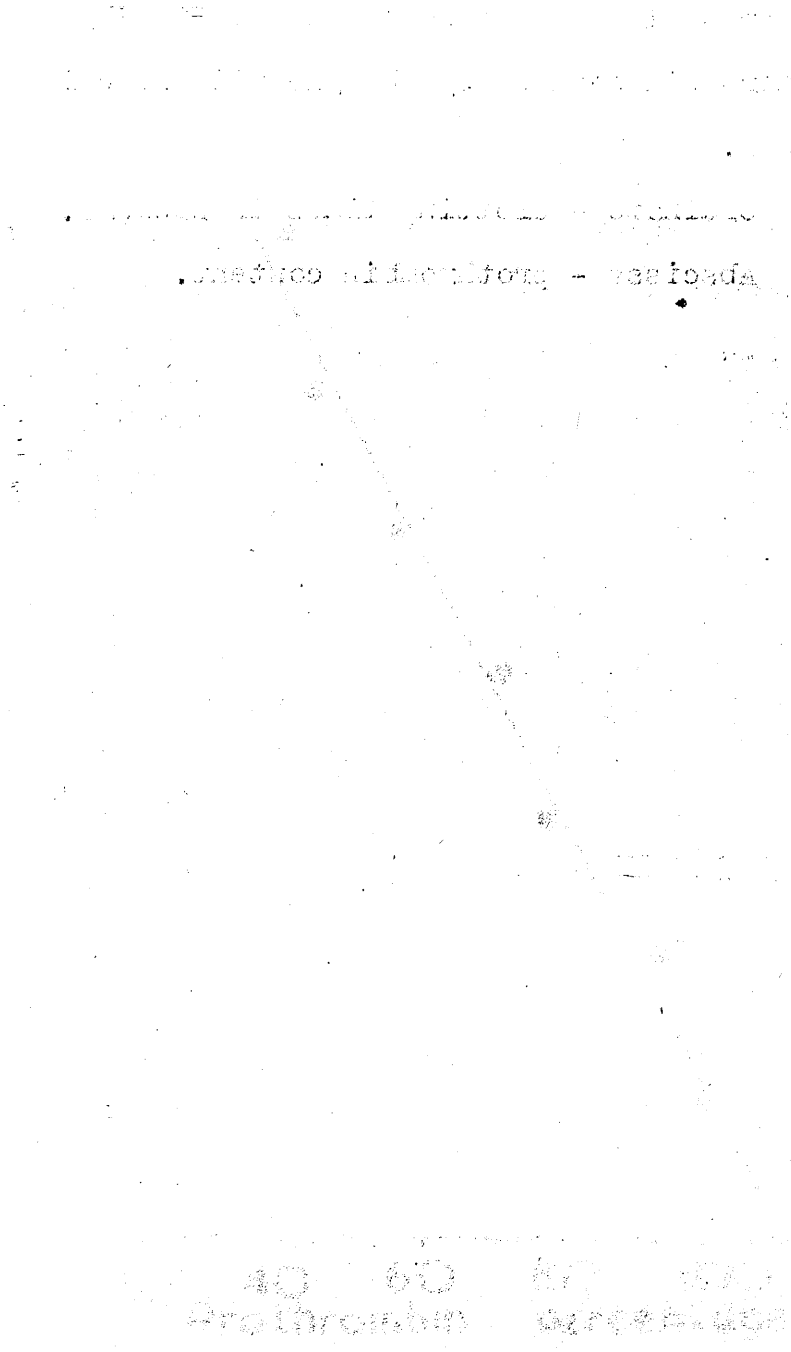


Figure (34)

Clotting times of tromexan plasma using prepared plasma thromboplastin plotted against prothrombin content by the globulin fraction technique - Case A.

Ordinate - clotting times in seconds.

Abscissa - prothrombin content.

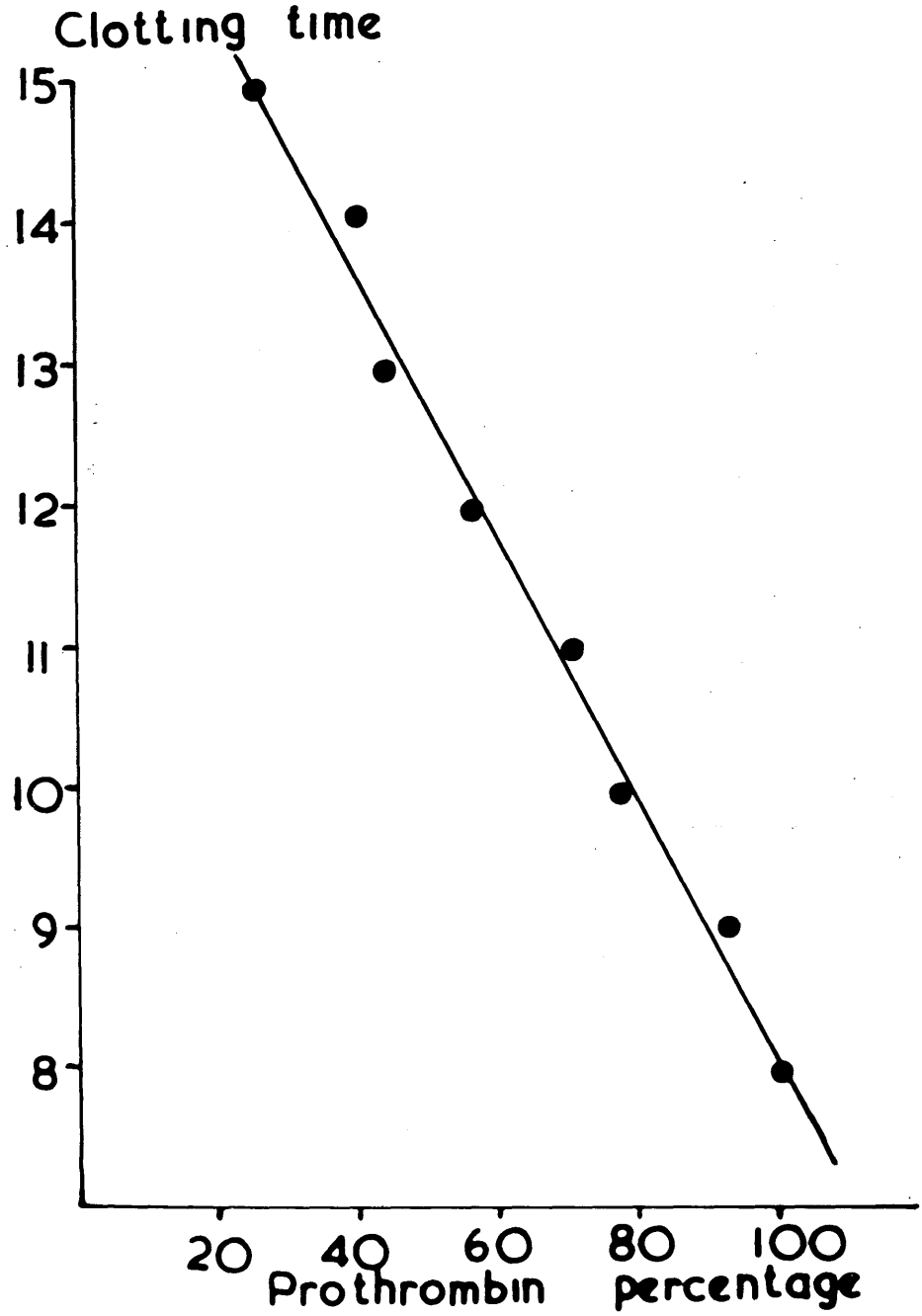

Clotting time
15
14
13
12
11
10
9
8
20
40
60
80
100
Prothrombin percentage

Figure (55)

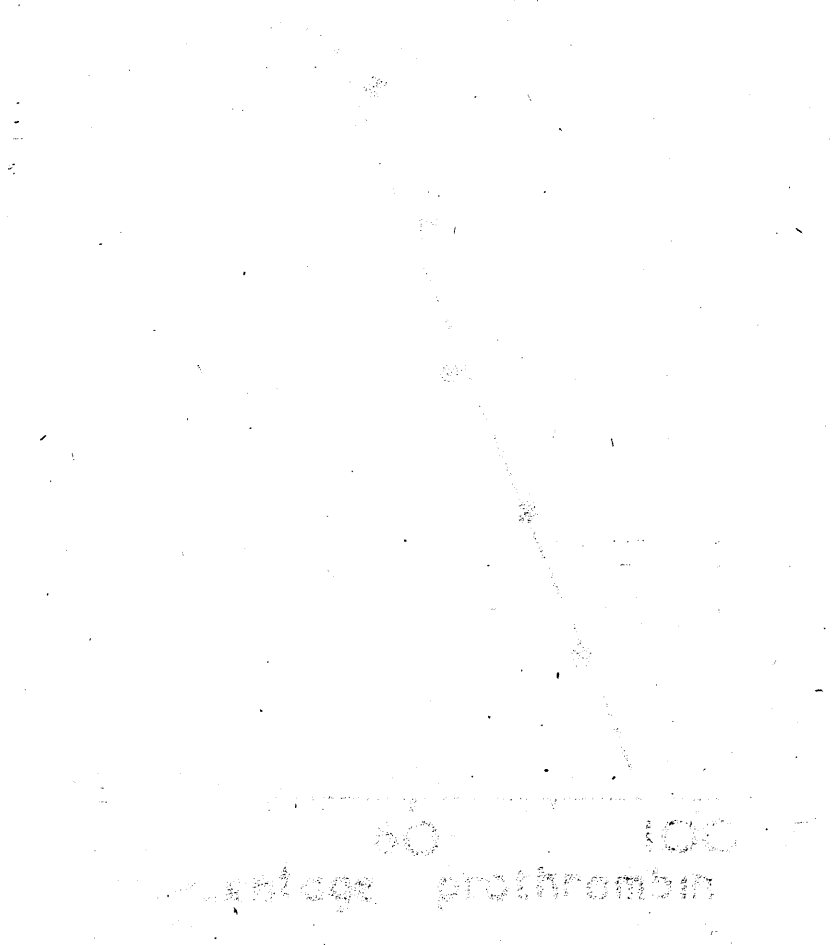


Figure (55)

Clotting times of tromexan plasma using prepared plasma thromboplastin plotted against prothrombin content by the globulin fraction technique - mean of cases C and D.

Ordinate - clotting times in seconds.

Abscissa - prothrombin content.

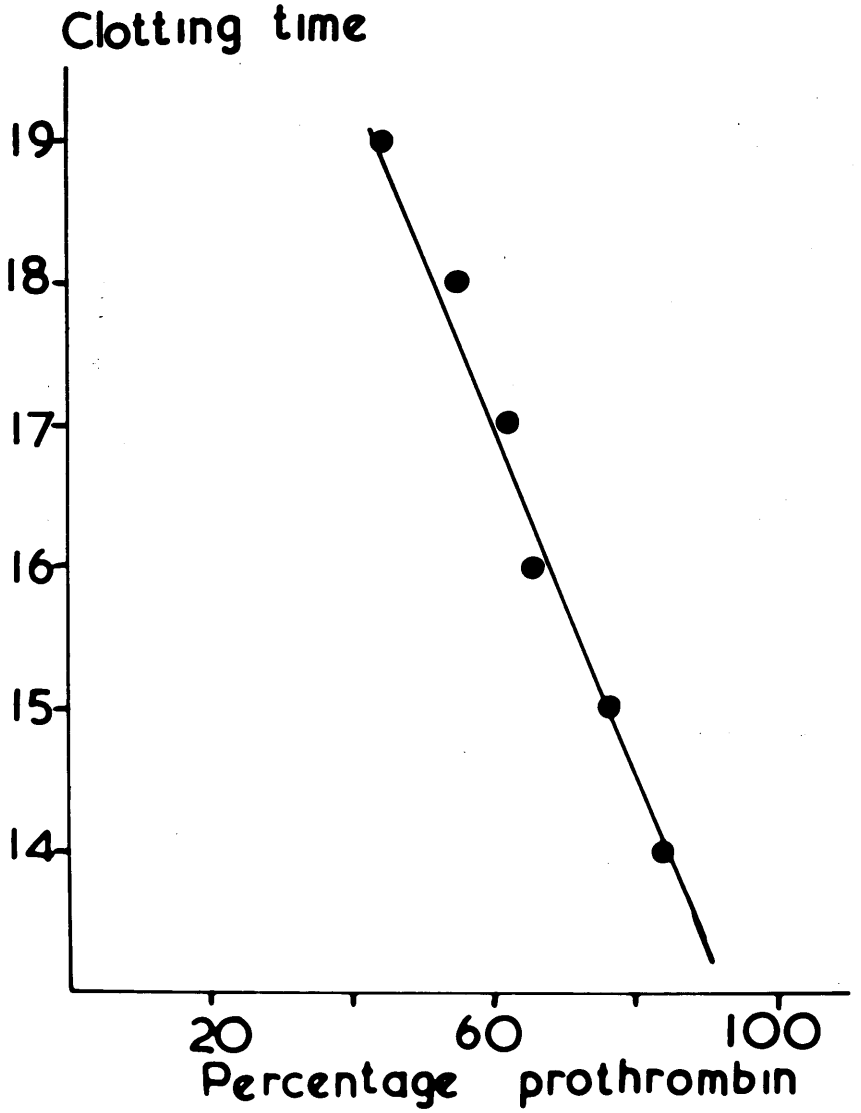

Clotting time
19
18
17
16
15
14
20
60
100
Percentage prothrombin

Figure (56)

Clotting times of tromexan plasma using prepared plasma thromboplastin plotted against prothrombin content by the globulin fraction technique - case F.

Ordinate - clotting times in seconds.

Abscissa - prothrombin content.

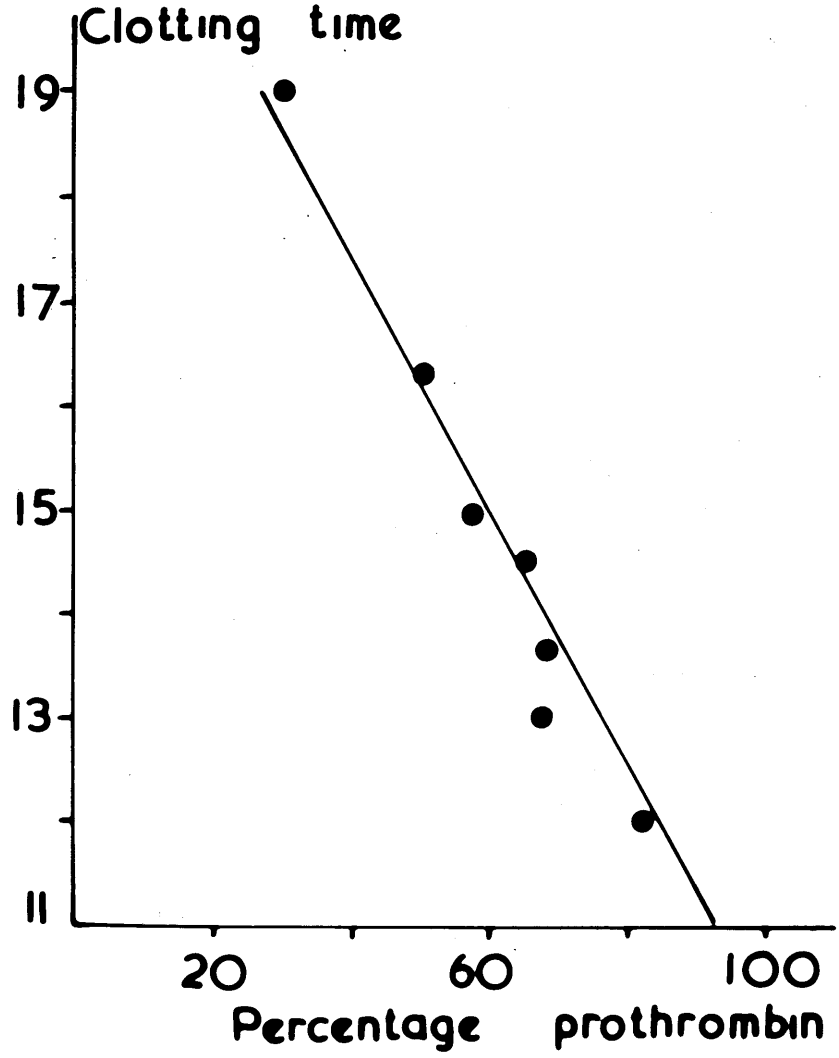

Clotting time
19
17
15
13
11
20
60
100
Percentage prothrombin

Figure (57)

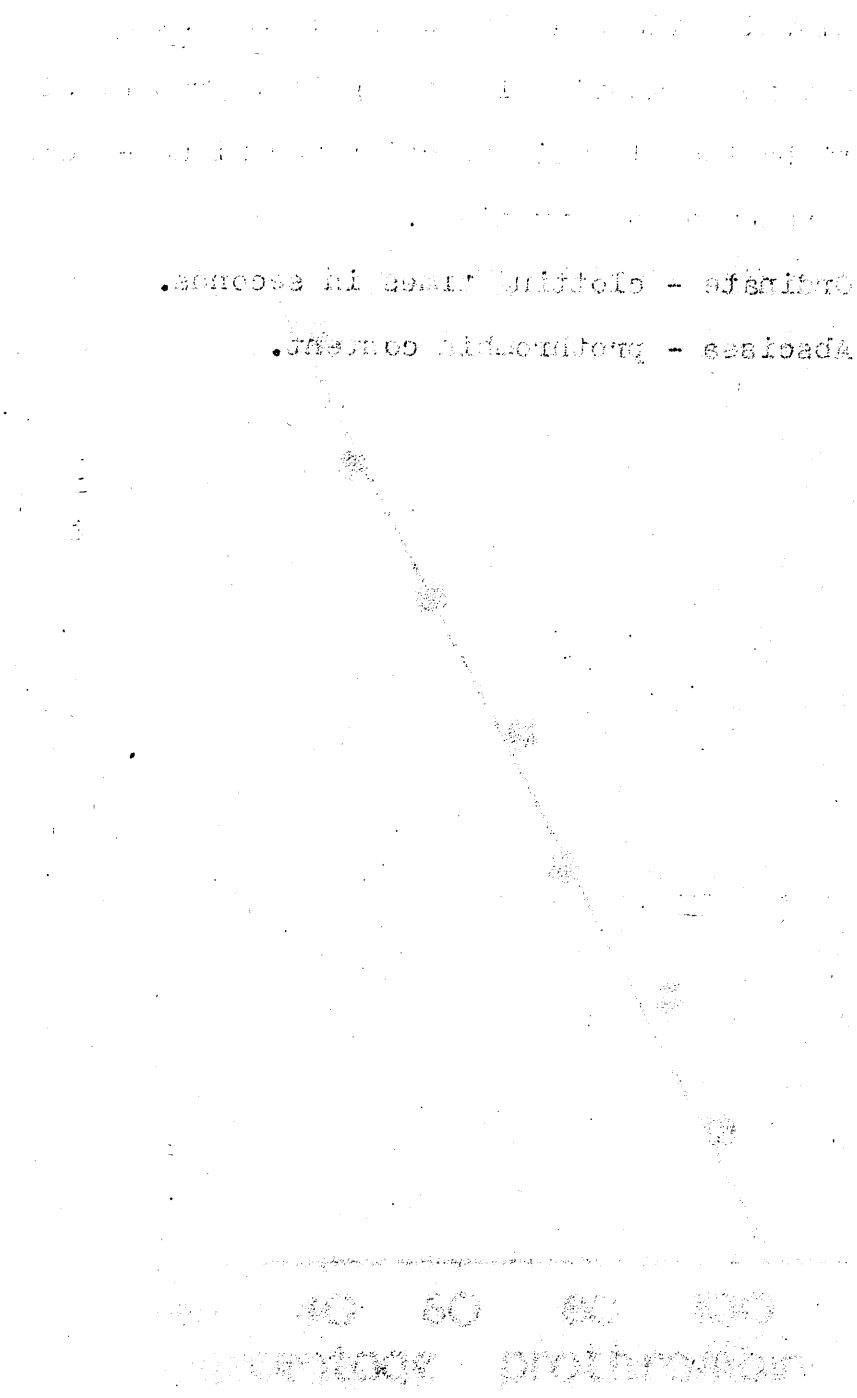


Figure (57)

Clotting times of tromexan plasma using prepared plasma thromboplastin plotted against prothrombin content by the globulin fraction technique - mean of the total number of observations.

Ordinate - clotting times in seconds.

Abscissa - prothrombin content.

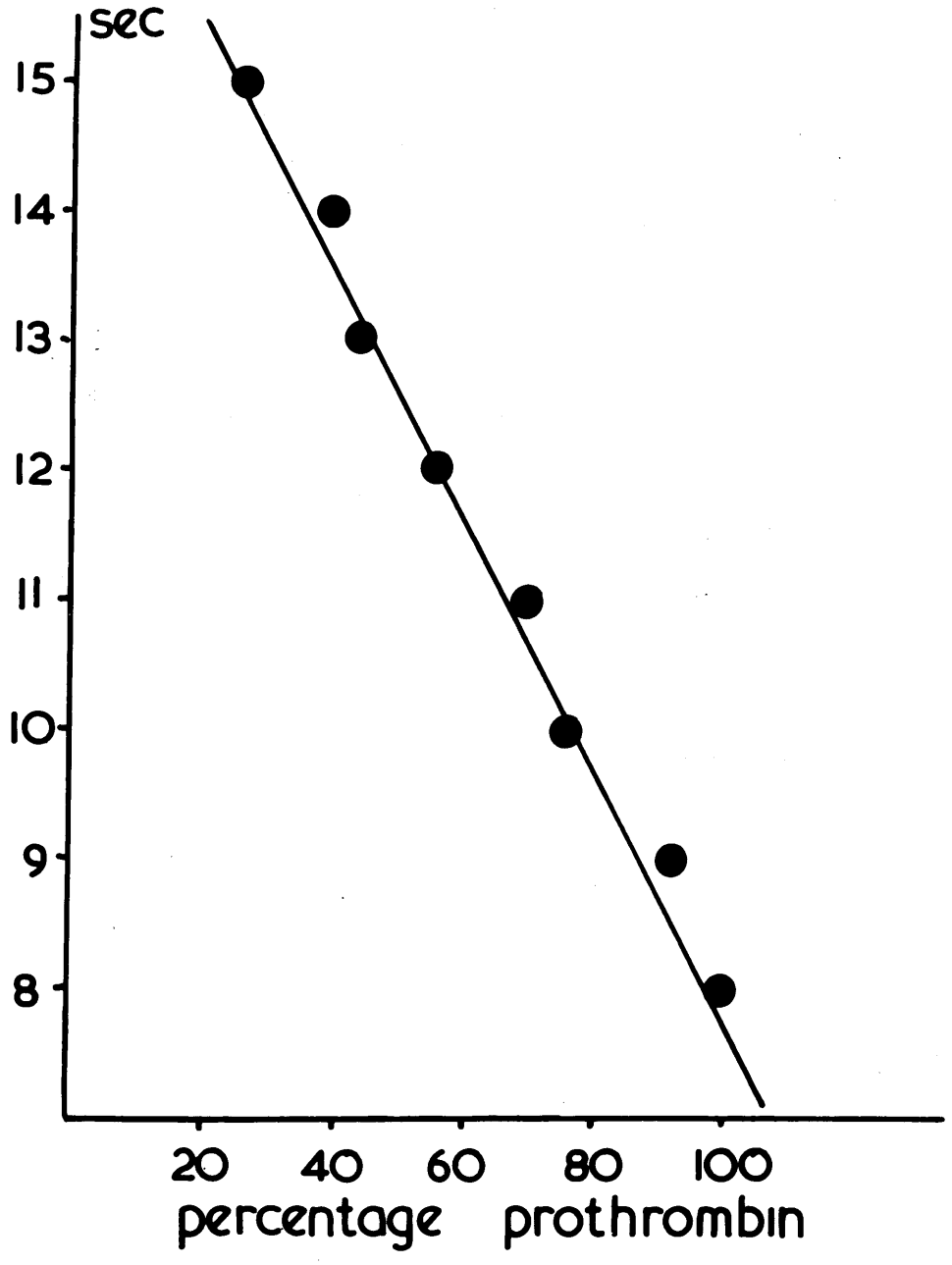

sec
15
14
13
12
11
10
9
8
20
40
60
80
100
percentage prothrombin

Figure (58)

Figure (58)

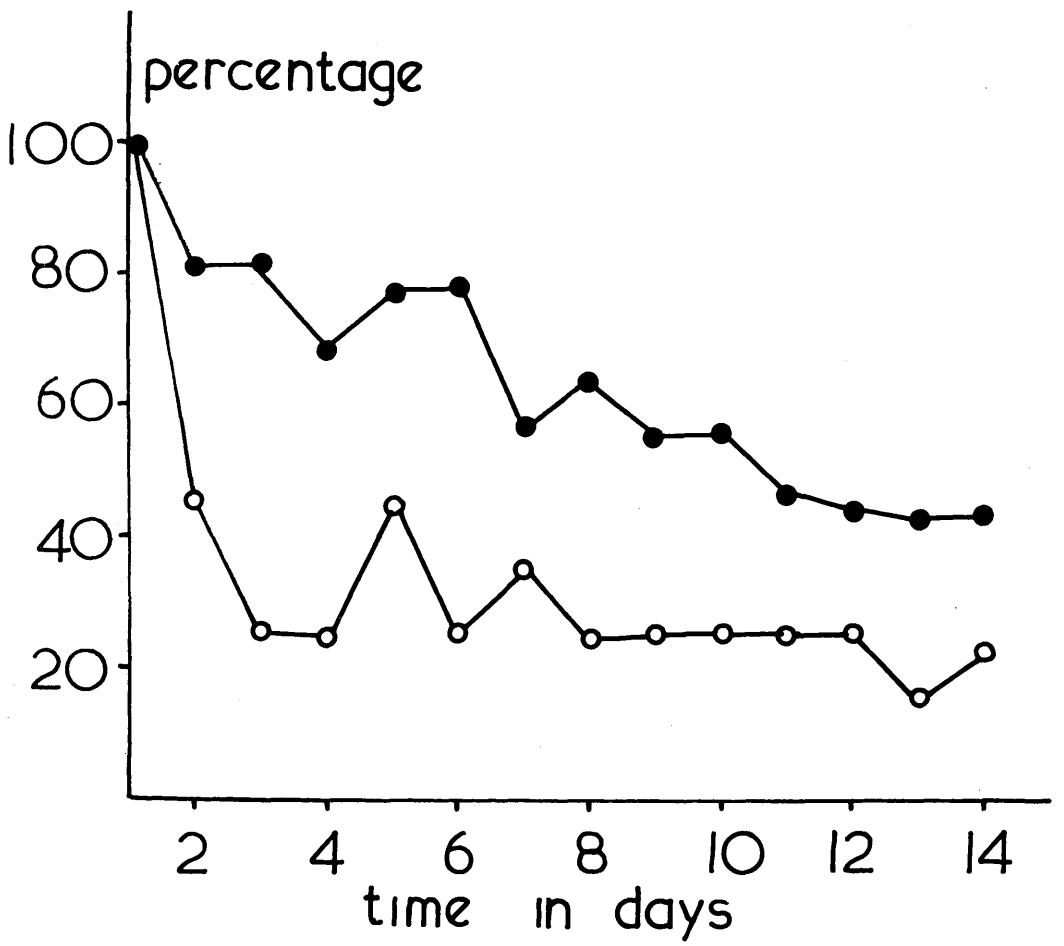
Comparison of prothrombin assay during 14 days of tromexan therapy, when the prothrombin was assayed by the globulin fraction method and on a one-stage technique with plasma thromboplastin.

Ordinate - percentage prothrombin.

Abscissa - time in days.

●——● - percentage prothrombin by the globulin fraction technique.

O——O - percentage prothrombin using a one-stage technique with plasma thromboplastin and a dilution curve with adsorbed plasma as a diluent.


percentage
100
80
60
40
20
2
4
6
8
10
12
14
time in days

fraction technique (see appendix). When the clotting times by this method are plotted against the prothrombin content as estimated by the globulin fraction technique the points lie on a straight line (Figures 54, 55, 56, 57). This suggests that the method does reflect the variation in prothrombin content, but the result cannot be recorded from a dilution curve. The results in one patient followed in this way from a dilution curve are shown in figure 58 . It will be seen that the assay is low as compared with the true prothrombin assay by the globulin fraction method.

(d) Russell's Viper Venom and Lecithin method.

Lamb (1903) observed that Russell's viper venom was almost devoid of thrombin-like activity and the immensely powerful thromboplastin-like action of the venom was largely overlooked until Macfarlane and Barnett (1934) re-investigated its possibilities. It has found general use as a haemostatic and was made available commercially by Burroughs Welcome under the trade name of Stypven. At first such preparations were thought to have some advantages over the tissue thromboplastins in Quick's technique. The venom was stable when dried and readily available commercially. The preparation was of relatively uniform potency whereas tissue thromboplastins were variable. Fullerton (1940) described its use in a one-stage technique, and its use subsequently reported by

Page et al (1941 a & b), Page and de Beer (1943) Shapiro et al (1942).

Trevan and Macfarlane (1936) found that the action of the venom was greatly potentiated by the use of lecithin. Macfarlane (1938) observed that plasma deprived of platelets by high speed centrifugation was only slowly clotted by venom, while Macfarlane et al (1941) showed that the removal of lipoid from plasma inhibited its coagulation by venom. This could be restored by the addition of various lipoid substances including lecithin. In 1942 Witts and Hobson suggested the use of venom and lecithin mixture as a thromboplastin for the one-stage method. Such a mixture shortens the prothrombin time to 6-7 seconds. With the advent of dicumarin therapy it became more obvious that venom and lecithin did not give results comparable to those obtained with brain. Witts (1942) estimated the prothrombin time in a dog following a large dose of dicoumarin and found that with venom and lecithin the apparent decrease in prothrombin was less than was the case if brain was used for the estimations. Evidence was produced by Biggs and Macfarlane (1949) to show that Russell's viper venom with lecithin was a less sensitive estimation of the effect of dicoumarin therapy than was brain.

In 1948 Lempert reported two cases under treatment with dicumarol where brain recorded 10 per cent prothrombin whereas

Figure (59)

Figure (89)

Clotting times of tromexan plasma using Russell's Viper Venom with lecithin as the source of thromboplastin plotted against prothrombin content by the globulin fraction technique. (64 observations on 5 cases on tromexan).

Ordinate - clotting times in seconds.

Abscissa - prothrombin content.

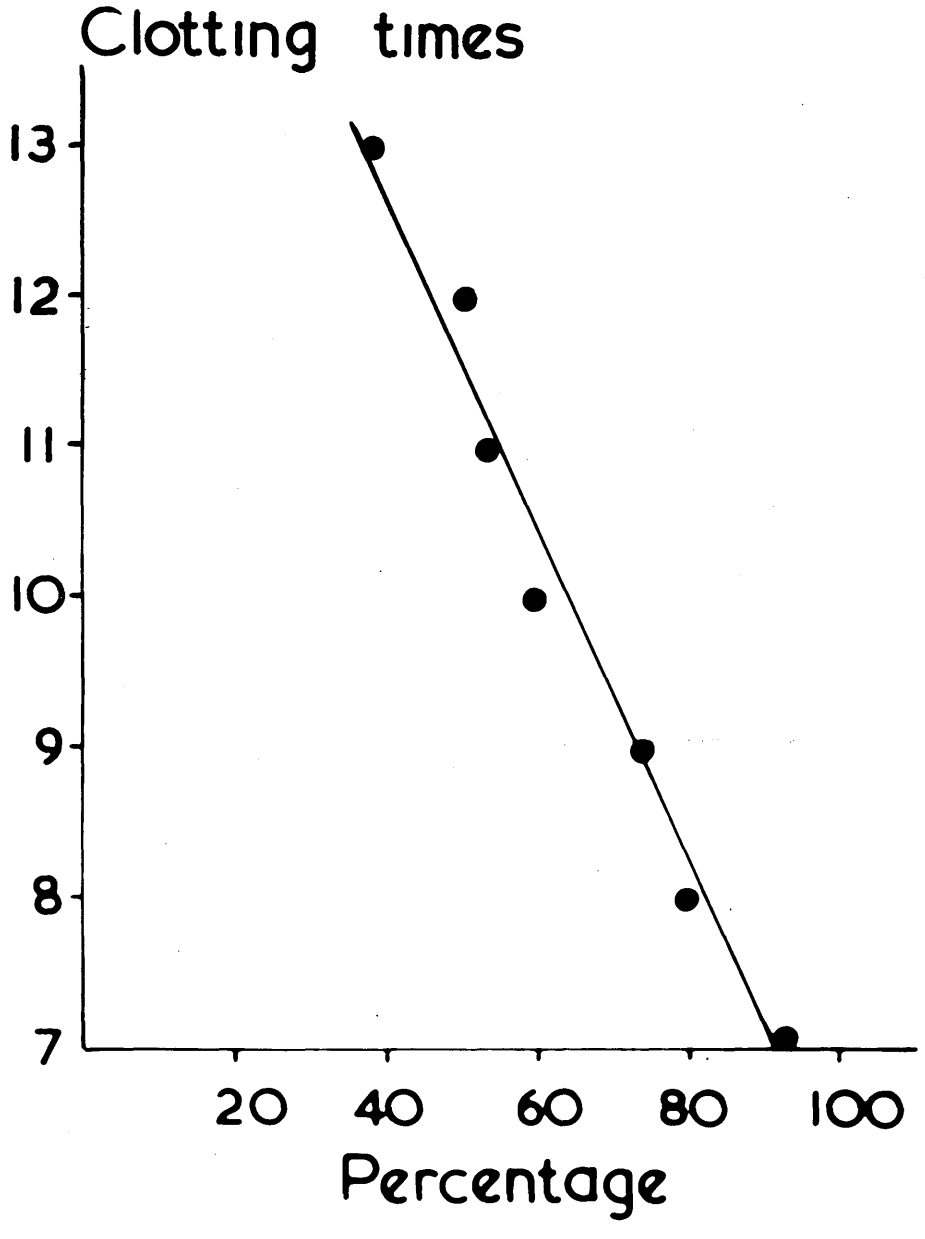

Clotting times
13
12
11
10
9
8
7
20
40
60
80
100
Percentage

Figure (60)

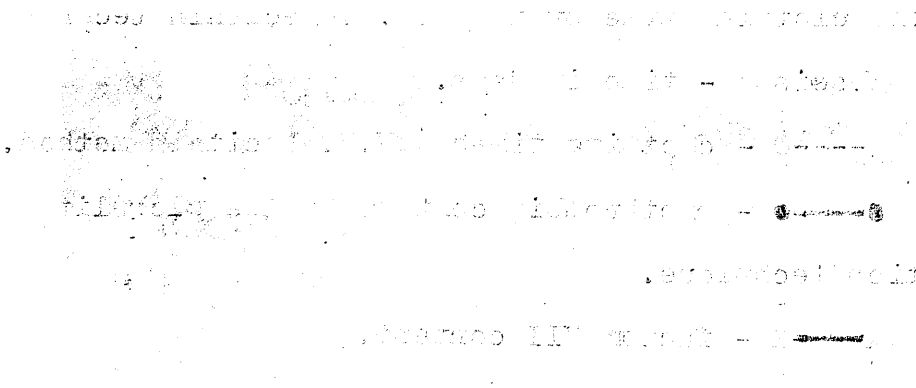


Figure (60)

Comparison of prothrombin assay during 14 days of tromexan therapy with the clotting times on the Russell's Viper Venom-Lecithin technique.

Ordinate - percentage prothrombin or factor VII and the clotting time by the R.V.V.-Lecithin technique.

Abscissa - time in days.

O----O - clotting times R.V.V.-Lecithin method.

●——● - prothrombin content by the globulin fraction technique.

X——X - factor VII content.

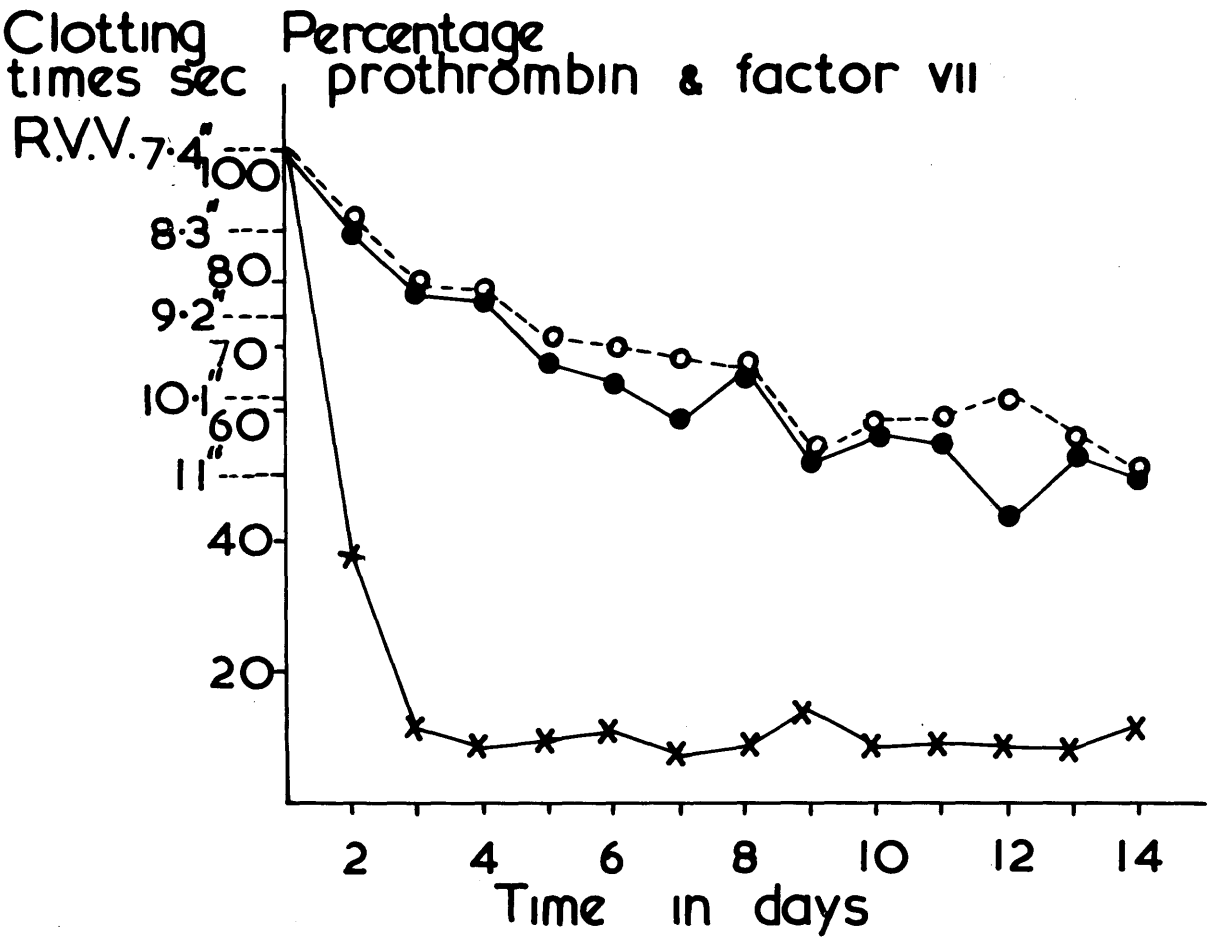

Clotting times sec
Percentage prothrombin & factor VII
R.V.V.
7·4"
8·3"
9·2"
10·1"
11"
100
80
70
60
40
20
2
4
6
8
10
12
14
Time in days

Figure (61)

Figure (31)

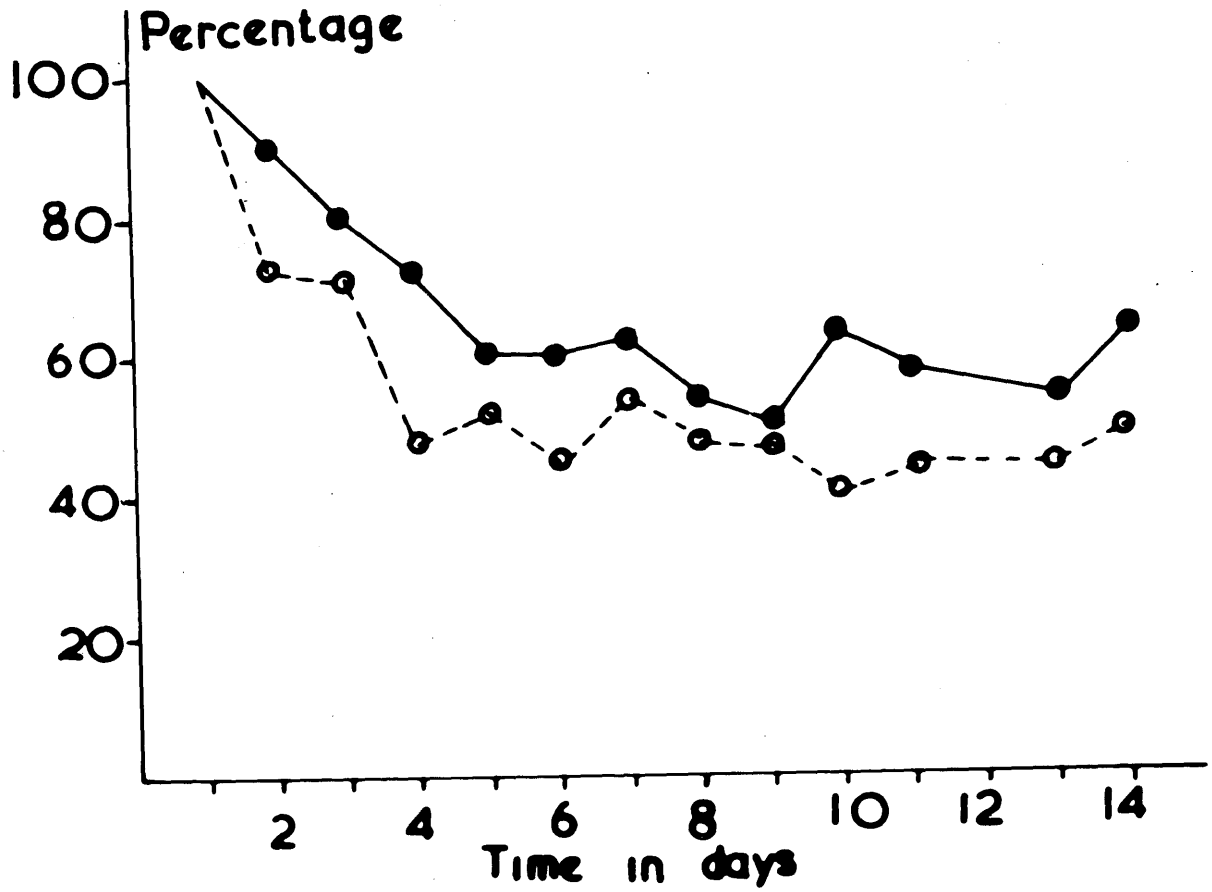
Comparison of prothrombin assay during 14 days of tromexan therapy with the clotting times on the Russell Viper Venom-Lecithin technique read from a dilution curve where the diluent was adsorbed normal plasma.

Ordinate - percentage prothrombin.

Abscissa - time in days.

●——● - prothrombin content by the globulin fraction technique.

O----O - prothrombin content by the Russell's Viper Venom-Lecithin method, read from a dilution curve where the diluent was adsorbed normal plasma.


Percentage
100
80
60
40
20
2
4
6
8
10
12
14
Time in days

Fullerton's technique recorded 25 and 35% respectively.

Five cases on tromexan were followed daily for fourteen days and some 64 observations made on the globulin fraction method of measuring prothrombin, and the one-stage test using brain and Russell's viper venom with lecithin. A mean prothrombin content as estimated by the globulin fraction method was then made and related to the respective clotting time by the R.V.V.-lec.technique. When these were plotted against each other a straight line relationship was established. This is reasonable evidence that the venom lecithin method reflects the prothrombin content (Figure 59 and Table 10). When the mean clotting time for each day of therapy was plotted with the prothrombin content for that day the similarity of the pattern is very striking (Fig. 60).

TABLE 10

	Globulin Fraction	Dilution Curve
7"	93	100
8"	80	50
9"	74	35
10"	58	30
11"	53	25
12"	50	20
13"	39	17

A dilution curve of normal plasma by alumina-adsorbed plasma was prepared and this gave results usually somewhat lower than the true prothrombin reading as determined by the globulin fraction technique.

The results of these investigations indicate that, like blood thromboplastin, the R.V.V. and lecithin method, reflects the change in prothrombin content but that this cannot be assayed from a dilution curve. Nevertheless, of all the one-stage techniques the R.V.V. and lecithin result, read from a dilution curve made with adsorbed plasma, was nearest to giving a true reading of prothrombin content. In one experiment using this method the mean prothrombin content of 13 tromexan plasma samples by the R.V.V. lecithin technique was 70% as compared with the mean value by the globulin fraction method of 80%. (see appendix pages 717-73). Fig. 61 illustrates one example where the result was followed daily by this technique. Mawson (1949) suggested that the venom-lecithin method might give a measure of prothrombin. It is probable from these experiments that brain thromboplastin is sensitive to factor VII deficiency, the main defect in coumarin therapy and it is for this reason of great value in controlling therapy with these drugs. In comparison the venom-lecithin method and formed blood thromboplastin are insensitive to the factor VII and they mainly reflect the lesser deficiency of prothrombin.

<u>The effect of Russell's viper venom on the shape of the two-stage curve on tromexan therapy</u>.

When Russell's viper venom is added to tromexan plasma and the two-stage test with brain carried out it will be seen

Figure (62)

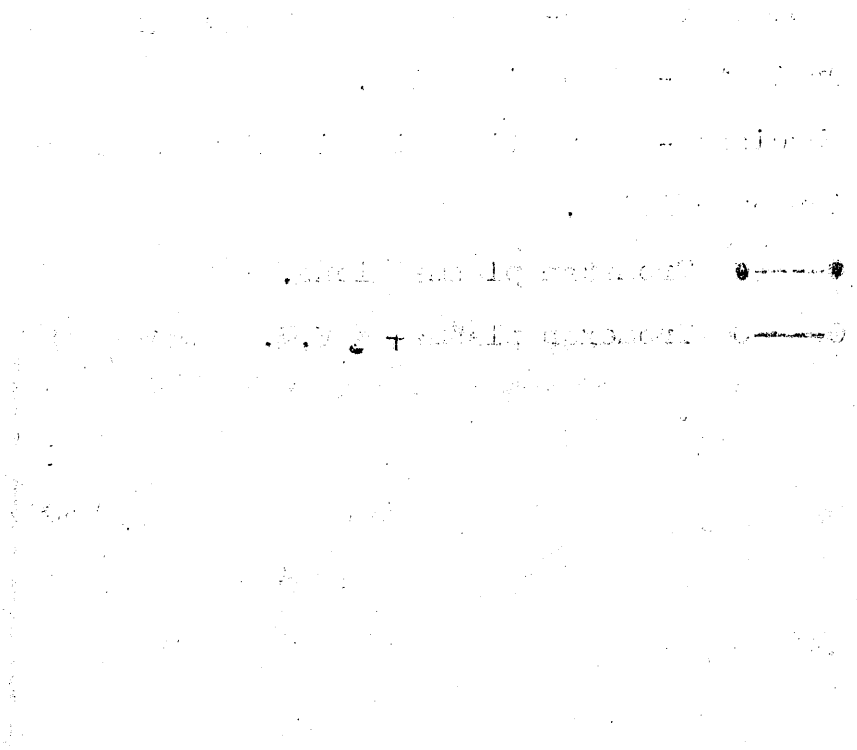


Figure (42)

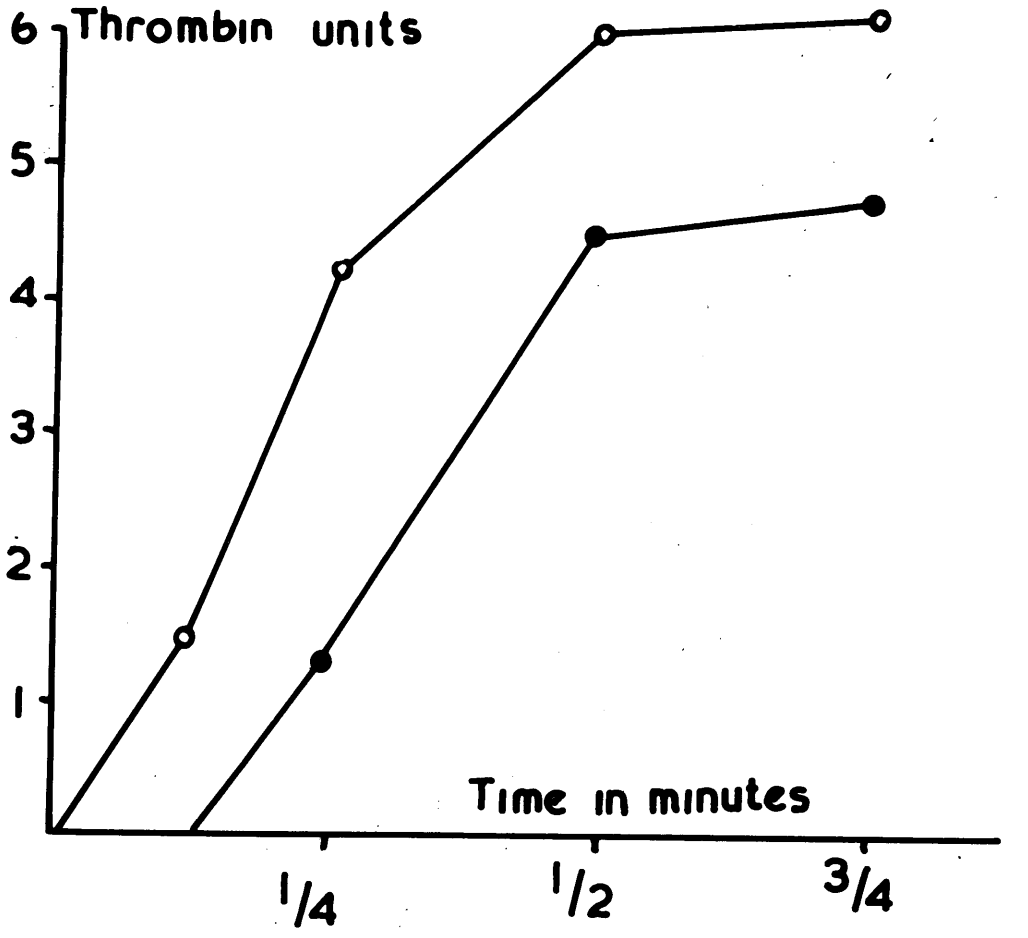
The effect of Russell's Viper Venom (R.V.V.) on the shape of the two-stage curve on tromexan therapy.

Ordinate - Thrombin units.

Abscissa - Incubation time in minutes after addition of calcium.

●——● Tromexan plasma alone.

O——O Tromexan plasma + R.V.V.

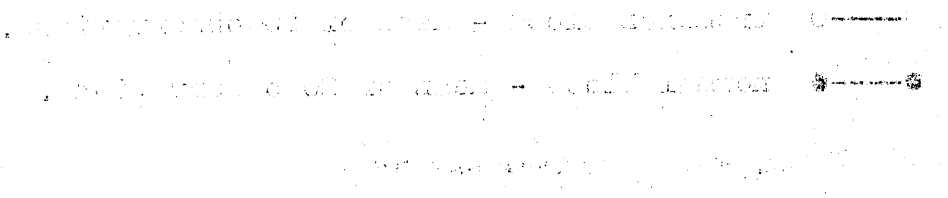

6
Thrombin units
5
4
3
2
1
Time in minutes
1/4
1/2
3/4

that the initial delay in prothrombin appearance is corrected. This explains the relatively short one-stage clotting time of tromexan plasma with Russell's viper venom. The results of one observation on this are shown in Fig.62 and given in detail in the experimental appendix page 749.

Blood Thromboplastin in coumarin therapy.

When it was believed that the coumarin drugs produced a very marked reduction in prothrombin the mechanism of the coagulation defect was easy to understand. Now that it is clear that the deficiency of prothrombin is limited, it has become necessary to reassess the nature of the interference produced by tromexan. It is clear that there is a marked deficiency of factor VII and that this substance is essential for the action of brain thromboplastin. The evidence that tromexan interferes with tissue thromboplastin in virtue of depression of factor VII, gives an incomplete indication of the disturbance with physiological clotting. The relative importance of the tissue mechanism as compared with the blood's own thromboplastin system in physiological haemostasis is uncertain. It is probable that the blood's own thromboplastin system is at least as important as the tissue mechanism. The nature of any interference by tromexan with the clotting of blood under its own or intrinsic thromboplastin had to be determined. There is available one

Figure (63)



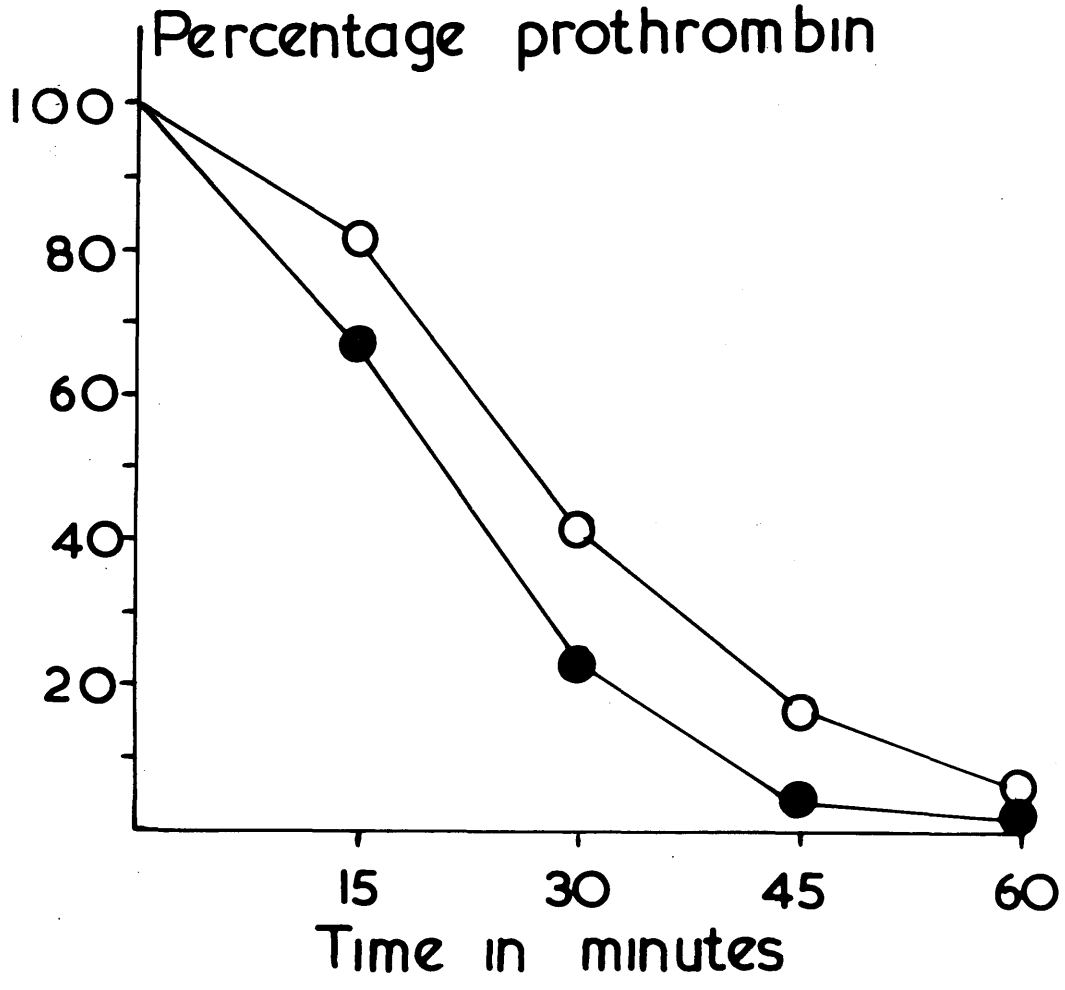
Prothrombin consumption in tromexan blood.

Ordinate - percentage prothrombin.

Abscissa - time in minutes after withdrawal of the blood.

O——O tromexan blood - mean of 20 observations.

●——● normal blood - mean of 20 observations.


Percentage prothrombin
100
80
60
40
20
15
30
45
60
Time in minutes

technique for studying directly the interference with thromboplastin formation and a number of other less specific and less sensitive procedures which reflect an overall deficiency of intrinsic thromboplastin. The direct method of approach is the thromboplastin generation test and the others include the whole blood clotting time, calcium clotting time, prothrombin consumption and the thrombin generation test. These latter tests give no information about individual factors but record the degree of abnormality regardless of its cause.

Whole Blood Clotting Time.

In Table 11 is shown a comparison between the whole blood clotting times (Lee and White method) in normal blood and in tromexan blood.

Table 11

Normal		Tromexan	
Glass	Silicone	Glass	Silicone
6	11	7'	28'

(Mean of 17 observations).

The whole blood clotting time in silicone in tromexan therapy is prolonged in some instances very markedly, blood being virtually incoagulable in silicone. (see page 752 of the

appendix) - technique (1) was employed.

Calcium Clotting Time.

In one patient followed daily the calcium clotting time became prolonged particularly so towards the end of the second week of therapy. In a further experiment on the routine specimens collected on the one day from patients on tromexan the prolongation of the calcium clotting time is demonstrated (pages 753-4).

Prothrombin Consumption Test.

If the prothrombin consumption test is used as an index of thromboplastin efficiency there is a slight delay in prothrombin consumption demonstrable as an average of 20 tests on normal blood and 20 tests on the blood of patients treated with tromexan (Fig. 63), but no significant difference can be demonstrated on single samples. The prothrombin consumption technique is that described by Douglas and Biggs 1953 and is given in detail in the appendix.

The specimens chosen for this investigation were selected at random from patients on routine therapy. No attempt was made to select persons most markedly under the effect of these drugs. The technique used is the only one which is suitable for the assessment of prothrombin under the particular circumstances. It allows sufficient time for the deficiency

Figure (64)

Figure (64)

Thrombin generation from normal blood and tromexan blood.

Ordinate - Thrombin units.

Abscissa - Time in minutes.

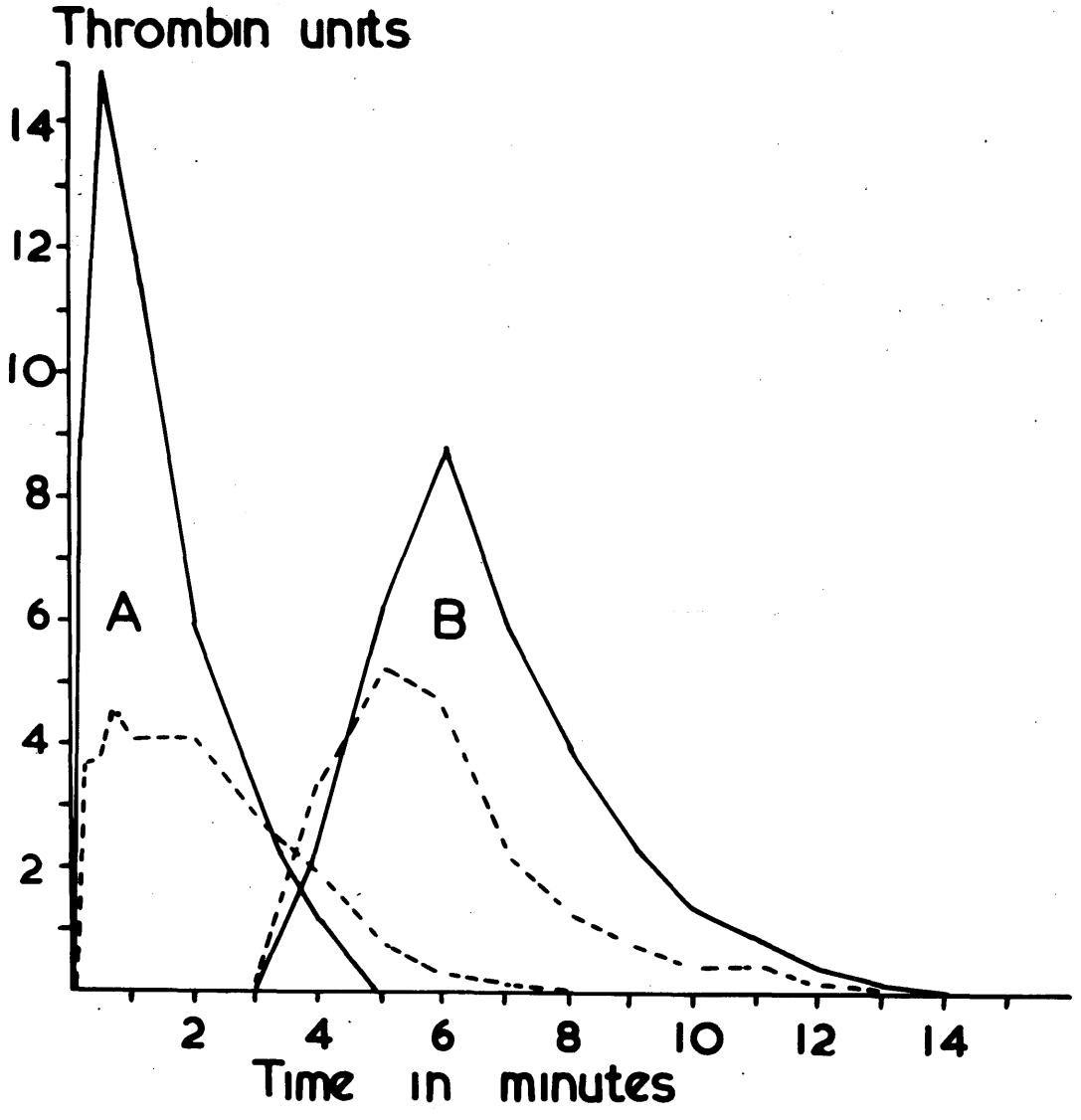
Continuous line = normal.

Discontinuous line = tromexan.

A = release of thrombin from prothrombin in plasma under the influence of brain extract and calcium.

B = release of thrombin from prothrombin in whole blood under the influence of the blood's own thromboplastin - in the thrombin generation technique.

(Mean of 5 observations).


Thrombin units
14
12
10
8
6
4
2
A
B
2
4
6
8
10
12
14
Time in minutes

of factor VII to cause full activation of the prothrombin.

Thrombin Generation Test.

Five patients were examined by the area two-stage test and by the thrombin generation test. The results are shown in Fig. 64 . It will be seen that allowing for the deficiency of prothrombin there was no great difference between the tromexan blood and the normal blood. It may be concluded from this that there was no delay in the formation of intrinsic thromboplastin, demonstrable by this test.

This experiment also makes an interesting comparison between the release of thrombin under the influence of brain thromboplastin and under the action of intrinsic thromboplastin. The areas enclosed by the tromexan curves when compared with its corresponding normal show astonishing agreement.

Area - by brain in two-stage test = 57% normal.
" " thrombin generation " = 60% "

Thromboplastin Generation Technique.

As previously described a powerful blood thromboplastin can be prepared by incubating together normal adsorbed plasma, platelets and normal serum in the presence of calcium chloride.

Figure (65)

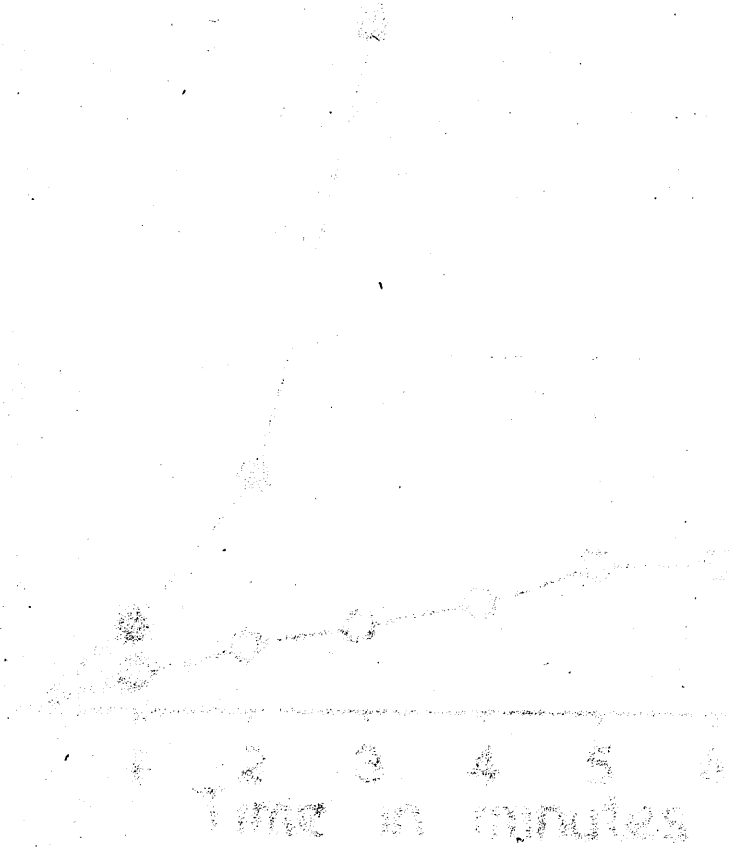


Figure (6C)

Thromboplastin generation using tromexan serum.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation test with normal adsorbed plasma and platelets constant and the serum variable. Mean of five observations.

●——● normal serum.

O——O tromexan serum.

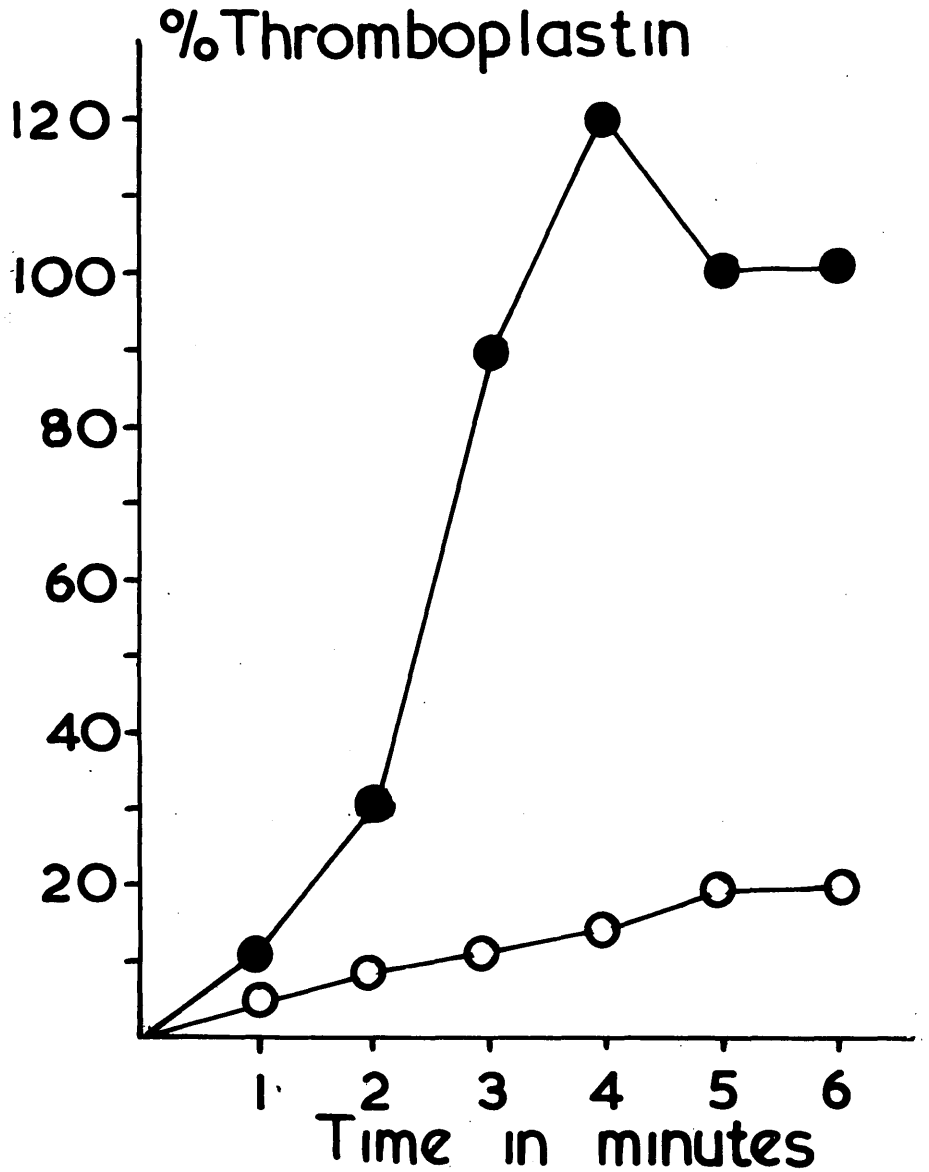

%Thromboplastin
120
100
80
60
40
20
1
2
3
4
5
6
Time in minutes

Figure (66)

Figure (36)

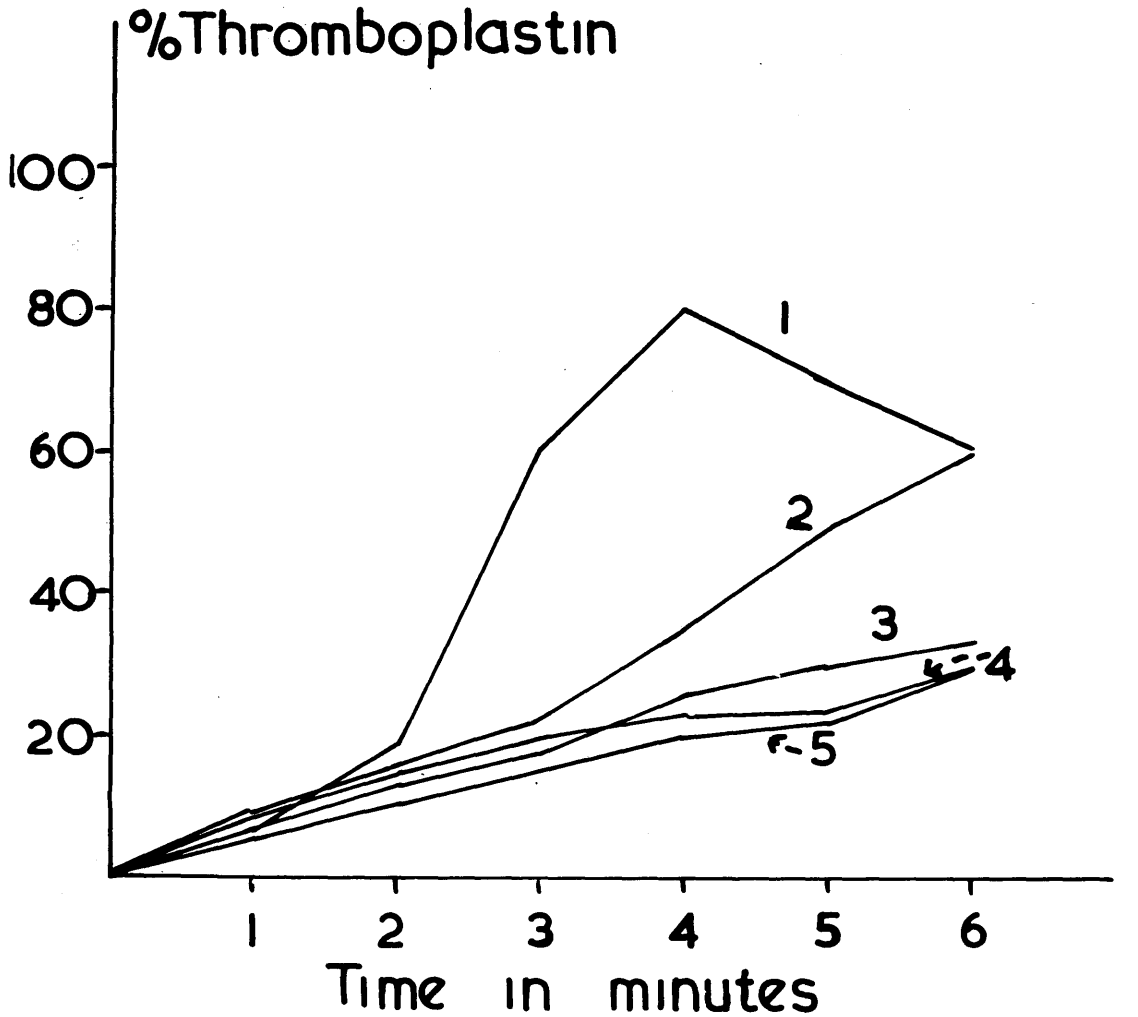
Thromboplastin generation using tromexan serum.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation test with normal adsorbed plasma and platelets constant and the serum variable.

1 - 5 : - represent Days 1 - 5 of therapy with tromexan. Mean of six observarions.

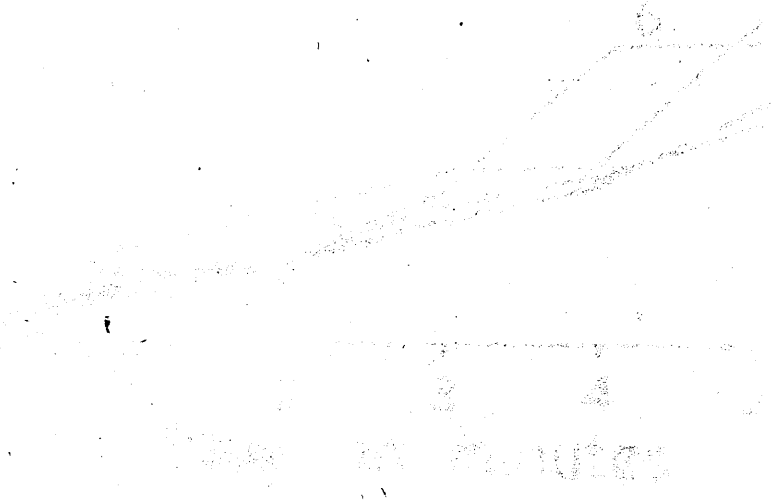

%Thromboplastin
100
80
60
40
20
1
2
3
4
5
1
2
3
4
5
6
Time in minutes

Failure of tromexan serum to form blood thromboplastin.

If in this system the normal serum is replaced by serum from a patient treated with tromexan the ability of the mixture to form thromboplastin is usually much less than that of the mixture containing normal serum. The mean result obtained with five normal sera and five sera from patients receiving tromexan is shown in Figure 65. The results are expressed in terms of percentage of blood thromboplastin. The sera were collected under identical conditions and each was examined in a dilution of one in ten. It will be seen that the tromexan samples are much less efficient in producing thromboplastin than are the normal samples.

In figure 66 is shown the progressive effect of therapy on successive days as judged by the effect of the respective sera on thromboplastin generation. The quantitative assay of this property of tromexan serum is not easy but an approximate measure can be obtained by comparing the effect of a one-in-ten dilution of tromexan serum with the effect of a range of dilutions of normal serum varying from one-in-ten to one-in-five hundred. Thus if the one-in-ten dilution of tromexan serum corresponds in its effect with the one-in-one hundred dilution of normal serum the tromexan serum would be said to have 10 per cent of the normal ability

Figure (67)



Thromboplastin generation using tromexan serum.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation test with normal adsorbed plasma and platelets constant and the serum variable.

6 - 9 :- represent Days 6 - 9 of therapy with tromexan. Mean of six observations.

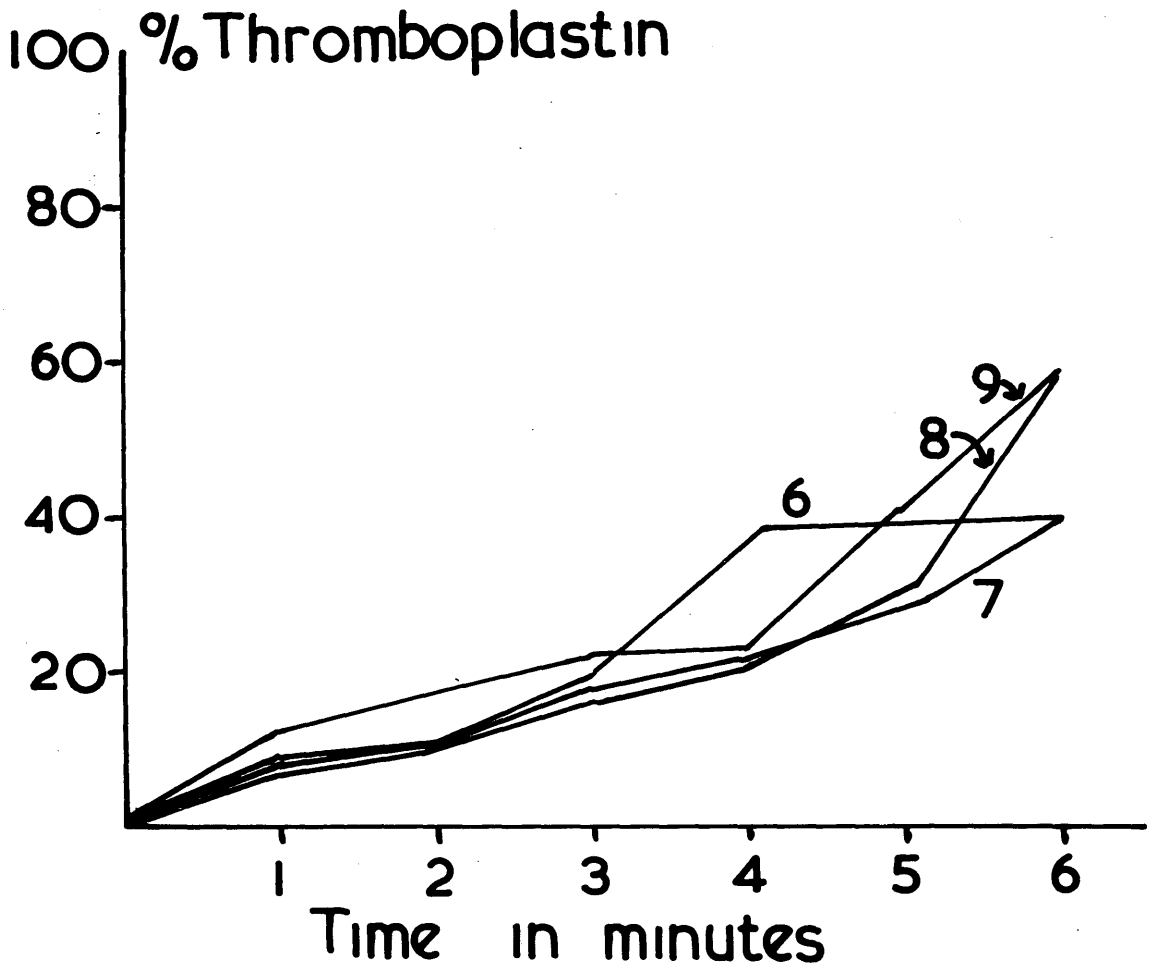

100
% Thromboplastin
80
60
40
20
9
8
6
7
1
2
3
4
5
6
Time in minutes

Figure (68)

Thromboplastin generation using tromexan serum.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation test with normal adsorbed plasma and platelets constant and the serum variable.

10 - 12 :- represent Days 10-12 of therapy with tromexan. Mean of six observations.

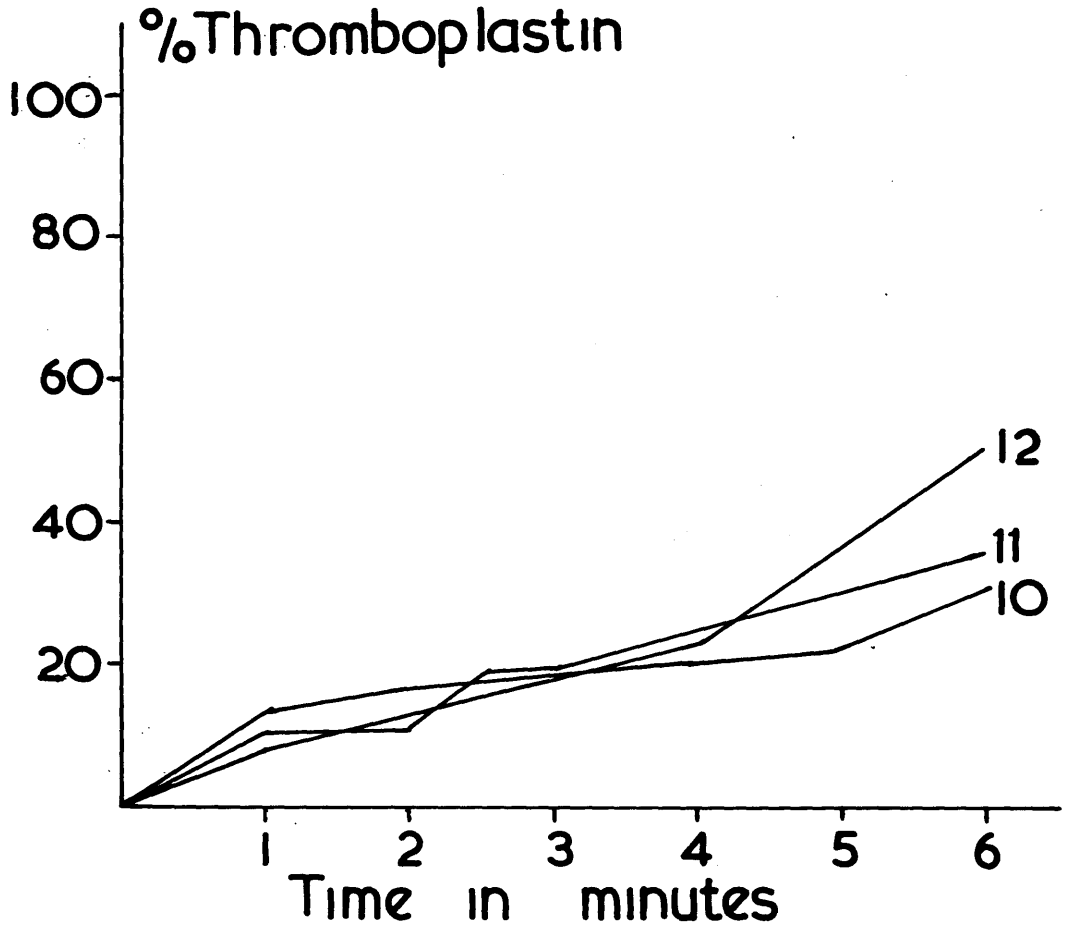
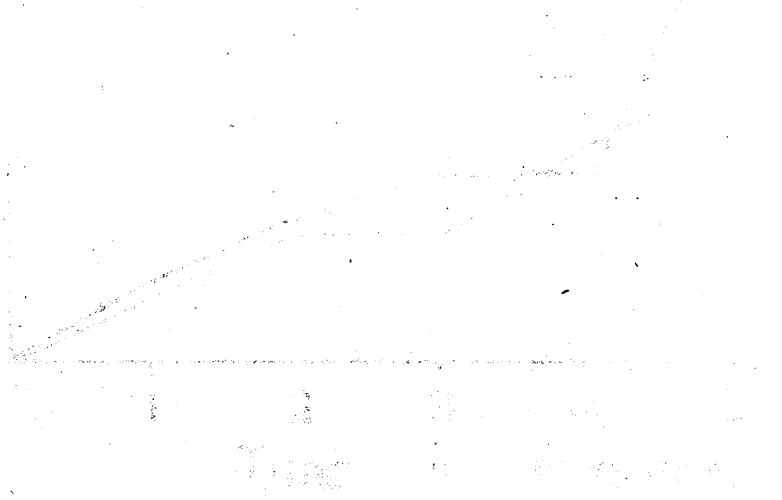

%Thromboplastin
100
80
60
40
20
12
11
10
1
2
3
4
5
6
Time in minutes

Figure (69)



Thromboplastin generation using tromexan serum.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation test with normal adsorbed plasma and platelets constant and the serum variable.

13 - 14 :- represent Days 13-14 of therapy with tromexan. Mean of six observations.

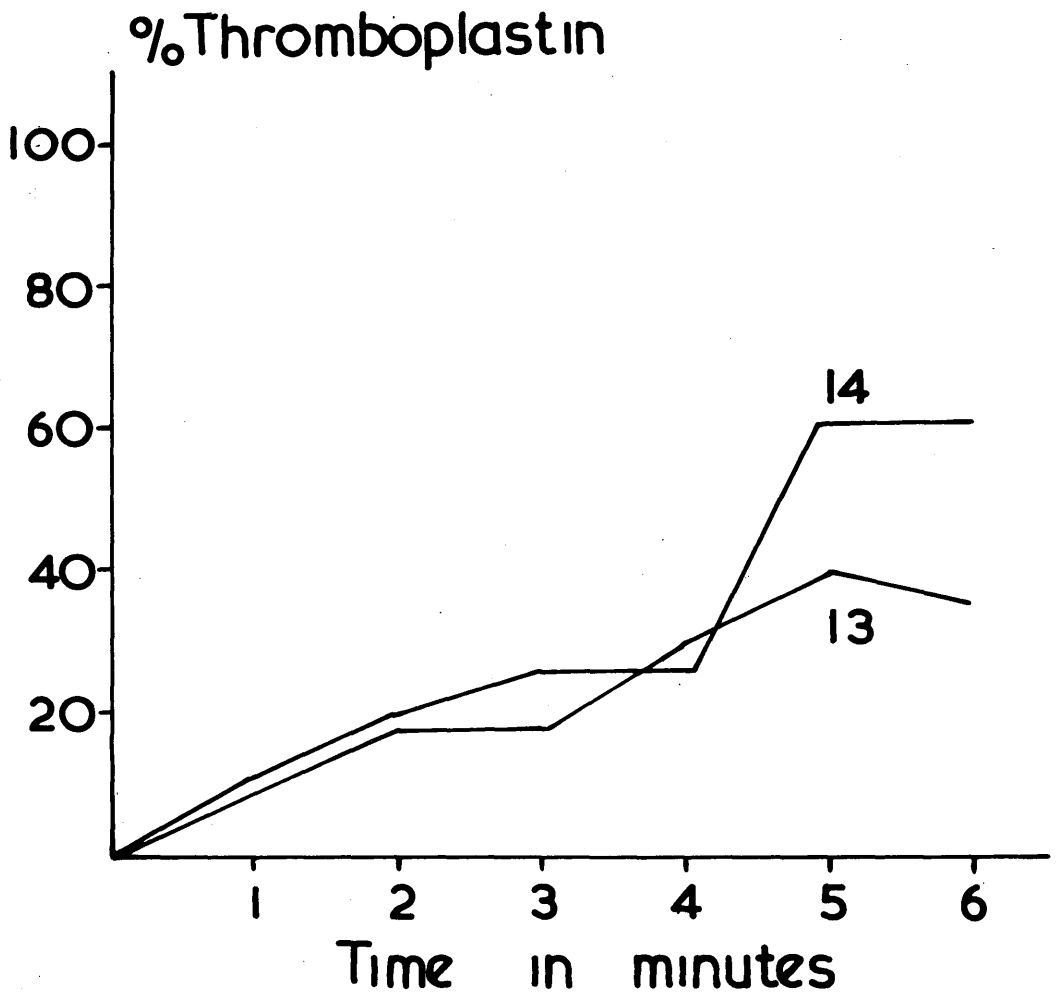

%Thromboplastin
100
80
60
40
20
14
13
1
2
3
4
5
6
Time in minutes

Figure (70)

Figure (70)

Thromboplastin generation on saline dilutions of normal serum.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

This figure exemplifies the series of dilution curves required to give a quantitative assay of the defective thromboplastin formation by the tromexan serum. With each tromexan serum to be assayed, a series of curves was prepared in the same experiment.

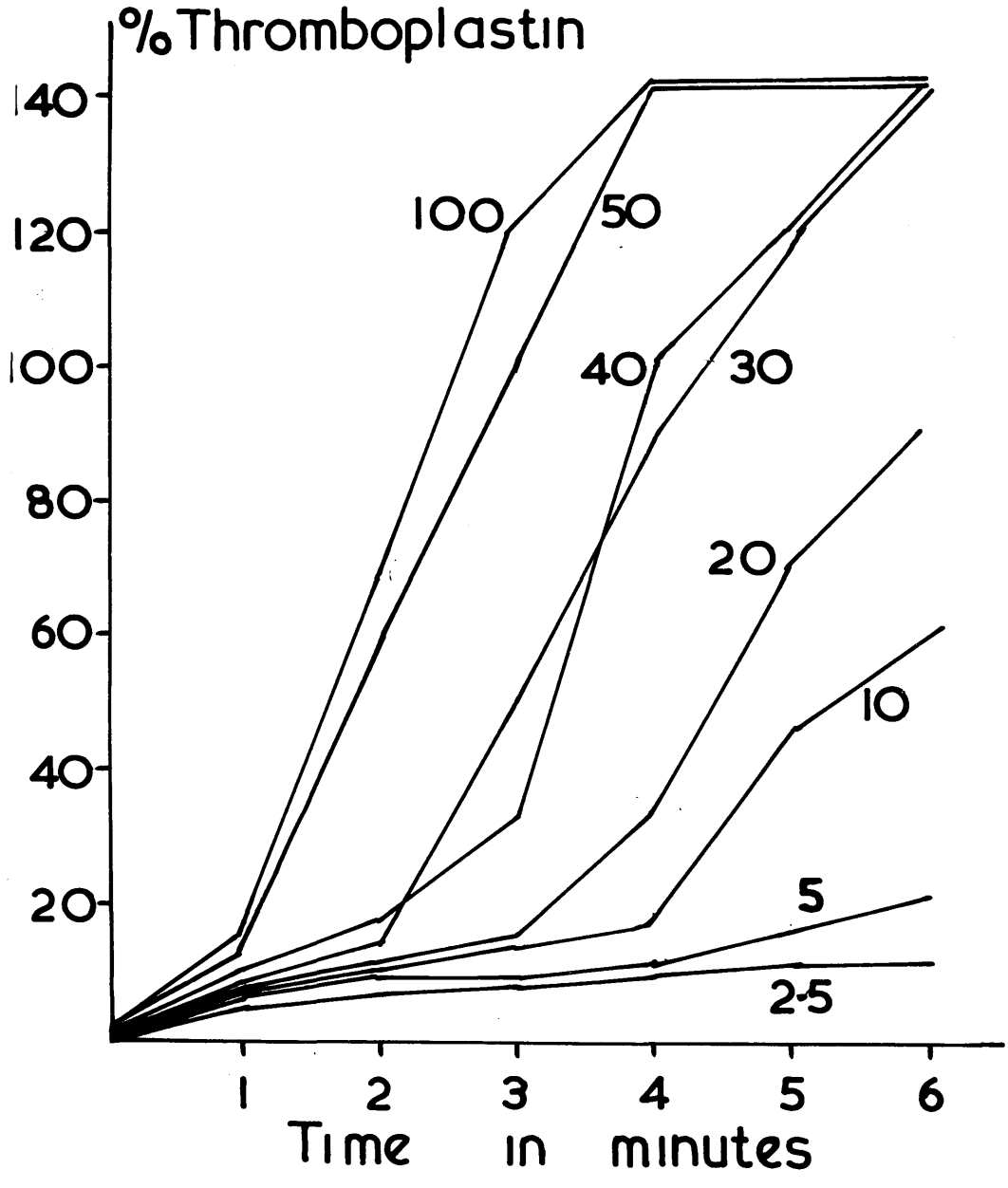

% Thromboplastin
140
120
100
80
60
40
20
100
50
40
30
20
10
5
2·5
1
2
3
4
5
6
Time in minutes

Figure (71)

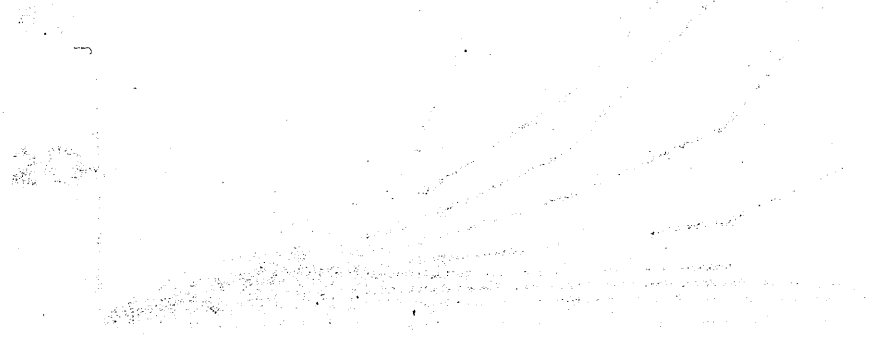


Figure (71)

Thromboplastin generation on saline dilutions of normal serum.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

This figure exemplifies the series of dilution curves required to give a quantitative assay of the defective thromboplastin formation by tromexan serum. With each tromexan serum to be assayed, a series of curves was prepared in the same experiment.

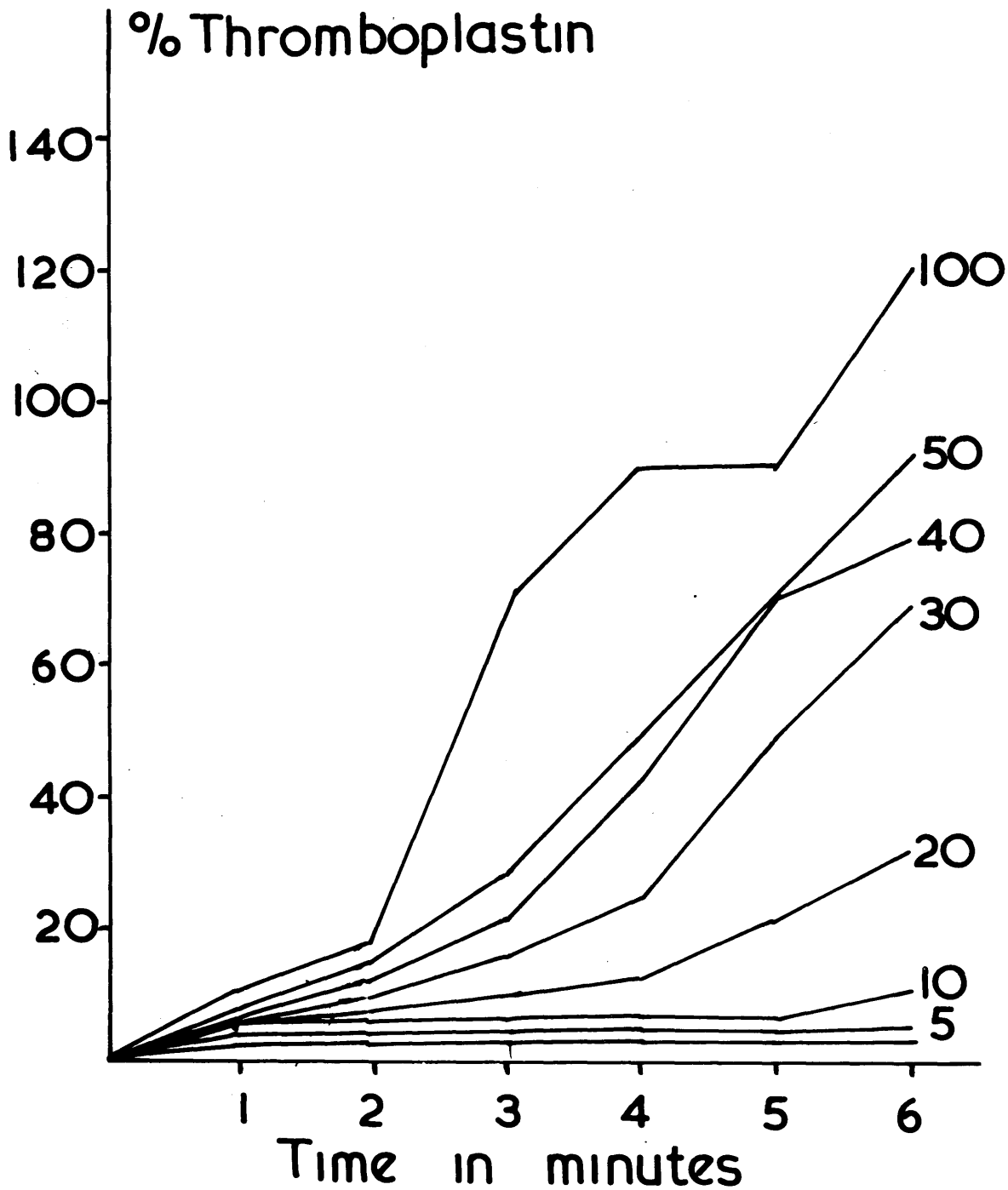

% Thromboplastin
140
120
100
80
60
40
20
100
50
40
30
20
10
5
1
2
3
4
5
6
Time in minutes

Figure (72)

Figure (7)

The effect of 14 days tromexan therapy on serum thromboplastic activity.

Ordinate - percentage - prothrombin, factor VII or serum thromboplastic activity.

Abscissa - time in days.

X——X prothrombin.

●——● serum thromboplastic activity.

O——O factor VII.

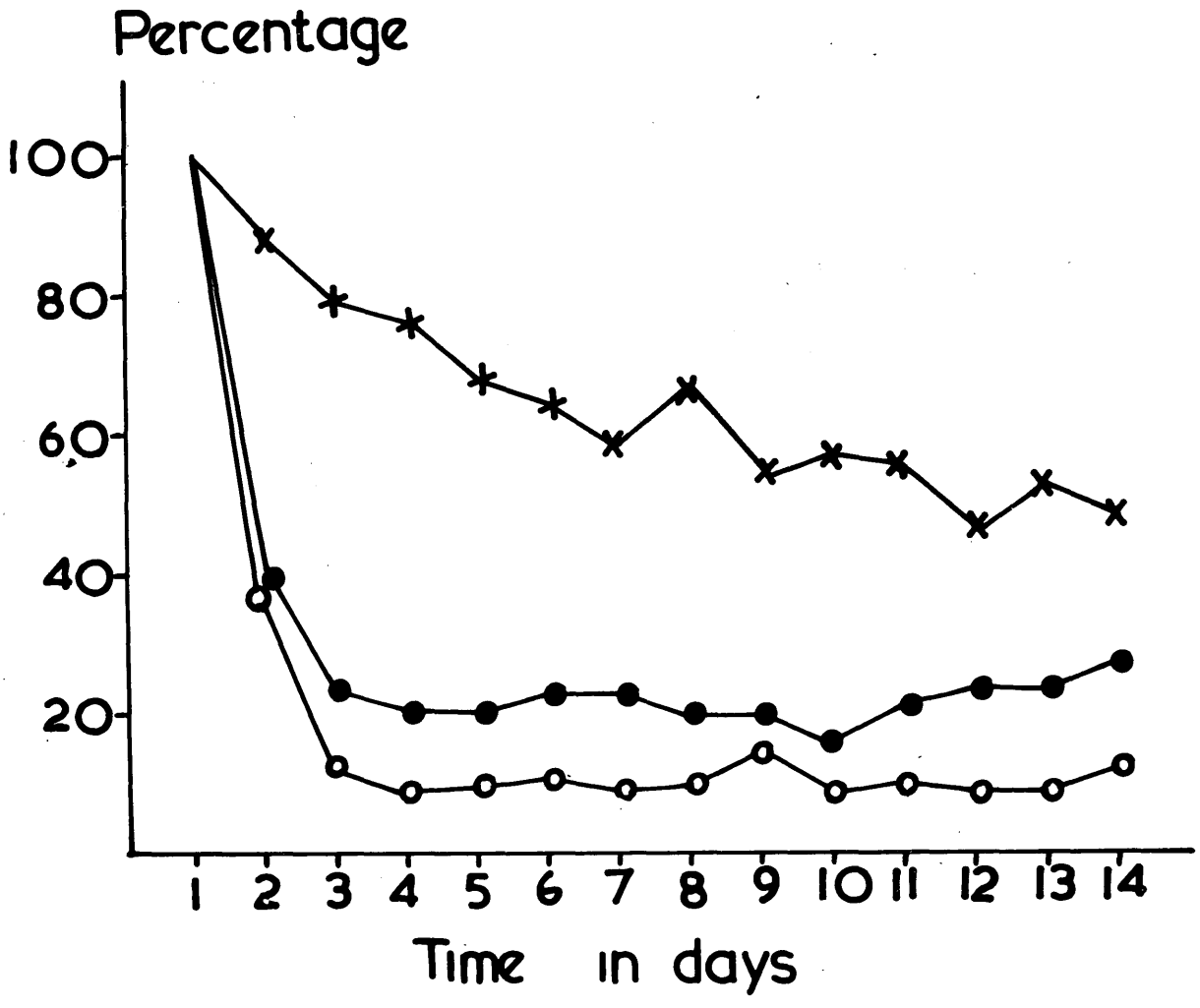

Percentage
100
80
60
40
20
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Time in days

Figure (73)

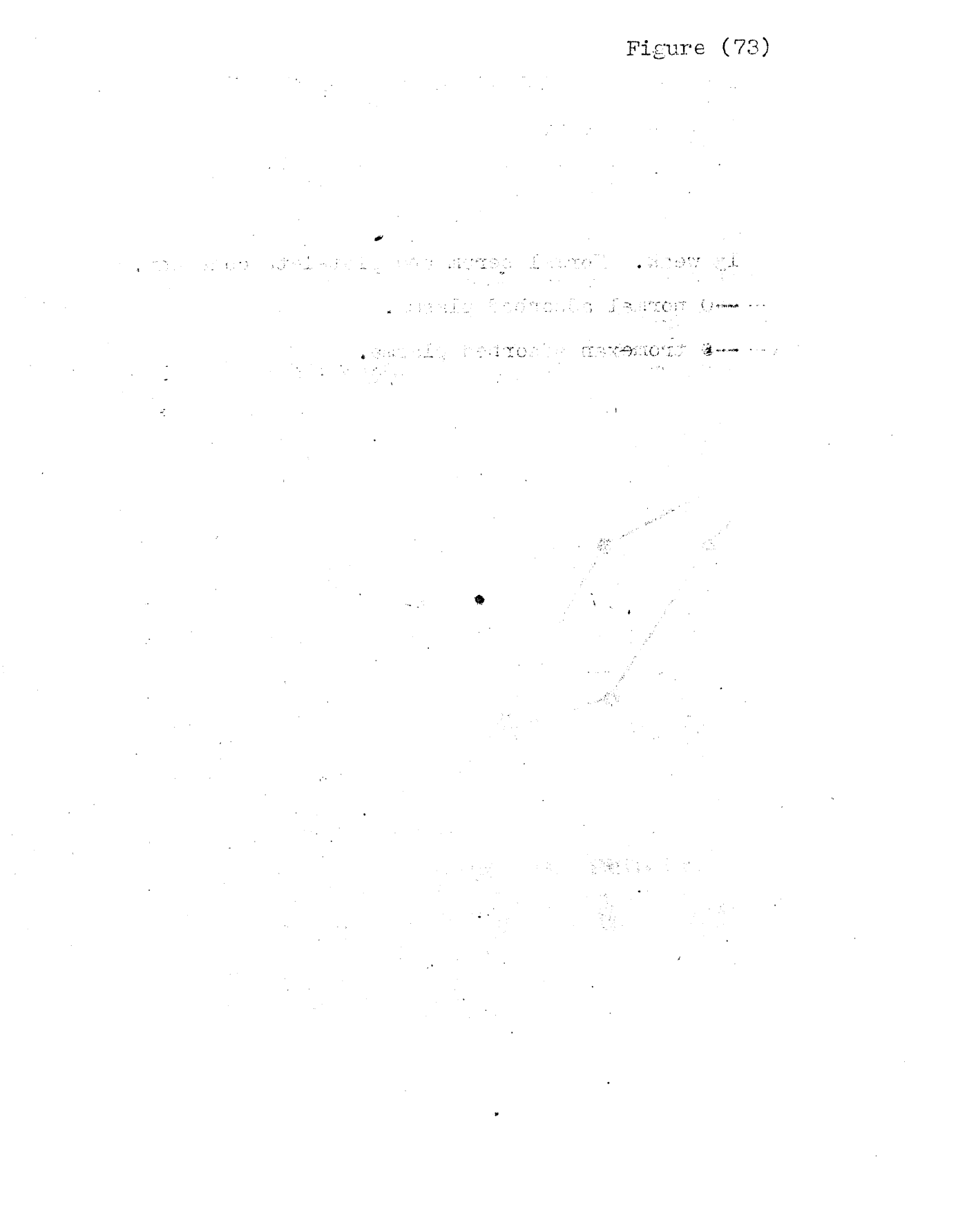


Figure (78)

Antihaemophilic globulin content of tromexan plasma.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation technique - system relatively weak. Normal serum and platelets constant.

O——O normal adsorbed plasma.

●——● tromexan adsorbed plasma.

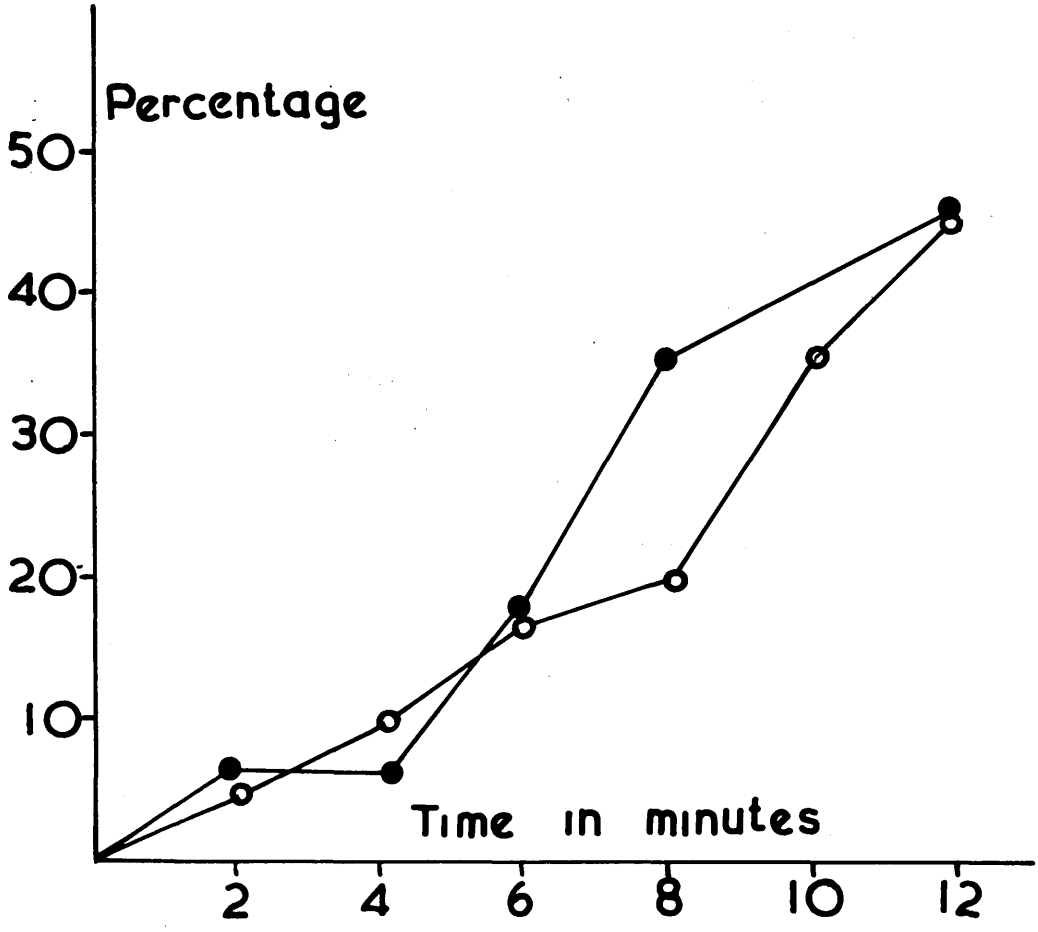

Percentage
50
40
30
20
10
Time in minutes
2
4
6
8
10
12

Figure (74)

Figure (74)

Comparison of Christmas serum and tromexan serum on thromboplastin generation.

- Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation technique with normal adsorbed plasma and platelets constant and serum variable.

●——● normal serum.

X——X Christmas serum.

O——O Tromexan serum.

This figure demonstrates that the serum defects in thromboplastin generation of Christmas disease and tromexan can be of equal severity.

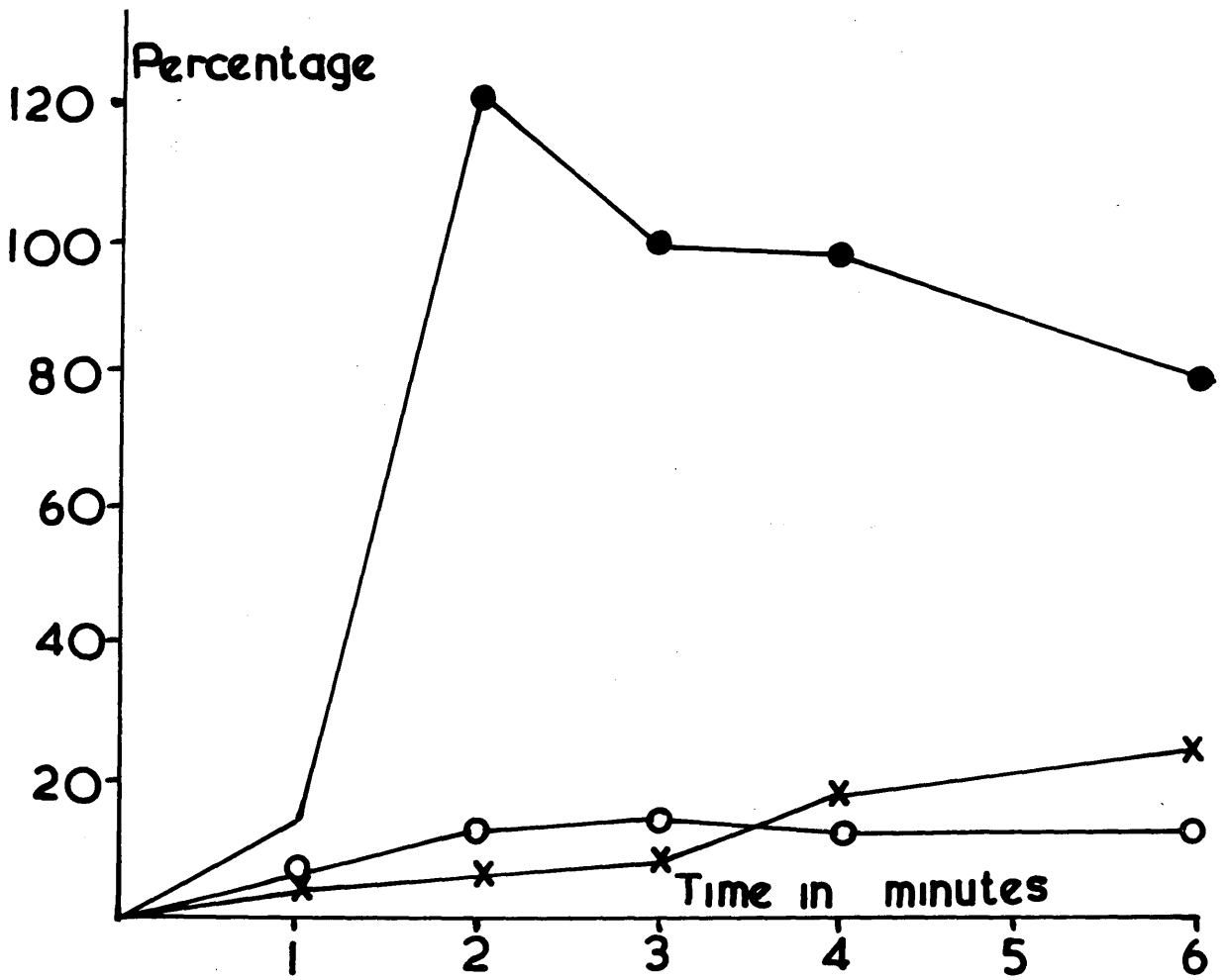

Percentage
120
100
80
60
40
20
Time in minutes
1
2
3
4
5
6

Figure (75)

Figure (75)

Comparison of Christmas serum and tromexan serum on thromboplastin generation.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation technique with normal adsorbed plasma and platelets constant and serum variable. The sera are indicated in the figure - normal, tromexan, tromexan and Christmas.

The experiment represented in this figure again demonstrates that the serum defects in thromboplastin generation of Christmas disease and tromexan can be of equal severity.

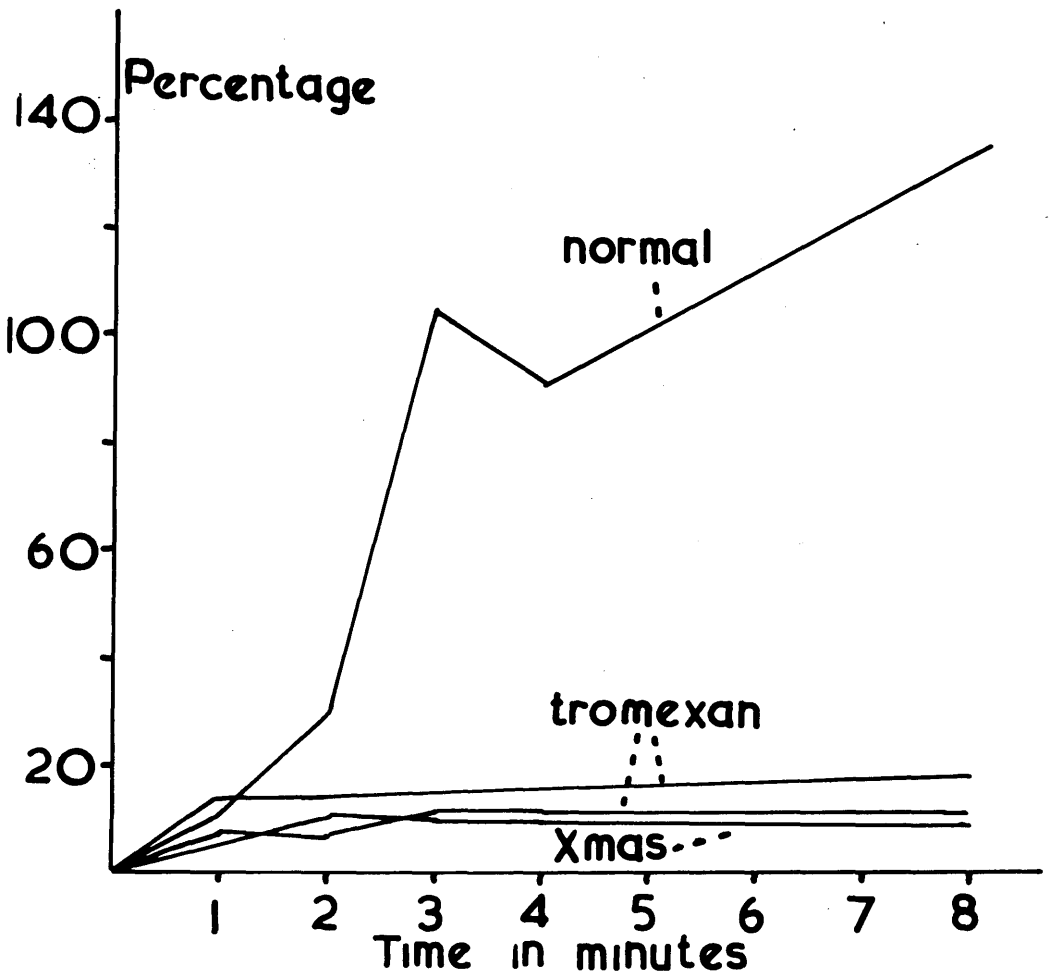
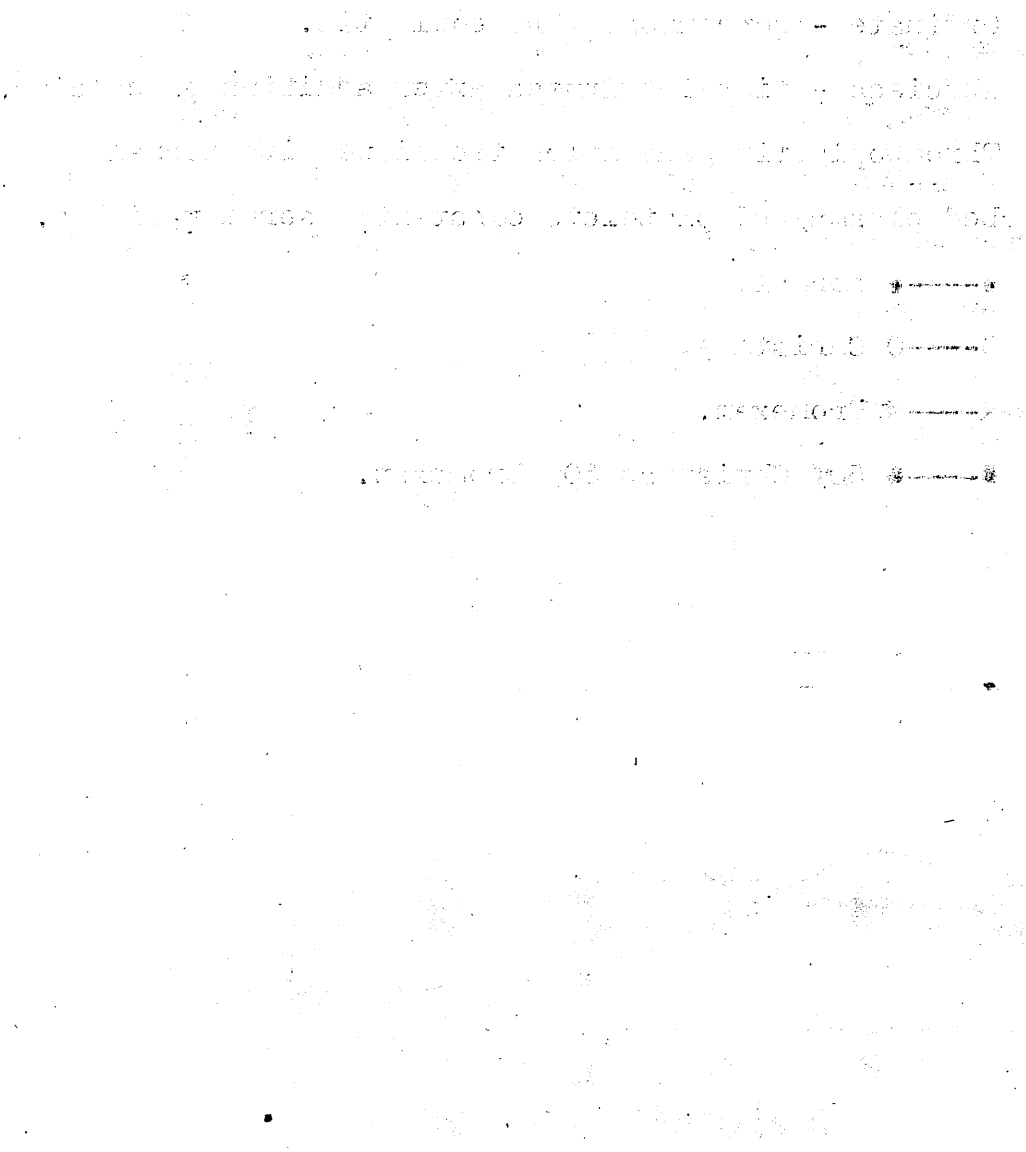

Percentage
140
100
60
20
normal
tromexan
Xmas
1
2
3
4
5
6
7
8
Time in minutes

Figure (76)



Failure of tromexan serum and Christmas serum to be mutually corrective in the thromboplastin generation technique.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation technique with normal adsorbed plasma and platelets constant; serum variable.

●——● normal.

O——O Christmas.

X——X Tromexan.

●——● 50% Christmas 50% Tromexan.

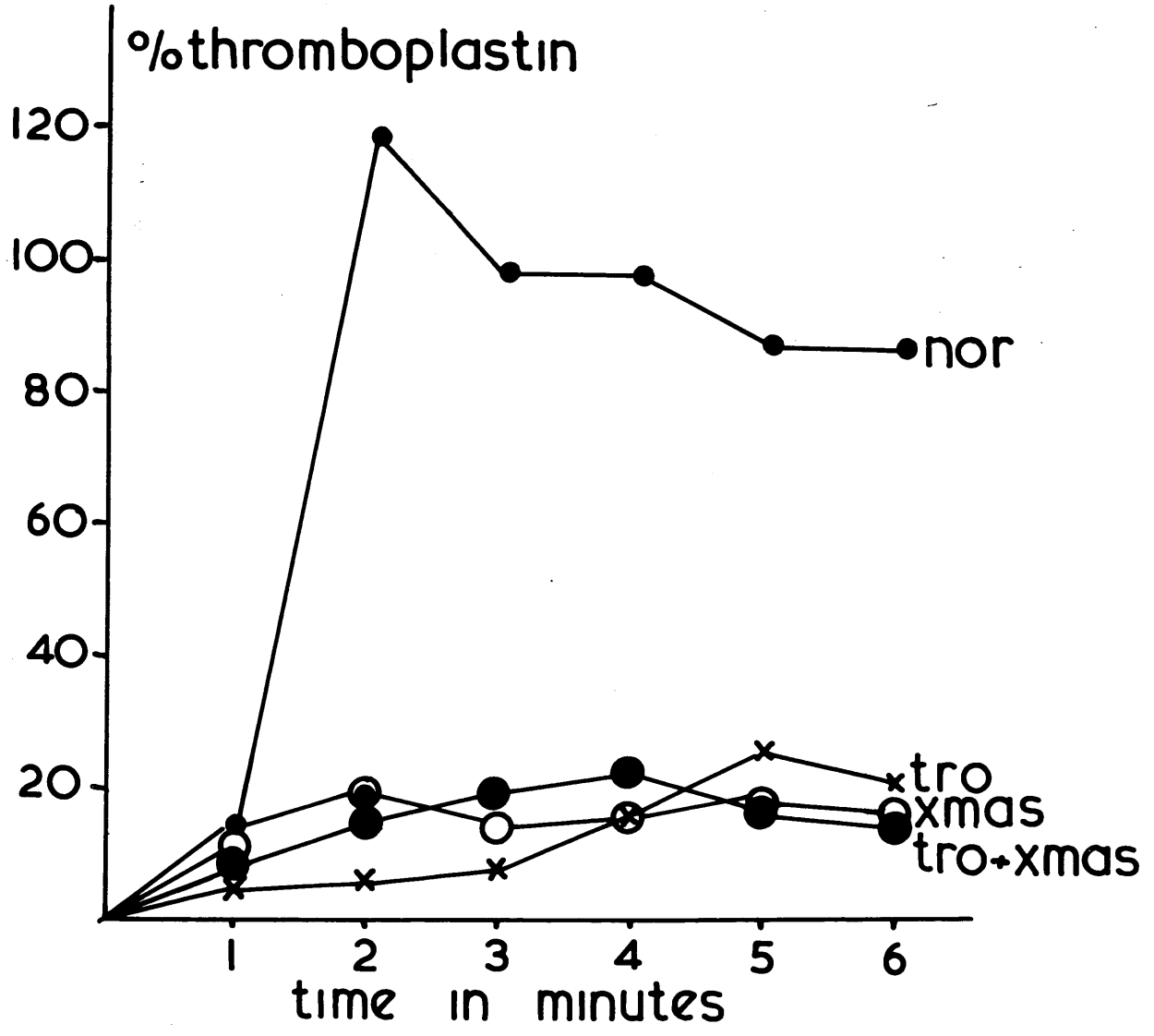

%thromboplastin
120
100
80
60
40
20
nor
tro
xmas
tro+xmas
1
2
3
4
5
6
time in minutes

Figure (77)

Figure (77)

The effect of dindevan plasma on the prothrombin consumption of Christmas plasma.

Ordinate - clotting time of fibrinogen in seconds.

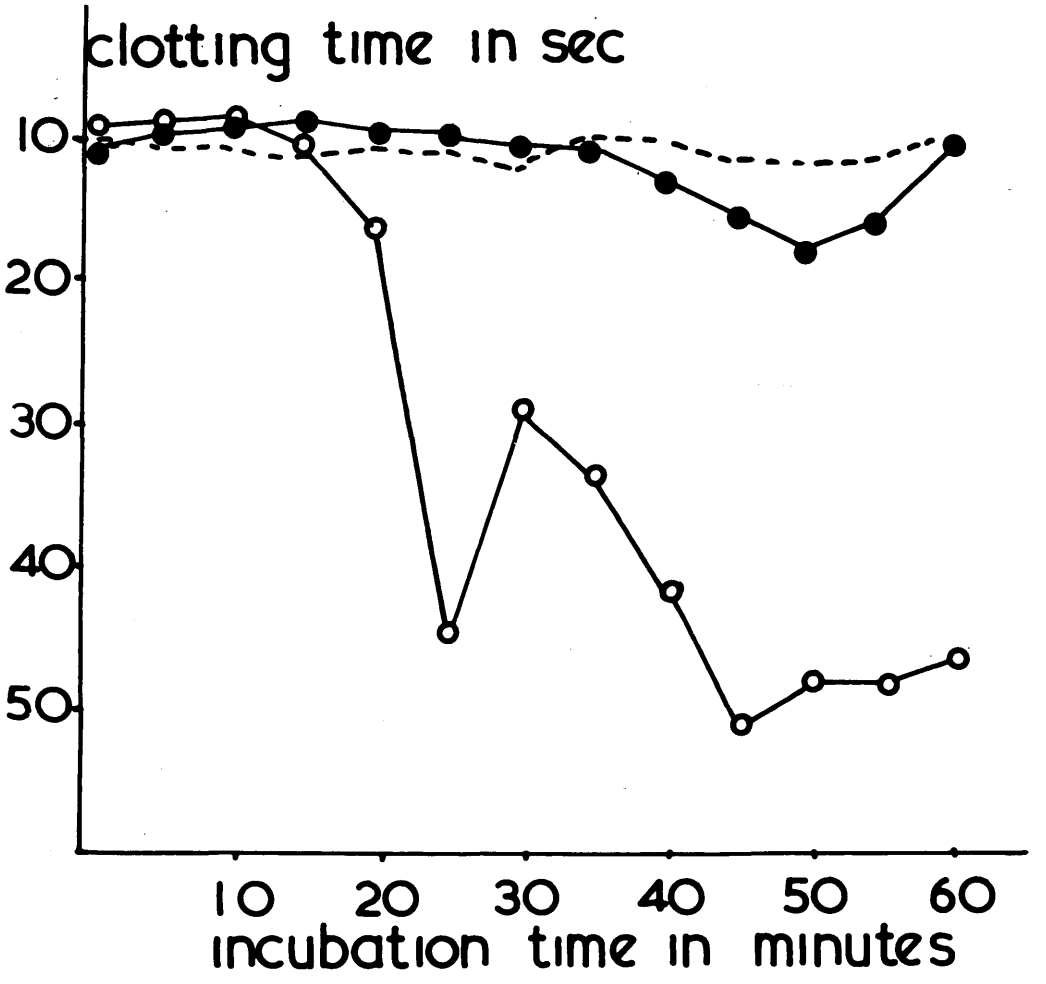
Abscissa - incubation time in minutes after recalcification.

Prothrombin consumption of recalcified plasma.

---- Christmas plasma alone.

●——● Christmas plasma + dindevan plasma.

O——O Christmas plasma + Normal plasma.


clotting time in sec
10
20
30
40
50
10
20
30
40
50
60
incubation time in minutes

to form blood thromboplastin. The details of this technique are described in the appendix (pages 764-5) Figures 70, 71, illustrate two series of curves obtained by dilution of normal serum. The blood of 6 patients treated with tromexan was tested daily for the ability of the serum to form blood thromboplastin and for its factor VII content using brain thromboplastin. (see figures 66,67,68 and 69). From Figure 72 it will be seen that the two properties are reduced together. (appendix pages 765-770).

This does not of course mean that the two tests are necessarily reflecting the same deficiency.

Antihaemophilic Globulin Content of Tromexan Plasma

Three normal plasmas and three tromexan plasmas were equally treated with alumina and the adsorbed plasma used as the source of antihaemophilic globulin in the plasma thromboplastin test. The results are shown in Fig.73 and given in detail in the experimental appendix page 773 . From these results it may be concluded that there is no deficiency of antihaemophilic globulin in the tromexan plasma.

Comparison of coumarin defect with that in Christmas disease.

(a) Relationship between the serum thromboplastin defect in coumarin therapy and that in Christmas disease.

In Christmas disease the serum behaves similarly to tromexan serum in that it is unable to form thromboplastin (see Figs.74 & 75

in the presence of normal alumina-treated plasma and platelets. These two coagulation defects, however, are in other respects very different for in Christmas disease there is a normal one-stage "prothrombin" time and the whole blood clotting time in glass is not infrequently prolonged. When the tromexan serum and the Christmas disease serum are mixed and used in the thromboplastin generation test there is failure to produce satisfactory mutual correction. (Figure 76)

(b) The effect of dindevan plasma on the prothrombin consumption of Christmas plasma.

The details of this experiment are given in the appendix. The prothrombin consumption of Christmas plasma over one hour was studied and the effect observed of additions of normal plasma and dindevan plasma. The results are shown in figure 77 . It will be seen that as compared with the effect of additions of normal plasma, the dindevan plasma was very defective in its ability to correct the prothrombin consumption of Christmas plasma. This is very convincing evidence of a Christmas factor deficiency in coumarin drug therapy. The results of only one experiment are shown and this aspect will be described in greater detail in subsequent publication.

(c) A comparison of the property of normal or Christmas plasma and of normal and Christmas serum to correct the one-stage clotting time of tromexan plasma.

The results of this are given in the appendix - page 78 . The normal and Christmas plasma are equally capable of correcting the defect in the tromexan one-stage clotting time. The Christmas serum is as good as its plasma but is not so good as normal serum in this property. The Christmas serum does not show the enhancement of normal serum over normal plasma.

For example:

Ability of normal and Christmas plasma to correct one-stage test.

Tromexan plasma	0	+ 1/10 normal plasma	+ 1/10 Xmas plasma
	28	21	21

Ability of normal and Christmas sera to correct one-stage test.

Tromexan plasma	0	+ 1/10 normal serum	+ 1/10 Xmas serum
	34	15½	22½

Figure (78)

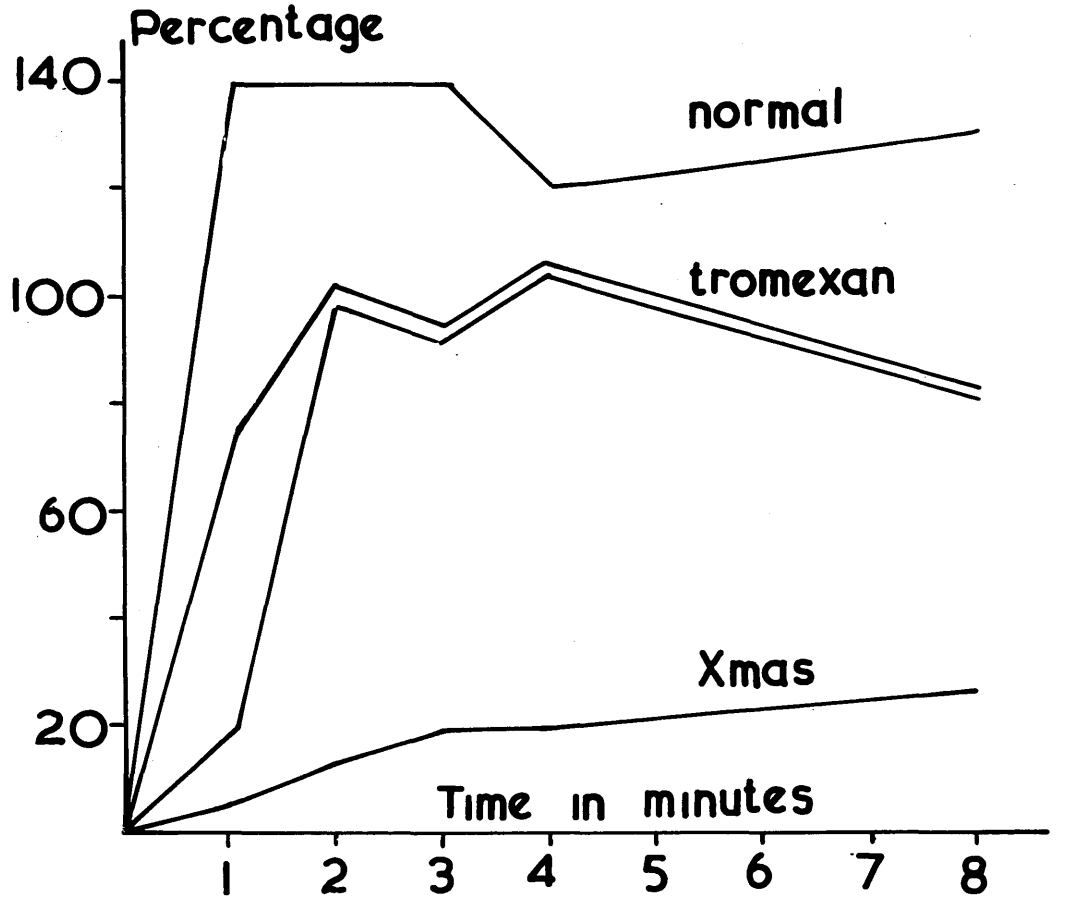
Figure (78)

Thromboplastin generation from Christmas disease plasma with additions of normal, tromexan and Christmas disease serum.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

The graphs are labelled on the figure. There are two results using tromexan sera.


Percentage
140
100
60
20
normal
tromexan
Xmas
Time in minutes
1
2
3
4
5
6
7
8

(d) Separation of Christmas factor from factor VII by heating at 56° C.

It is probable that some measure of separation can be obtained by heating of serum to 56° C. as described by Aggeler et al (1952). The results of an experiment designed to test this are shown in the appendix. The results are not conclusive, but it is suggestive that in undiluted serum factor VII and Christmas factor are both largely destroyed by heating while when diluted, factor VII is more destroyed than is the Christmas factor. The result of heating experiments as a method of separation of coagulation components is unreliable, and no final conclusions are drawn from this observation.

(e) When the thromboplastin generation from Christmas disease plasma is studied with the addition of normal serum, tromexan serum and Christmas disease serum the results are as shown in figure 78 . The tromexan serum is not so effective as the normal serum in correcting the Christmas disease defect. The results of this experiment are in part a manifestation of thrombin as well as thromboplastin production.

In the appendix (page 785) is described the result of a similar experiment from sera collected daily from a patient on tromexan. The progressive impairment of this property

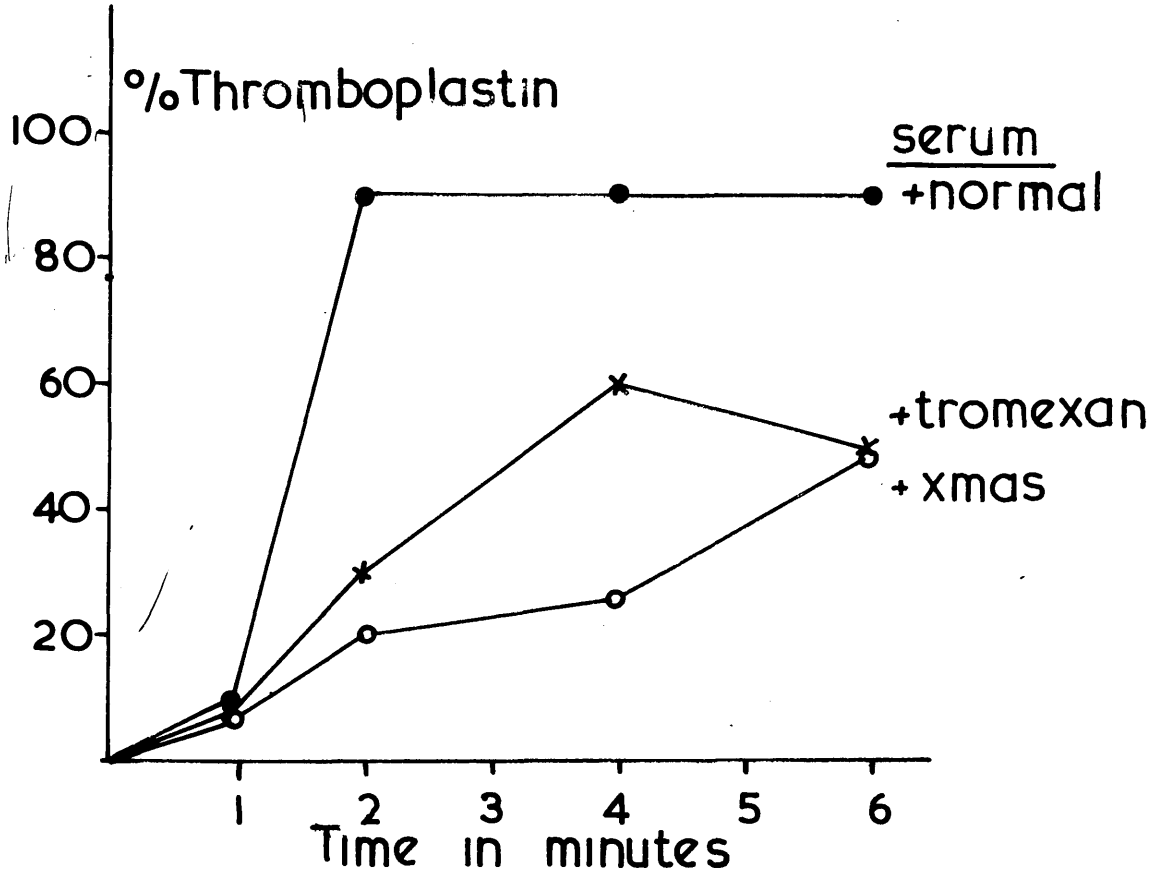
Figure (79)

Thromboplastin generation from Tromexan plasma with additions of normal, tromexan and Christmas disease sera.

Ordinate-percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

The graphs are labelled on the figure.


%Thromboplastin
100
80
60
40
20
serum
+normal
+tromexan
+xmas
1
2
3
4
5
6
Time in minutes

of serum to correct the Christmas defect is demonstrated.

(f) Similar experiments were carried out using tromexan plasma with the addition of Christmas serum, normal serum and tromexan serum. The results are shown in figure 79 . The Christmas disease serum does not produce any correction of the thromboplastin generation from the tromexan plasma - see figure 79 .

The following conclusions can be drawn:-

(a) Christmas disease serum and tromexan serum are both defective in their ability to form blood thromboplastin in the thromboplastin generation technique. There is no mutual correction of these sera in this test.

(b) Dindevan plasma is defective in its ability to correct the prothrombin consumption of Christmas disease plasma.

(c) The Christmas disease serum does not show the enhancement of normal serum over its corresponding plasma in the ability to correct the one-stage clotting time of tromexan plasma.

(d) Separation of Christmas factor from factor VII by the heating of normal serum was not satisfactory.

(e) The thromboplastin generation from Christmas disease plasma is corrected by normal serum but not so effectively by tromexan serum.

(f) The thromboplastin generation from tromexan plasma is corrected by normal serum but not by Christmas disease serum.

Discussion.

These observations indicate that the main defect causing the prolongation of the one-stage clotting time in coumarin therapy is the deficiency of factor VII. The depression of prothrombin is less and it is unlikely that the degree of prothrombin deficiency which occurs in tromexan therapy is of much consequence in interfering with the coagulation system. Factor VII is one of the components required for the completion of tissue thromboplastic activity.

Evidence has also been produced of interference by these drugs with blood thromboplastic activity. The serum from patients under the effect of these drugs is defective in its ability to form blood thromboplastin. When followed daily this serum thromboplastic activity follows the pattern of factor VII deficiency. Hicks (1955), however, has produced evidence indicating that factor VII is not required in blood thromboplastin formation. Using a patient with a constitutionally determined factor VII deficiency, he showed that there was normal blood thromboplastin formation using the serum. It was likely therefore that the serum thromboplastic defect in coumarin therapy was not due to the deficiency of factor VII. The experiments described here suggest that this serum defect is due to a Christmas factor deficiency. Christmas disease serum and tromexan serum

are not mutually corrective in the thromboplastin generation technique. Dindevan plasma is poor in its ability to correct the defective prothrombin consumption of Christmas disease plasma.

It is likely, therefore, that these drugs interfere with extrinsic thromboplastin by virtue of depression of factor VII and with the intrinsic mechanism by depression of the Christmas factor. There is also some deficiency of prothrombin.

It is possible that these coumarin drugs have actions other than those demonstrable by the available in vitro techniques. Jewell, Pilkington and Robinson (1954) have shown that the administration of tromexan to rabbits is even more effective than heparin in preventing chemical thrombosis. There is evidence that adequate therapy with coumarin compounds is beneficial in promoting recanalization of arteries and veins occluded by thrombosis produced experimentally in rabbits. (Kubik & Wright 1950).

Quite apart also from the 'in vitro' evidence of interference with coagulation there is the ever growing support for the belief that the coumarin drugs have therapeutic value in the management of coronary thrombosis. Many series of cases have been reported where these drugs have been used. Some of these are open to criticism. Others however are

carefully controlled on alternate case basis and there can be little doubt that the use of these drugs has been of value. (Biggs and Macfarlane 1953, Tulloch and Gilchrist 1950, Tulloch and Gilchrist 1951, Wright, Marple and Beck 1948).

SUMMARY

(1) The prolongation of the one-stage clotting time of coumarin plasma in response to brain thromboplastin has been studied. Comparison of coumarin plasma with that from a patient with idiopathic prothrombin deficiency indicates that marked prothrombin deficiency, in the presence of normal amounts of other coagulation components, produces only very slight prolongation of the one-stage clotting time. Plasma from the patient with idiopathic prothrombin deficiency was capable of correcting the prolonged one-stage clotting time of coumarin plasma as efficiently as did normal plasma.

(2) Normal serum, which contains no prothrombin, was often able to restore the one-stage clotting time of tromexan plasma to normal. This action of normal serum is called its factor VII property.

(3) From (1) and (2) it was confirmed that tromexan plasma is deficient in factor VII. It remained to assess the prothrombin content. 324 specimens were examined by the area method and the globulin fraction method.

		Mean prothrombin content
Area method	(140)	69%
Globulin fraction method	(184)	64%

(4) Factor V was found to be normal.

(5) The progress of factor VII and prothrombin under the influence of continued tromexan administration was studied throughout the first fourteen days of therapy. In view of the high prothrombin content the one-stage test expressed as a percentage from a dilution curve was used as a measure of factor VII. The factor VII falls at the start of therapy to a low level at which it remains. The prothrombin falls much more gradually and to a less extent.

(6) The response of tromexan plasma to one-stage techniques using as thromboplastin brain-serum mixtures, Russell's viper venom and lecithin, and plasma thromboplastin, has been studied. It is probable that these methods reflect the prothrombin content but this cannot be assayed from a dilution curve. All the methods when read from a dilution curve give results which are too low. The Russell's viper venom method gives results nearest to the true prothrombin reading.

(7) Interference with blood thromboplastin

The evidence for this is:

(a) prolongation of whole blood clotting time in silicone.
(b) prolongation of calcium clotting time.
(c) delay in prothrombin consumption.
(d) failure of coumarin serum to replace normal serum in the thromboplastin generation technique. When this failure to form blood thromboplastin is assayed quantitatively, the effect of therapy is parallel to the depression of factor VII.
(e) Christmas disease serum and tromexan serum are not mutually corrective in the thromboplastin generation test.
(f) The prothrombin consumption of Christmas disease plasma is not significantly corrected by additions of dindevan plasma.

It is likely that the interference with blood thromboplastin produced by this group of drugs is a consequence of Christmas factor deficiency.

REFERENCES

Aggeler, P.M., White, S.G., Glendening, M.B., Page, E.W., Leake, T.B. and Bates, G. (1952). Plasma thromboplastin component (P.T.C.) deficiency: a new disease resembling haemophilia. Proc.Soc.exp.Biol. & Med. 79, 692.

Alexander, B., Goldstein, R. and Landwehr, G.(1951). The labile factor of prothrombin conversion; its consumption under normal and pathological conditions affecting blood coagulation. J. clin. Invest.30,252.

Biggs, R. and Douglas, A.S.(1953). The measurement of prothrombin in plasma. J. clin. Path. 6, 15.

Biggs, R. and Macfarlane, R.G. (1949). Estimation of prothrombin in dicoumarin therapy. J.clin.Path.2,33.

Biggs, R. and Macfarlane, R.G. (1953). Human blood coagulation and its disorders. Blackwell Scientific Publications, Oxford.

Burke, G.E. and Wright, I.S. (1951). Tromexan - 3, 3' - Carboxy methylene bis (4 - hydroxy coumarin) ethyl ester. Experimental and clinical properties. Circulation 3, 164.

Burt, C.C., Wright, H.P. and Kubik, M. (1949). Clinical tests of a new coumarin substance. A report to the Medical Research Council. Brit. med. J. 2,1250.

Douglas, A.S., and Biggs, R. (1953). The consumption of some components involved in physiological blood coagulation. Glasg. med. J. 34, 329.

Fullerton, H. (1940). Estimation of prothrombin; simplified method. Lancet, 2, 195.

Hicks, N.D. (1955). A coagulation disorder due to a factor VII-like defect. Med. J. Aust. 331.

Hunter, R.B. and Shepherd, D.M.(1955). Chemistry of coumarin anticoagulant drugs. Brit. med. Bull.11,56.

Jewell, P., Pilkington, T. and Robinson, B. (1954). Heparin and ethyl biscoumacetate in prevention of experimental venous thrombosis. Brit. med. J. 1, 1013.

Kaulla, K.N. von and Pulver, R. (1948). Tier experimentalle untersuchungen, mit dem neuen antithromboticum tromexan. Schweiz, med. Wschr. 78, 806.

Koller, F., Loeliger, A. and Duckert, F.(1951). Experiments on a new clotting factor (Factor VII). Acta haemat, 6, 1.

Kubik, M. and Wright, H.P. (1950). Coumarin treatment of experimental thrombosis. Proc. internat Soc. Haemat.477.

Lamb, G.(1903). On the actions of venoms of the cobra (Naja Tripudians) and of the Daboia (Daboia russellii), on the red blood corpuscles and on the blood plasma. Sci. Mem. med. San. Depts. India No.4.

Lempert, J.H. (1948). "Dicumarol". Letter to the editor. Brit. med. J. 1, 125.

Macfarlane, R.G.(1938). "The normal haemostatic mechanism and its failure in the haemorrhagic states". Thesis for the degree of Doctor of Medicine, University of London. (see Biggs and Macfarlane 1953.)

Macfarlane, R.G. and Barnett, B. (1934). The haemostatic possibilities of snake venom. Lancet 2, 985.

Macfarlane, R.G., Trevan, J.W. and Attwood, A.M.P. (1941). Participation of a fat soluble substance in the coagulation of the blood. J. Physiol. 99, 79.

Mawson, C.A. (1949). The use of Russell viper venom and lecithin as thromboplastin in the estimation of prothrombin. J. Lab. clin. Med. 34, 458.

Merskey, C. (1951). The consumption of prothrombin during coagulation; the defect in haemophilia and thrombocytopenic purpura. J. clin. Path. 3, 130.

Olwin, J.H. (1949). The one-stage and two-stage methods in the control of dicumarol therapy, with remarks on ac-globulin. J. Lab. clin. Med. 34, 806.

Owren, P.A. and Aas, K. (1951). Scand. The control of dicumarol therapy and the quantitative determination of prothrombin and pro-convertion. J. clin.lab. Invest. 3, 201.

Page, R.C. and de Beer, E.J. (1943). Prothrombin studies using Russell viper venom. Amer.J. med. Sci.206,336.

Page, R.C.,de Beer, E.J. and Orr, M.L. (1941).
(a) Prothrombin studies using Russell Viper Venom. J. Lab. clin. Med. 27,197.
(b) Prothrombin studies using Russell Viper Venom. J. Lab. clin. Med. 27, 830.

Reinis, Z. and Kubik, M. (1948). Kinische erfahrungen mit einem neuen praparat der cumarinreihe. Schweiz. med. Wschr. 78, 785.

Shapiro, S., Sherwin,B. and Gardener, H. (1942). Post-operative thrombo-embolization. Ann. Surg. 116,175).

Stirling, M. and Hunter, R.B. (1951). Pharmacology of Bis 3-3'- (4 oxy coumarinyl) ethyl acetate (Tromexan) Lancet 2, 611.

Trevan, J.W., and Macfarlane, R.G. (1936). Med. Res.Coun. Ann. Rep. p. 143.

Tulloch, J.A. and Gilchrist, A.R. (1950). Anticoagulants in treatment of coronary thrombosis. Brit. med. J. 2, 965.

Tulloch, J.A. and Gilchrist, A.R. (1951). Tromexan in the treatment of coronary thrombosis. Amer.Heart J. 42, 864.

Witts, L.J. (1942). Disturbances in the coagulation of the blood. Glasg. med. J. 137, 57.

Witts, L.J. and Hobson, F.C.G. (1942). Estimation of prothrombin with venom and lecithin. Brit. med. J. 2, 862.

Wright, I.S., Marple, C.D. and Beck, D.F. (1948). Report of the committee for the evaluation of anticoagulants in the treatment of coronary thrombosis with myocardial infarction.

CHAPTER 8

THE ACTION OF HEPARIN

CONTENTS.

CHAPTER 8

INTERFERENCE WITH THE THROMBIN-FIBRINOGEN REACTION

There is good experimental evidence that heparin, while acting together with a co-factor in the albumin fraction of plasma interferes with the thrombin-fibrinogen reaction (Mellanby 1934, Quick 1938). Plasma was collected in citrate before and immediately after the intravenous administration of a single dose of 10,000 units of heparin. Dilutions of thrombin were prepared and added in 0.1 ml. amounts to 0.1 ml. of normal plasma and the clotting time recorded.

Thrombin concentration.	1/1	1/2	1/4	1/8	1/16	1/32
Plasma before heparin	11	20	38	95	3'+	3'+
Plasma after heparin	3'+	3'+	3'+	3'+	3'+	3'+

In the presence of heparin there was considerable prolongation of the clotting time. Since these plasmas were clotting as a consequence of added thrombin and not in response to the conversion of their own prothrombin this observation can be accepted as confirmation of interference with the thrombin-fibrinogen reaction.

It has also been suggested that heparin interferes with the interactions resulting in thrombin formation (Howell & Holt 1918, Brinkhous et al 1939). Investigations on the

action of heparin in preventing thrombin formation are difficult on account of the interference by the heparin with the thrombin-fibrinogen reaction.

Addition of heparin to the thromboplastin generating system.

It can be shown (appendix, page 794) that, in a mixture containing all the requirements for the formation of blood thromboplastin, there is prevention of formation in the presence of low concentrations of heparin. In the presence of heparin, at a concentration as low as 1/30 unit/ml. in the mixture, the formation of heparin is markedly depressed (see Figure 80).

Destruction of formed thromboplastin.

In figure 81 is shown the effect on formed thromboplastin of the addition of heparin. The progress of destruction of thrombin can be followed. The heparin is considerably less powerful in destroying formed thromboplastin than in preventing its formation.

Removal of heparin by adsorption on alumina.

When specimens of citrated plasma and uncitrated blood are collected from patients on therapeutic doses of heparin the adsorbed plasma reacts normally in the thromboplastin generating system whereas there is an abnormal reaction

Figure (80)

Figure (20)

Thromboplastin generation in the presence of various concentrations of heparin.

Abscissa - percentage thromboplastin.

Ordinate - time in minutes after addition of calcium.

The figures opposite the graphs show the concentration of heparin in the incubation mixtures.

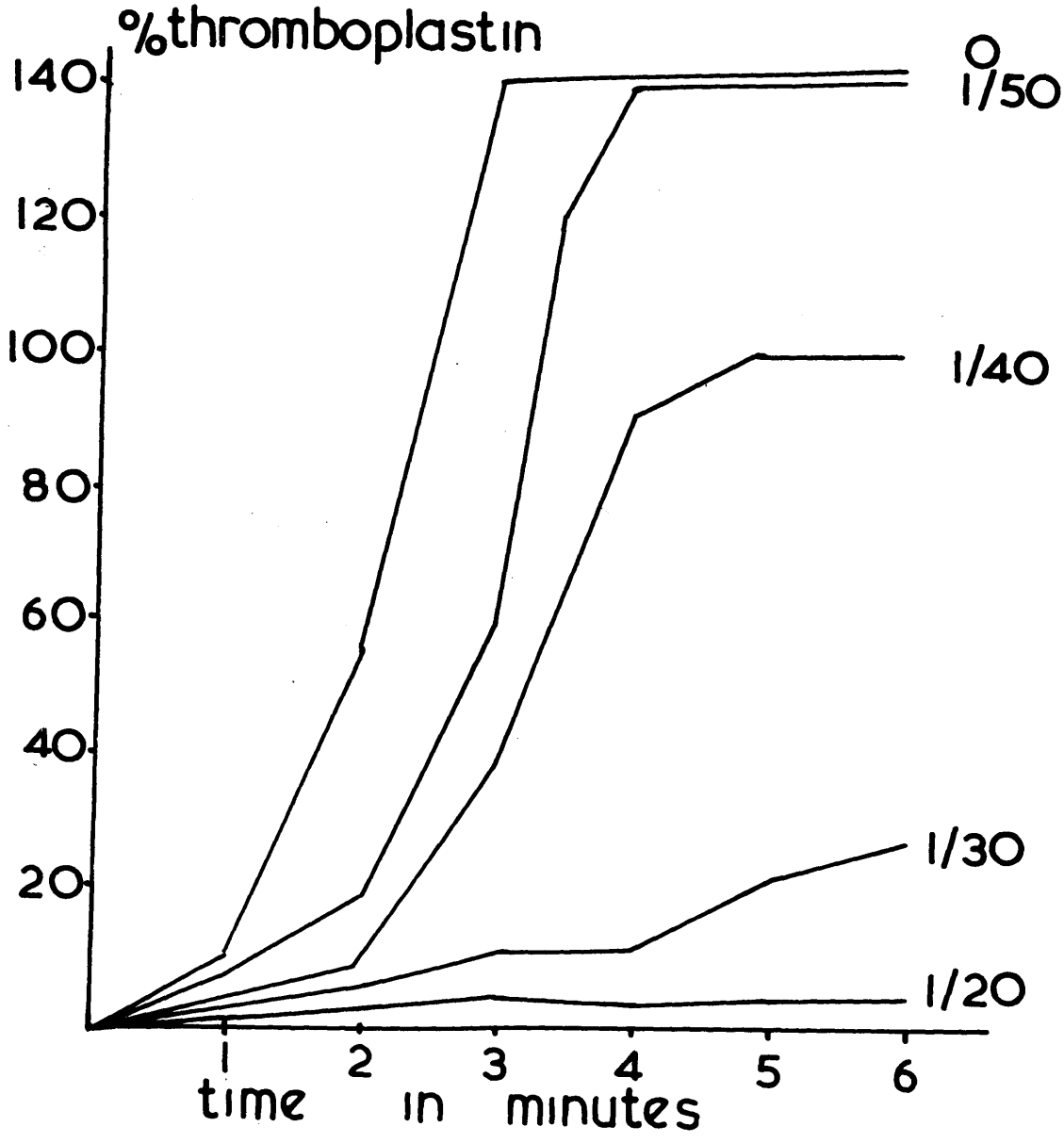

%thromboplastin
140
120
100
80
60
40
20
0
1/50
1/40
1/30
1/20
1
2
3
4
5
6
time in minutes

Figure (81)

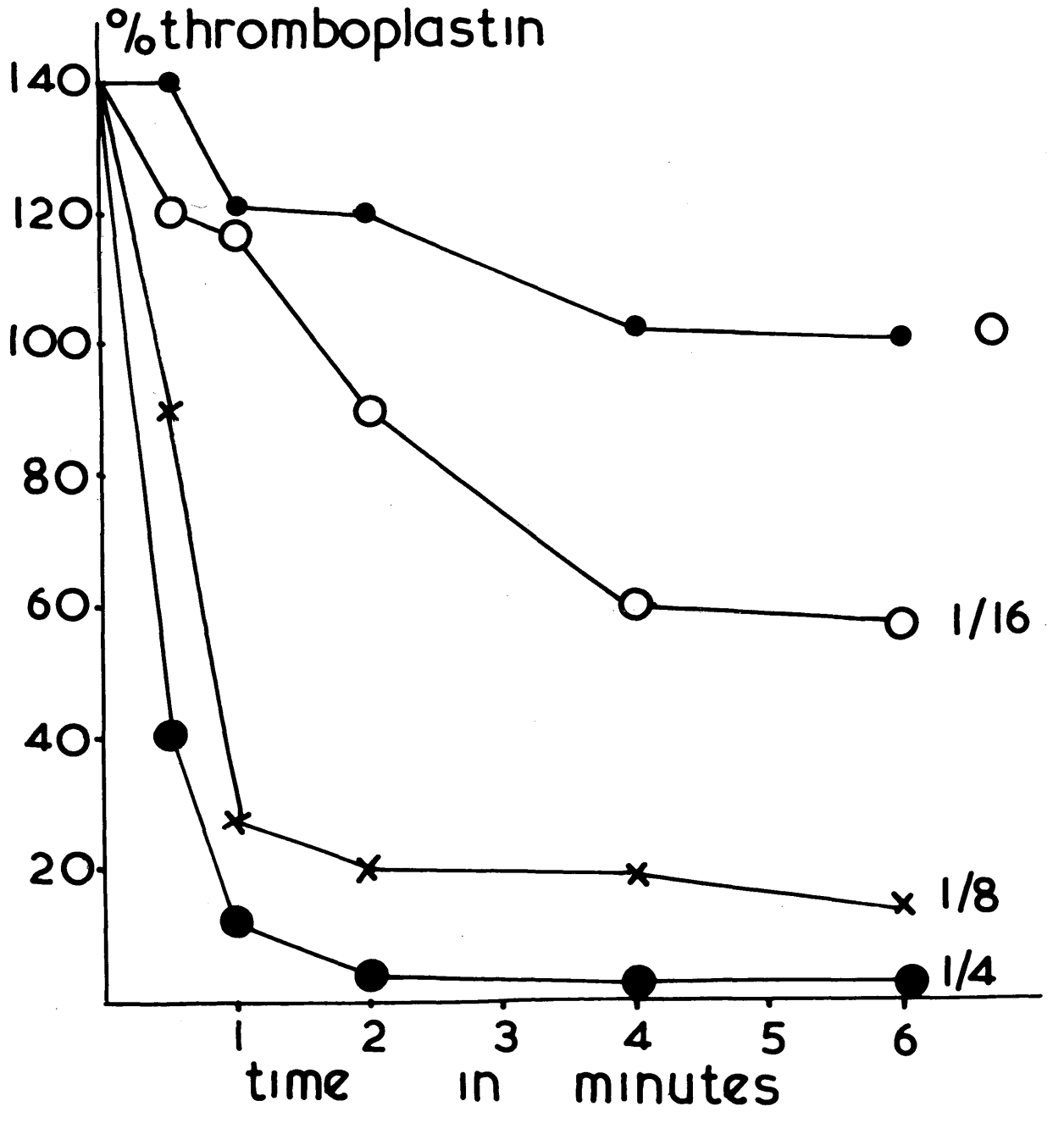
Figure (71)

Destruction of formed thromboplastin after the addition of heparin.

Abscissa - percentage thromboplastin.

Ordinate - time in minutes after addition of calcium.

The figures opposite the graphs show the concentration of heparin in the incubation mixture.


% thromboplastin
140
120
100
80
60
40
20
1/16
1/8
1/4
1
2
3
4
5
6
time in minutes

when the serum is tested (such an experiment is given in detail on page 793 of the appendix). It may be concluded from this that heparin is removed by the adsorbing agent.

It was described above how the presence of heparin in a thromboplastin generating system apparently inhibits the formation of thromboplastin. It could be argued that the results are in part or in total merely a reflection of the heparin interfering with the thrombin-fibrinogen reaction in the test substrate.

The investigations now to be described are concerned with the earlier stages of blood coagulation when subjected to the influence of heparin, using techniques where the heparin does not interfere with the final indicator system. Douglas and Biggs (1953) have demonstrated that during blood coagulation under the influence of its own thromboplastin factor V_1 antihaemophilic globulin and prothrombin are used up in the reaction. It is assumed that this consumption of factor V and antihaemophilic globulin is due to their utilization in blood thromboplastin formation. Prothrombin consumption is the outcome of blood thromboplastin formation and the resulting conversion to thrombin.

Collection of specimens: The investigation was carried out on patients starting heparin therapy. Needles and syringes of identical size were used throughout. By venepuncture using

a wide bore needle (S.W.G. 18), 30 ml. of blood were collected into a large syringe care being taken to avoid frothing. This syringe was detached, the needle being left in situ and 10,000 units of heparin contained in another syringe, injected intravenously. Five minutes after the injection of heparin an identical specimen was collected by separate venepuncture from the other arm. The specimens of blood collected before and immediately after giving heparin were treated as follows. A mixture was made immediately of 4.5 ml. of blood with 0.5 ml. of 3.8% sodium citrate and further 4.5 ml. volumes of blood delivered into 4 identical graduated centrifuge tubes. These tubes were placed in a water bath at 37° C. and at intervals of 15 minutes after collection 0.5 ml. of 3.8% sodium citrate added and the contents of the tube mixed with a wooden applicator stick. In this way the process of clotting was arrested at fifteen minute intervals after withdrawal of the blood. The tubes were left in the water bath for an hour to allow for the neutralization of any thrombin formed. After this the specimens were tested to determine the amounts of prothrombin, factor V and antihaemophilic globulin present. In this way the pattern of utilization of these factors before and after the administration of heparin could be studied. Three observations were made on each of these coagulation components.

Measurement of prothrombin.

The method used is described in the appendix in the technical section. This is the globulin fraction technique. It is dependent on the separation of prothrombin from antithrombin and its activation thereafter by brain thromboplastin and calcium. On evidence to be described below, the precipitate containing prothrombin was found to be freed not only from antithrombin, but also from heparin activity. There was, in consequence, no evidence of interference by heparin with the thrombin-fibrinogen reaction.

Measurement of factor V.

The method used was as mentioned earlier in this thesis and is described in detail in the appendix. The only difficulty in applying this technique to the present problem was the removal of the heparin from the appropriate test specimens and the prevention of its interference with the thrombin-fibrinogen reaction. It was found that treatment with alumina, which was employed in the preparation of the factor V from the specimens, removed the heparin by adsorption. This adsorption of heparin on alumina confirms the observations made independently by MacMillan and Brown (1954) - see also page 793 of the appendix.

Figure (82)

Figure (12)

Prothrombin consumption before and after the intravenous administration of 10,000 units of heparin.

Abscissa - percentage prothrombin.

Ordinate - time in minutes after withdrawal of blood.

●——● Prothrombin consumption before the administration of heparin.

O——O Prothrombin consumption after the administration of heparin.

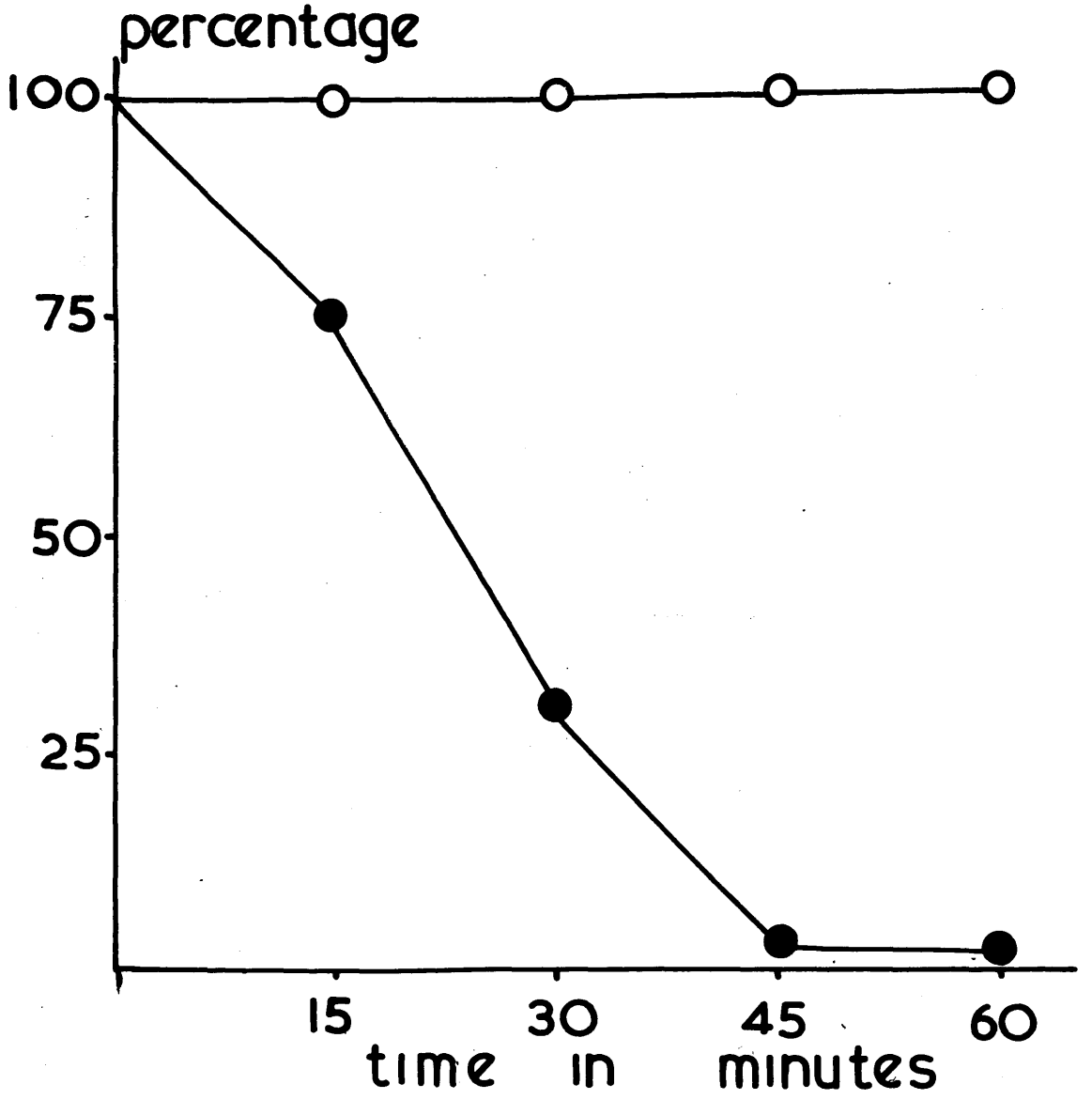

percentage
100
75
50
25
15
30
45
60
time in minutes

Figure (83)

Figure (28)

Factor V consumption before and after the intravenous administration of 10,000 units of heparin.

Abscissa - percentage factor V.

Ordinate - time in minutes after withdrawal of blood.

●——● Factor V consumption before the administration of heparin.

O——O Factor V consumption after the administration of heparin.

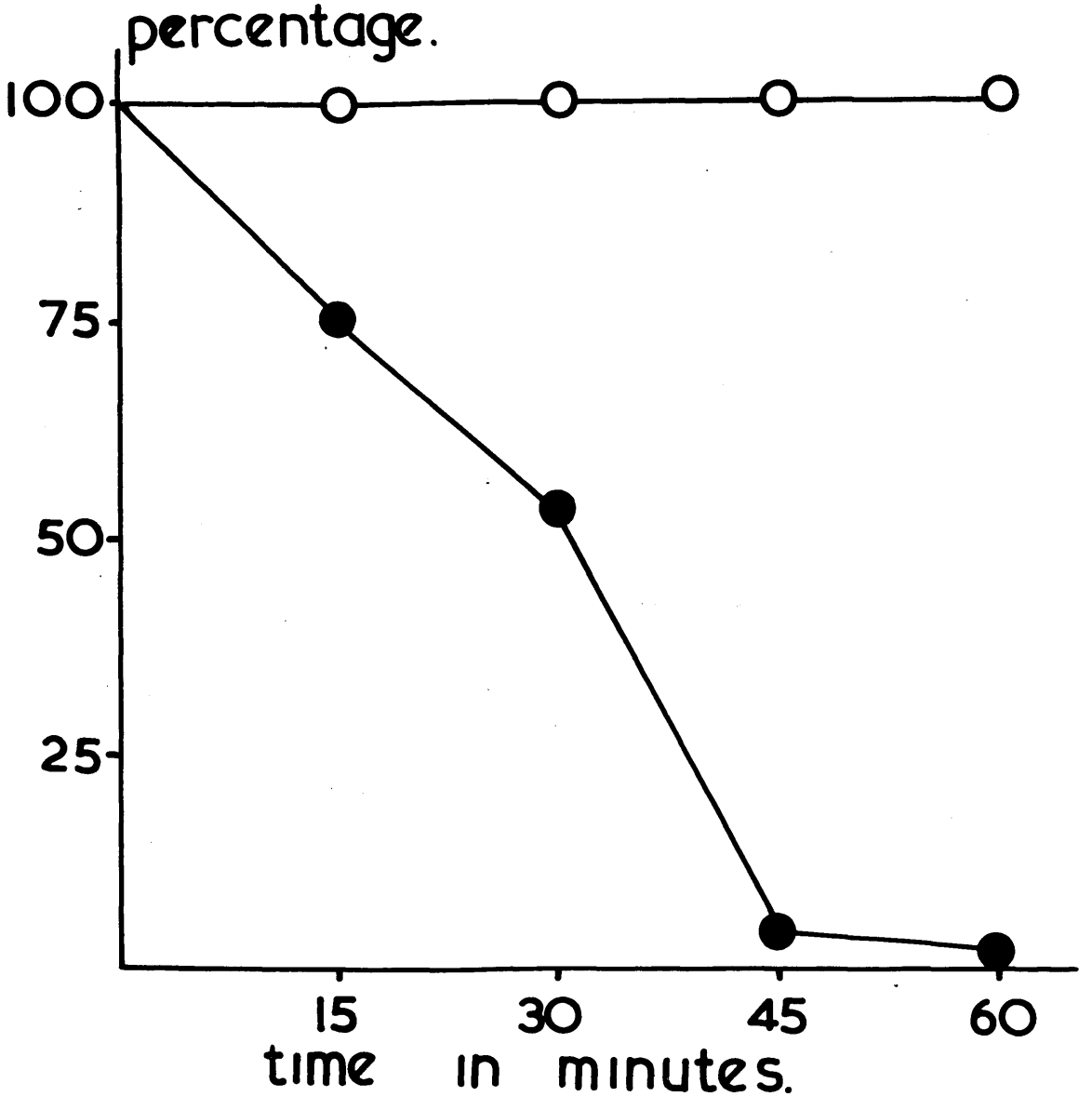

percentage.
100
75
50
25
15
30
45
60
time in minutes.

Figure (84)

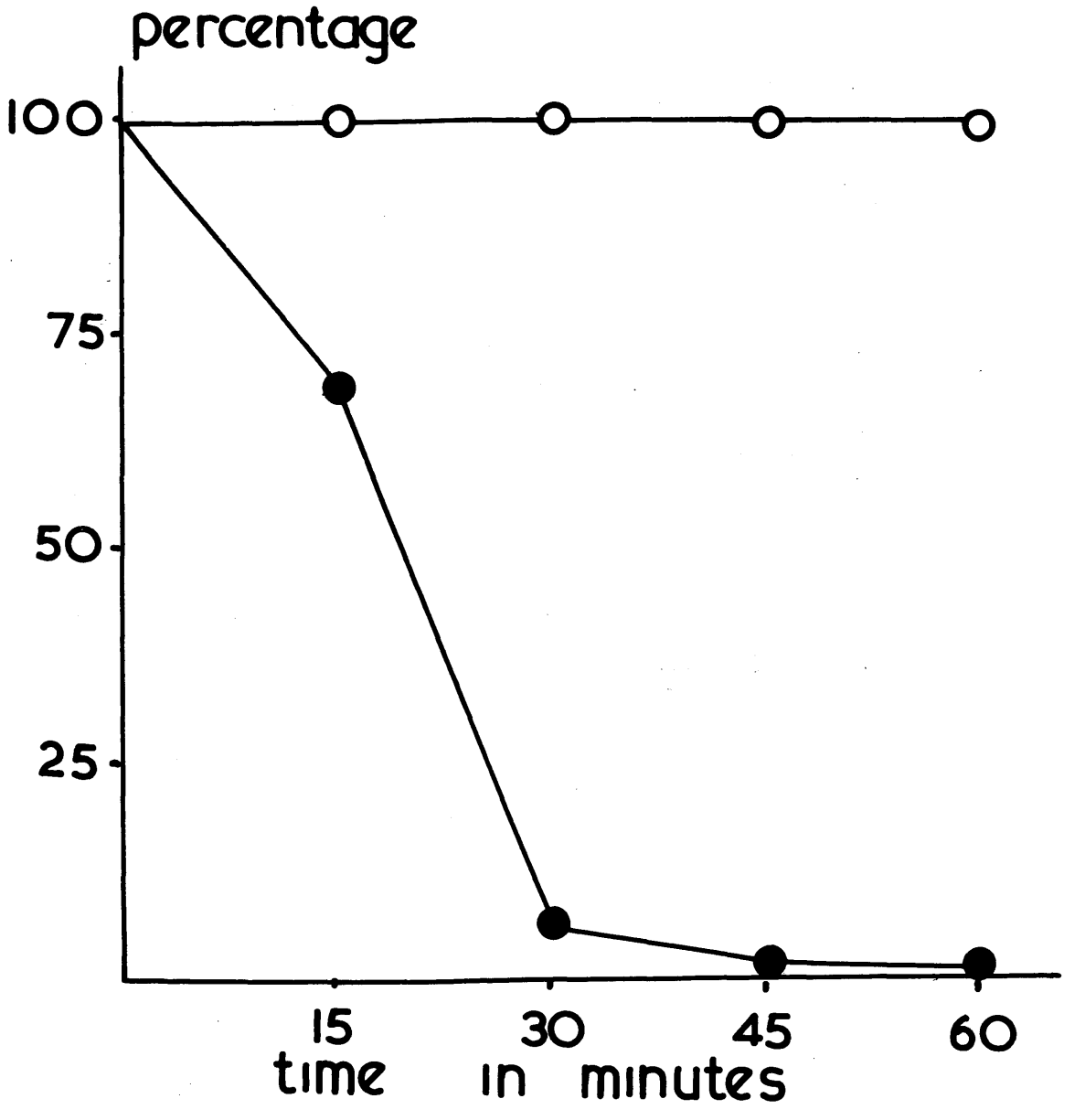
Antihaemophilic globulin (A.H.G.) consumption before and after the intravenous administration of 10,000 units of heparin.

Abscissa - percentage antihaemophilic globulin.

Ordinate - time in minutes after withdrawal of blood.

●——● Antihaemophilic globulin consumption before the administration of heparin.

O——O Antihaemophilic globulin consumption after the administration of heparin.


percentage
100
75
50
25
15
30
45
60
time in minutes

Measurement of antihaemophilic globulin.

This is described in the technical section - it is dependent on the correction of thromboplastin generation of adsorbed haemophilic plasma by dilutions of adsorbed normal and test plasmas. A supply of haemophilic plasma was kept frozen at -20° C. in a deep freeze. The heparin was removed by the process of adsorption on alumina.

Results:

The mean of the results on the utilization of prothrombin, factor V and antihaemophilic globulin before and after the administration of the heparin are shown respectively in Figures 82, 83, & 84 . The results are also shown in Table 12 . It will be seen that after the administration of the heparin there was no utilization of any of these components up to one hour, whereas in the specimens collected before giving the heparin, all had been used up by the end of the hour.

TABLE 12

		BEFORE HEPARIN				AFTER HEPARIN			
		TIME AFTER VENEPUNCTURE							
	Plasma	15 mins.	30 mins.	45 mins.	60 mins.	15 mins.	30 mins.	45 mins.	60 mins.
Pro-thrombin	100	79	51	10	0	100	100	100	100
	100	73	24	0	0	100	100	100	100
	100	74	10	0	0	100	100	100	100
Mean	100	75	28	3	0	100	100	100	100
Factor V	100	85	60	10	0	100	100	100	100
	100	70	40	0	0	100	100	100	100
	100	70	55	5	0	100	100	100	100
Mean	100	75	53	5	0	100	100	100	100
Antihae-mophilic globulin	100	100	15	0	0	100	100	100	100
	100	50	5	0	0	100	100	100	100
	100	60	5	0	0	100	100	100	100
Mean	100	70	8	0	0	100	100	100	100

Evidence has been described in Chapter 4 to suggest that factor V and antihaemophilic globulin are essential components of blood thromboplastin. During the clotting of blood under the influence of its own thromboplastin these factors disappear presumably in consequence of their utilization in the formation of blood thromboplastin. The failure to consume factor V and antihaemophilic globulin in the presence of heparin can be interpreted as a consequence of interference with thromboplastin formation.

The investigation has shown that in the presence of heparin there is defective consumption of prothrombin. This is a further, non specific, indication of failure in the blood thromboplastin system.

The techniques used in the investigation of the consumption of coagulation components have employed methods for the removal of heparin from the incubation mixtures. There was in consequence no interference by heparin with the indicator system, the thrombin-fibrinogen reaction. The efficacy of the methods for the removal of heparin was confirmed by the demonstration that all the specimens collected after the administration of the heparin contained as much prothrombin, factor V and antihaemophilic globulin as the plasma before the administration of heparin. Any traces of heparin left in the incubation mixtures would have resulted in lower

readings being obtained. The evidence of complete interference with prothrombin conversion and utilization of blood thromboplastin components may possibly indicate that this represents the main action of heparin rather than interference with the thrombin-fibrinogen reaction.

SUMMARY

(1) It has been confirmed that heparin interferes with the thrombin-fibrinogen reaction.

(2) Using the thromboplastin generation technique with additions of heparin, there is apparent interference with blood thromboplastin formation and to a lesser extent, there is evidence of its destruction by heparin. The interpretation of this evidence is difficult on account of possible interference with the thrombin-fibrinogen reaction.

(3) Using techniques which have involved the removal of heparin there is defective utilization of factor V, antihaemophilic globulin and prothrombin in the blood of patients given therapeutic doses of heparin. The failure to utilize factor V and antihaemophilic globulin is interpreted as interference with blood thromboplastin formation and in consequence there is defective prothrombin conversion.

REFERENCES

Brinkhous, K.M., Smith, H.P., Warner, E.D., and Seegers, W.H. (1939). The inhibition of blood clotting: an unidentified substance which acts in conjunction with heparin to prevent conversion of prothrombin into thrombin. Amer. J. Physiol. 125, 683.

Douglas, A.S. and Biggs, R. (1953). The consumption of some components involved in physiological blood coagulation. Glasg. med. J. 34, 329.

Howell, W.H. and Holt, E. (1918). Two new factors in blood coagulation-heparin and proantithrombin. Amer. J. Physiol. 47, 328.

MacMillan, R.L. and Brown, K.W.G. (1954). Heparin and thromboplastin. J. lab. clin. Med. 44, 378.

Mellanby, J. (1934). Heparin and blood coagulation. Proc. roy. Soc. London, S.B.116, 1.

Quick, A.J. (1938). The normal antithrombin of the blood and its relation to heparin. Amer. J. Physiol. 123, 712.

DISORDERS OF HAEMOSTATIC FUNCTION

by

A. S. DOUGLAS

Part IV. The Diagnosis of Haemorrhagic Disease

CHAPTER 9

INVESTIGATION OF HAEMORRHAGIC DISEASE

CONTENTS.

Confirmatory tests.

(13) Whole blood clotting time.
(14) Calcium clotting time.
(15) Prothrombin consumption test.
(16) Thrombin generation test.

Current Classification of Haemorrhagic Disease.

CHAPTER 9

INVESTIGATION OF HAEMORRHAGIC DISEASE

As a consequence of the advances in our understanding of blood coagulation, in particular of blood thromboplastin, it became necessary to design a scheme of investigation of the patient with undue tendency to haemorrhage. The diagnosis of such a patient rests on the results of clinical and laboratory assessment. It may often be possible from the history and examination to suspect that there is a haemorrhagic tendency but we are ultimately dependent on the laboratory investigation in order to establish this diagnosis and its exact nature.

CLINICAL INVESTIGATION.

Duration of the illness: In any of the constitutional disturbances of blood clotting the history of the condition will usually extend back to early childhood. This is not always so, as the mild haemophiliac may have his first incident of serious haemorrhage after the first extraction of a permanent tooth.

Age of the patient: Certain haemorrhagic diseases are prone to present at particular ages. Middle-aged women who develop a bleeding tendency are likely to be suffering from

thrombocytopenia. An elderly man who for the first time develops a disturbance of his clotting mechanism, will not be suffering from haemophilia but is more likely to have developed a circulating inhibitor, to be failing to produce coagulation factors as a consequence of parenchymatous liver disease or to have a coagulation disturbance in association with the development of dysproteinaemia.

Details of the liability to haemorrhage.

The initial suspicion as to the possibility of a haemostatic defect comes generally from the clinician in charge of the patient. Such a possibility may be the predominant feature of the case where a patient presents with purpura or with massive deep tissue haemorrhages in the absence of severe trauma. Occasionally the question of a haemostatic defect is raised only after investigation of the site of haemorrhage has failed to reveal any local lesion. The limitation of bleeding to one particular site is in general against the presence of a haemostatic defect but this is not always so. Patients have been seen where recurrent epistaxis or repeated gastro-intestinal bleeding have been the only manifestations of deficiency of antihaemophilic globulin. Other patients may present with symptoms which are not associated initially with an abnormal tendency to haemorrhage; for example a patient with Christmas disease was seen recently,

where the question of a haemostatic abnormality was raised only after the development of an obscure neurological lesion; the cause of this was pressure from haematoma formation.

The reaction of the patient to operative procedures provides the best test of haemostasis. Tooth extraction in particular provides a valuable test of the clotting mechanism. Even normal people may bleed continuously or intermittently up to 24 hours after a tooth extraction. It is probably abnormal if there is significant bleeding 36-48 hours after the extraction. The same is true of nose and throat operations and tonsillectomy provides an even more severe test. Any patient who requires blood transfusion as a consequence of tooth extraction is probably suffering from a haemostatic abnormality.

Family History: Because of the stigma attached to hereditary diseases there is often reluctance within a family to discuss the question of an inherited tendency to bleed. In the interrogation of the patient or his relatives a detailed chart should be drawn up with all members of the family shown and inquiry made as to each. In the case where an abnormality is in doubt clinically, for example, the very mild haemophiliac, the occurrence of a similar condition in other members of the family strengthens the diagnosis of a haemostatic defect. As will be described in Chapter 15

about one third of the patients with haemophilia have no positive family history.

Haemophilia in its various grades of severity is still the most common of the coagulation defects with Christmas disease as the next in frequency of incidence. Inherited deficiencies of factors V or VII or fibrinogen are very rare. Where the family history indicates that males only are affected and where in particular the family tree is sufficiently large to determine a sex-linked recessive type of inheritance then the condition is either haemophilia or Christmas disease. Where females are affected as well as males then it is not likely that the condition is haemophilia or Christmas disease but is more likely to be one of the other inherited abnormalities of the coagulation system. A very rare exception to this rule is the occurrence of homozygous females in haemophilic families as the result of the marriage of a transmitter female and an affected male. Factor VII deficiency and factor V deficiency appear to be inherited as non-sex-linked, probably dominant characters, and fibrinogenopenia as a recessive character has usually resulted from cousin intermarriage.

Associated features: These may on occasion give valuable indication of the diagnosis. If a patient develops a haemorrhagic tendency at the same time as symptoms and signs

of a severe anaemia it is likely that the condition is a non-regenerative thrombocytopenia possibly secondary to leukaemia or marrow aplasia. If the patient has joint changes in addition to other evidence of a haemorrhagic diathesis then the condition is probably haemophilia or Christmas disease. Enquiry as to the diet may suggest scurvy.

Circulating thromboplastin inhibitors are generally found associated with other recognised conditions; the development of a haemorrhagic state in association with these particular disorders would suggest such a circulating anticoagulant. Any of the coagulation components may be deficient in parenchymatous liver disease. Acute fibrinogen deficiency can arise as a complication of concealed accidental haemorrhage, or other obstetrical conditions and sometimes also as a consequence of pulmonary operations.

Site of haemorrhage: Generally the patient with a haemostatic defect bleeds from various sites, but the occurrence from one site alone does not exclude such a condition. In general the occurrence of petechiae and oozing from mucous membranes in the absence of trauma indicates that the condition belongs to the haemorrhagic purpuras rather than to the group of coagulation abnormalities.

Pathological haemorrhage due to disturbance of the coagulation mechanism is not limited to the skin and mucosae.

The bleeding often follows minor trauma or damage to tissue and when this affects the body surface obvious haemorrhage occurs usually as slow but continuous oozing which may persist for hours, days,or weeks. Where the skin or mucosal surface is unbroken the bleeding will often persist into tissues and tissue spaces until a haematoma of enormous size has resulted. These patients are frequently in greater danger from bleeding into tissue and pressure on vital structures than from external blood loss.

In the coagulation disturbances certain sites of haemorrhage are characteristic - haemarthroses, haematuria, epistaxis, haematemesis and melaena. As mentioned above sometimes the patient only bleeds from one site, e.g. recurrent epistaxis or recurrent haematemesis. In such patients where no demonstrable local lesion has been found it is necessary to assess for some abnormality of the haemostatic system.

Physical examination. The examination should include the scrutiny of the skin and the ocular fundus for evidence of capillary bleeding as petechiae or fundal haemorrhages. The distribution of any haematoma should be determined and the joints examined for evidence of chronic haemophilic arthritis. The tongue, buccal mucosa and fingers should be examined for telangiectatic areas which might indicate the

condition of hereditary teleangiectasia.

Perifollicular skin haemorrhages in association with deep tissue haematomata would suggest scurvy as the diagnosis.

LABORATORY INVESTIGATION.

From previous chapters it will be clear that there are many tests which can be applied to the investigation of haemorrhagic disorders. It is essential therefore to have available a scheme of differentiation. In the past reliance had to be placed on many tests which were really non-specific and threw little light on the exact nature of the disturbance and which in addition were insensitive. A procedure such as the whole blood clotting-time cannot compete either in specificity or sensitivity with the more modern techniques. It is clearly desirable, therefore, that a scheme of investigation should be based on whichever tests are most appropriate. A list of the tests is given in Table 13 and some account of these will be given in the text in the same order as they are enumerated in the table.

TABLE 13

Tests required in the diagnosis of haemorrhagic disorders with indication of the haemostatic defect detected by each procedure.

Tests	Haemostatic defect demonstrable
Essential	
Platelet count	Quantitative platelet deficiency.
Bleeding time.	Structural capillary defect. Quantitative platelet deficiency.
Capillary resistance test.	Scurvy. Quantitative platelet deficiency.
Quick's one-stage "prothrombin" test.	Factor V deficiency. Factor VII deficiency.
Thromboplastin generation test.	Haemophilia. Christmas disease. Circulating anticoagulants and heparin.
Occasionally required.	
Prothrombin assay	Prothrombin deficiency.
Fibrinogen assay.	Fibrinogen deficiency.
Thrombin-fibrinogen reaction.	Fibrinogen deficiency. Heparin.
Measurement of fibrinolytic activity.	Active fibrinolysis.
Capillary microscopy.	Structural capillary defect.
Confirmatory Tests. Whole blood clotting time. Calcium clotting time. Prothrombin consumption test. Thrombin generation test.	See text: abnormal in disorders of blood thromboplastin formation.

All these techniques are described in detail in the experimental appendix but some of the underlying principles will be discussed here.

The techniques enumerated above do not of course, exhaust the possible methods of diagnostic approach; the procedures mentioned are those which are usually of most value in the diagnosis of the individual patient.

Platelet count. In the earlier years of my interest in this subject Lempert's method for counting platelets was used. The technique is described in the appendix page 600 and was found to be reasonably satisfactory. The action of the solution in lysing the red cells was variable and the volume of blood used in the method is small. The larger the volume of blood in the diluting solution the more accurate the method. During the last four years a method has been used, which was demonstrated to me by Dr. Dacie in the Postgraduate Medical School, London, and is given in detail in the appendix. Venous blood is collected through a wide bore needle without frothing and some of this is delivered to a waxed watch glass. 0.1 ml. of the blood is transferred to 10 ml. of red cell diluting fluid, mixed and a sample transferred to a counting chamber. The platelet count is of course essential to the diagnosis of thrombocytopenia. The difficulty in counting platelets generally arises from extraneous material in the preparation giving the appearance of platelets. Dr. Dacie's method results in a very clean preparation.

Platelet counting is not a difficult procedure, but like so many of these techniques it requires practice before it can be used with confidence. The person who counts platelets only occasionally not infrequently records the platelets as numerous when in fact they are scanty.

Bleeding Time: The method used throughout this work is that described by Ivy et al (1935) and O'Brien (1951). This depends on the application of a sphygomanometer cuff at 40 mms. pressure round the upper arm and pricking the extensor aspect of the arm with an automatic pricker set at a constant depth. The bleeding time is prolonged in thrombocytopenia and in diffuse capillary defects.

Capillary Resistance Test: This is a procedure, which does not justify too much refinement, where markedly positive or quite negative it is of value. Unfortunately the results are often of doubtful significance. A sphygomanometer cuff set at 75 mms. for 5 minutes is a generally accepted standard. With this there should be normally not more than 10 petechiae on the forearm. This test is usually positive in thrombocytopenia and scurvy.

Coagulation Factors in Plasma and Serum. Adsorption on alumina. Normal plasma and serum differ in their content

of coagulation factors as a consequence of utilization of certain components during clotting. The distribution of the coagulation components can be further modified by treatment with alumina (Table 14).

On the treatment of plasma with alumina, prothrombin, factor VII and the Christmas factor are adsorbed; factor V and the antihaemophilic globulin remaining in the supernatant after centrifuging. Since prothrombin, factor V and antihaemophilic globulin are all consumed during clotting of normal blood, serum contains only factor VII and the Christmas factor.

TABLE 14

Properties of adsorption of coagulation factors and of their utilization during clotting. Those factors missing in adsorbed plasma are removed by alumina; those factors missing from serum are consumed during clotting. Factors present are indicated + and absent -

	Plasma	Serum	Adsorbed Plasma
Prothrombin	+	-	-
Factor V	+	-	+
Antihaemophilic globulin	+	-	+
Christmas factor	+	+	-
Factor VII	+	+	-

Quick's one stage "prothrombin" test.

This well known procedure was introduced by Quick in 1935 and is fully described by him (Quick 1938). The technique used in 1949 and 1950 differed slightly from that used thereafter. The details are contained in the appendix page 569. The principal difference was concerned with a change in the method of preparation of the brain thromboplastin. In the earlier years a phenol-saline extract of rabbit brain was used whereas later this was replaced by an acetone extracted preparation of human brain.

A marked prolongation of the clotting time obtained by this test is produced by a deficiency either of factor V or factor VII. Quick's test is not influenced by deficiency of the Christmas factor or antihaemophilic globulin or platelets; the brain extract replaces these components. Prothrombin deficiency alone is rarely of a severity sufficient to cause a prolongation of the one-stage clotting time and is measured by the two-stage techniques mentioned below.

Differentiation of deficiencies of factors V or VII.

The method of differentiation between deficiencies of factors V and VII, both of which cause prolongation of the one-stage clotting time is summarized in Table 15 . It is dependent upon the effect on the one-stage clotting time of the addition of factor V freed from factor VII or of factor VII freed from

factor V (Biggs and Douglas 1953a).

TABLE 15

Methods of differentiation between deficiencies of factors V and VII.

Deficiency of either factor V or factor VII will cause a prolongation of the one-stage "prothrombin" time. Factor V is diagnosed when there is significant shortening of the clotting time on the addition of 10% alumina-treated normal plasma but not of normal serum. The alumina-treated plasma contains factor V but not factor VII. Factor VII deficiency is diagnosed when there is shortening on the addition of serum but not adsorbed plasma. The serum contains factor VII but no factor V.

One-stage "prothrombin" time prolonged.

	Addition of 10% of alumina-treated plasma.	Addition of 10% normal serum.
Factor V deficiency	Shortened.	Not appreciably shortened.
Factor VII deficiency.	Not shortened.	Shortened.

If the deficiency is of factor V, then the one-stage clotting time is shortened by the addition of adsorbed plasma which contains factor V but not factor VII or prothrombin. If the deficiency is of factor VII then the one-stage clotting time is shortened by the addition of normal serum which contains factor VII but not factor V or prothrombin. The main causes of deficiency of factors V and VII are shown in Table 17 .

Thromboplastin Generation Test. This is probably the most important single procedure used at the present time in the diagnosis of haemorrhagic disease. The test in its original form was described by Biggs and Douglas (1953) and is given in the appendix, page 590. The only variation from the original technique is the treatment of the serum. As we originally described this test the blood, while clotting to produce serum, was shaken with glass beads. It was subsequently appreciated that this agitation with beads is not needed and the blood is now allowed to stand for two hours at 37° C. for neutralization of any thrombin or thromboplastin formed and for complete conversion of prothrombin.

The reagents required for the test are adsorbed plasma (i.e. after treatment with alumina), serum and platelets. The platelets are prepared by the differential centrifugation of plasma and the subsequent washing in saline followed by resuspension. It will be remembered that the adsorbed normal plasma contains factor V and antihaemophilic globulin and the normal serum contains Christmas factor and factor VII. The conditions for which the test is required in diagnosis all have a normal one-stage clotting time with Quick's test and therefore there is no deficiency of factor V or factor VII. A mixture containing adsorbed plasma (factor V and

antihaemophilic globulin) platelets and serum (factor VII and Christmas factor) together with calcium provides all the reagents required for intrinsic thromboplastin formation. The adsorbed plasma, serum and platelets are prepared from a normal subject and also from the patient and various combinations of these preparations are made as indicated in Table 16.

TABLE 16

Thromboplastin Generation Test.

The adsorbed normal plasma is tested with normal serum and normal platelets to ensure that the system is functioning satisfactorily. Thereafter the combinations as indicated in the table are made, and the results interpreted accordingly. Satisfactory formation of thromboplastin is indicated + and where this fails to form adequately it is shown - .

Components of the reacting mixture. Adsorbed Plasma / Serum / Platelets	Patient Normal Normal	Normal Patient Normal	Normal Normal Patient	
Resulting Thromboplastin Formation	+	+	+	Normal.
	-	+	+	Haemophilia.
	+	-	+	Christmas Disease.
	-	-	+	Circulating anticoagulants
	+	+	-	Qualitative platelet defect.

The patient's adsorbed plasma is tested with normal serum and patient's serum with normal adsorbed plasma. If the patient

Figure (85)

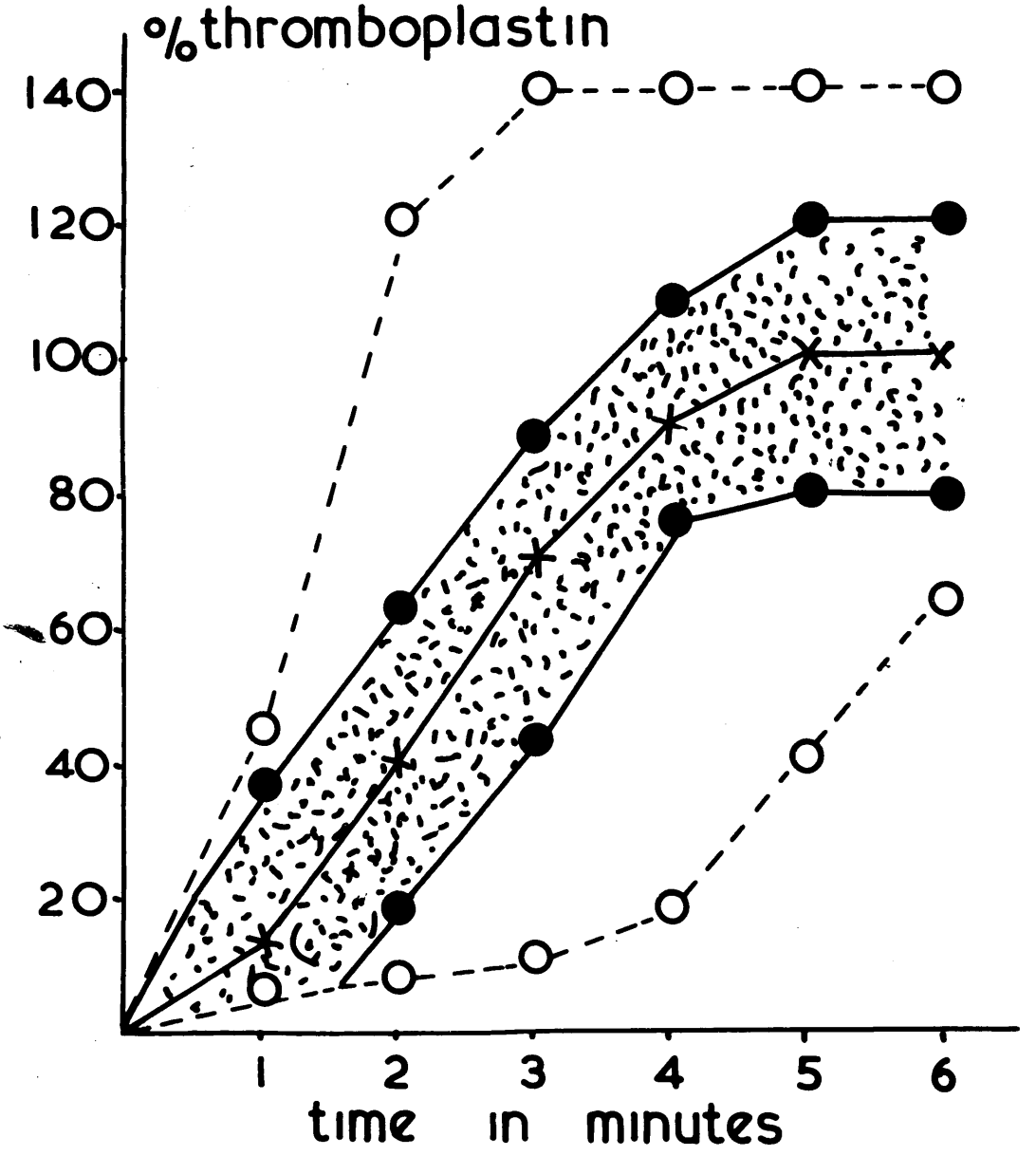
Figure (35)

The thromboplastin generation test - normal range.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

The central curve represents the average of 40 different tests. The extreme limits indicate the range of variation obtained when all of the thromboplastin components are varied simultaneously. The stippled area shows the range of variation obtained in 38 of 40 observations in which the alumina plasma was obtained from different normal subjects, but serum and platelets were from one subject.


% thromboplastin
140
120
100
80
60
40
20
1
2
3
4
5
6
time in minutes

has no defect in this system then thromboplastin is formed satisfactorily in both these combinations. Where the adsorbed plasma is defective and the serum satisfactory the disease is haemophilia. Where the serum is defective and the adsorbed plasma satisfactory the disease is Christmas disease. Where neither the adsorbed plasma nor the serum are effective the explanation is likely to be the presence of a circulating anticoagulant; when the patient's platelets fail to form thromboplastin, but the adsorbed plasma and serum are normal the condition is a qualitative platelet defect.

NORMAL VARIATION.

In figure 85 is shown the average result of carrying out this test on 40 different sets of reagents. It will be seen that a level of 100% of thromboplastin, indicating a plasma clotting time of 10 secs., is achieved on an average after an incubation time of five minutes. This very rapid clotting time cannot be due to the transfer of thrombin from the incubation mixture, because no significant amount of thrombin is transferred. This can be confirmed by the simultaneous transfer into fibrinogen as a substrate. From figure 85 it will be seen that there is a wide range of variability between tests made on different days in which all three reagents are different. At first it might be thought

Figure (86)

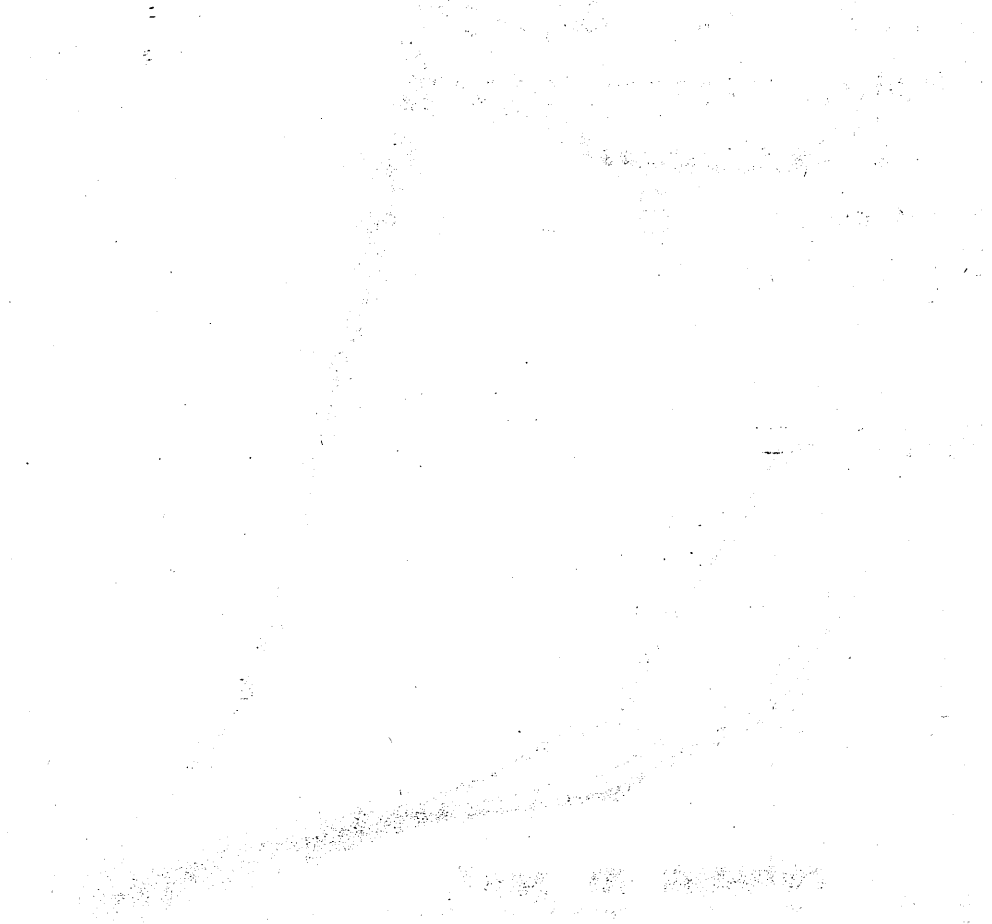


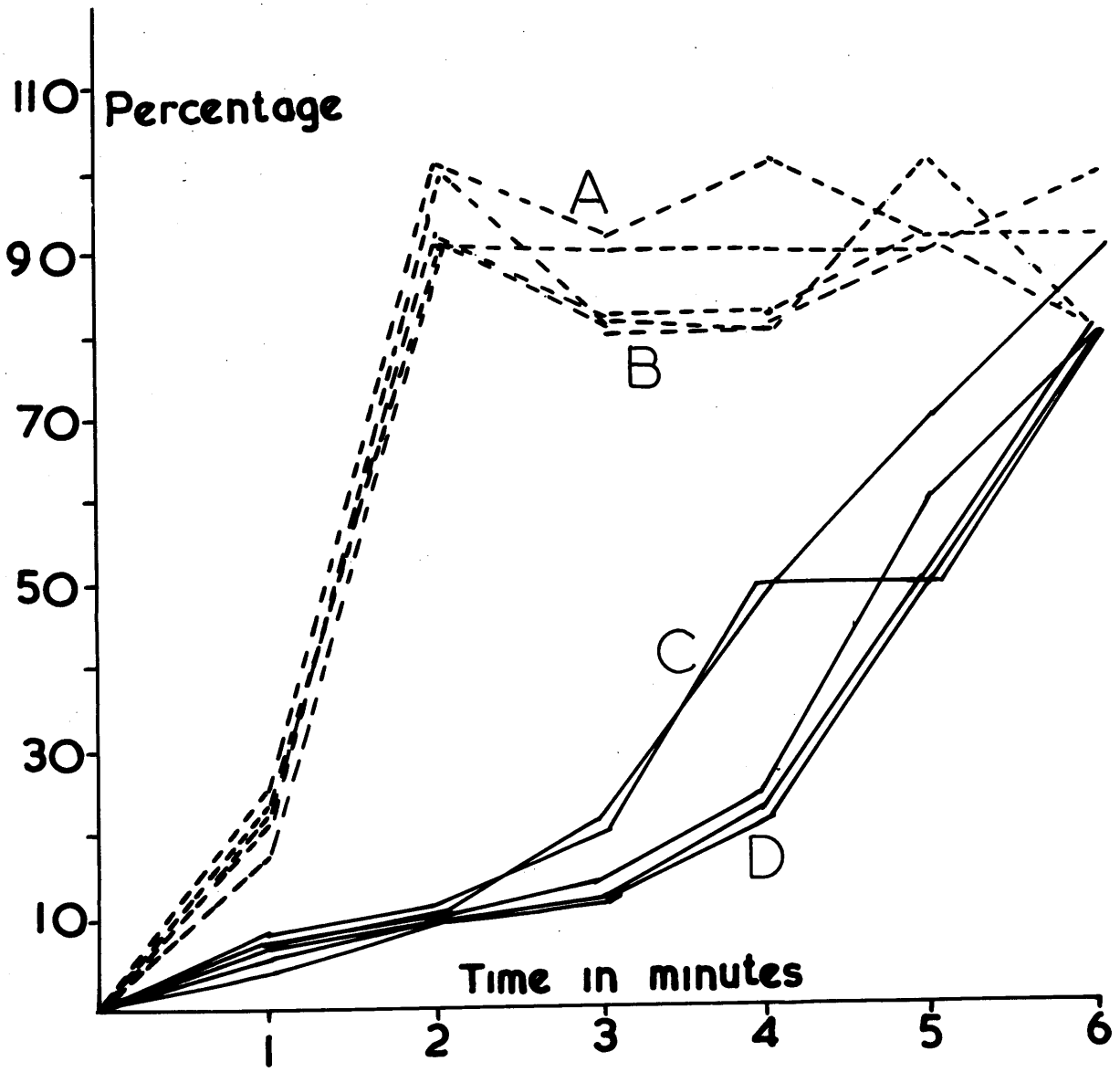
Figure (26)

The thromboplastin generation test - normal range.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

The curves show the range of variation in thromboplastin formation in two experiments. In each five samples of normal alumina plasma were tested with the same preparations of platelets and serum.


110
Percentage
90
70
50
30
10
A
B
C
D
Time in minutes
1
2
3
4
5
6

that this large variation greatly reduced the usefulness of the test but in practice the test is not used to measure differences between two sets of the three components. In the investigation of any particular case only one component is usually being studied - the adsorbed plasma in haemophilia or the serum in Christmas disease. For this limited objective the normal range is much less.

In tests on 40 samples in which the platelets and serum were constant, but the alumina plasma was prepared from different samples, the observations exceeded the range shown by shading in Figure 85 on only two occasions. Similar results were obtained in 15 samples in which the alumina plasma and platelet preparations were constant but the source of serum was varied.

The observed range in two experiments is shown in figure 86. In each of these experiments five different samples of adsorbed plasma were tested with the same preparation of serum and platelets. It will be seen that although the results in two experiments are different the range of observations in one experiment is not wide. To assess for example whether or not a preparation of adsorbed plasma is abnormal the alumina plasma is prepared simultaneously and identically from normal and patient's plasma and tested with the same preparations of normal serum and platelets. When expressed

Figure (87)

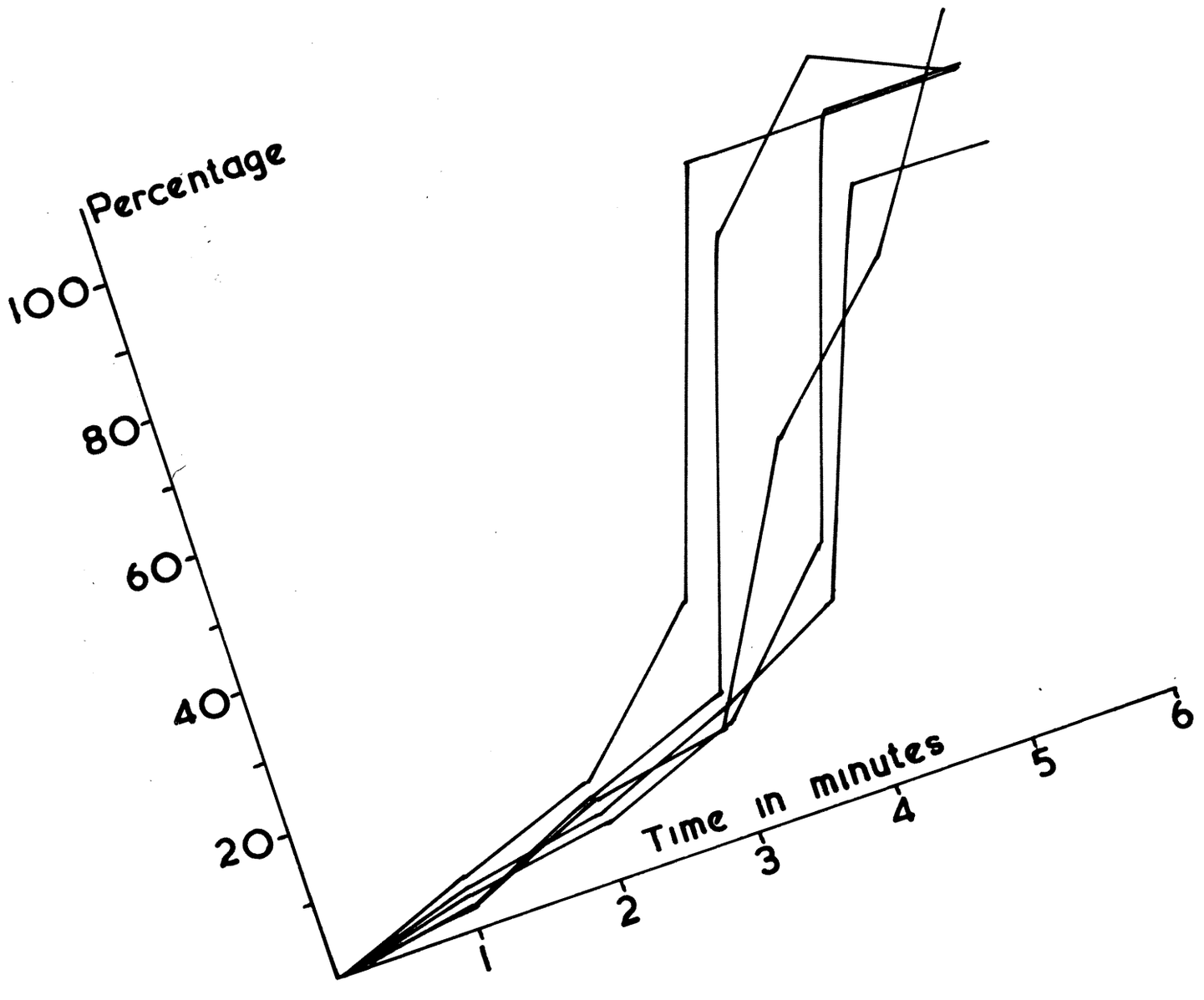
Figure (87)

Thromboplastin Generation Test - range of variation.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

In this experiment the alumina adsorbed plasma and platelets were kept constant and five different normal sera were tested.


Percentage
100
80
60
40
20
Time in minutes
1
2
3
4
5
6

graphically the difference between normal and abnormal should be wider than the total normal range shown in the shaded area of figure 85 . If the normal for example corresponds to the upper limit curve C in figure 86 then the abnormal should lie rather below curve D before a deficiency of A.H.G. could be deduced. This does not provide a quantitative assay but only provides an assessment between normal and abnormal.

The technique can be modified to give a quantitative assessment of the individual factors. For example, if a serum factor is thought to be deficient an approximate measure of the extent of the deficiency can be obtained by carrying out the thromboplastin generation test on a 1 in 10 dilution of the patient's serum and comparing the resulting curve with a series of curves prepared using dilutions of normal serum. If the 1 in 10 dilution of the patient's serum gives a curve similar to that of the 1 in 100 dilution of normal serum, the patient's serum may be said to have 10% of the normal thromboplastin-forming capacity.

A more satisfactory method of assaying A.H.G. or the Christmas factor is to make the dilutions of normal adsorbed plasma not in saline but in haemophilic adsorbed plasma for the A.H.G. assay and of normal serum in Christmas disease serum for the Christmas factor assay. This is described

elsewhere (see appendix page 594).

Haemophilia: In figure 88 are shown the results of carrying out this test on plasma samples from five haemophiliacs. In each of these experiments the alumina plasmas from the normal and the haemophilic subjects were compared in their ability to form blood thromboplastin, using preparations of platelets and serum from normal subjects. In these observations no fine distinction between normal and abnormal was required and the total observed range of normal variation is shown.

The test is more sensitive than other laboratory tests for haemophilia. As will be seen in the appendix, mild haemophiliacs with normal whole blood clotting time and normal prothrombin consumption gave very abnormal results on the thromboplastin generation technique. Not only is the technique sensitive to the defect but the thromboplastin generation test gives a more specific diagnosis than the other techniques, which only detect a thromboplastin abnormality irrespective of its cause.

The sensitivity of the thromboplastin generation test to the haemophilic defect is well illustrated by experiments on mixtures of normal and haemophilic adsorbed plasma when one part of normal is mixed with nine parts of haemophilic plasma the clotting time and rate of prothrombin consumption

Figure (88)

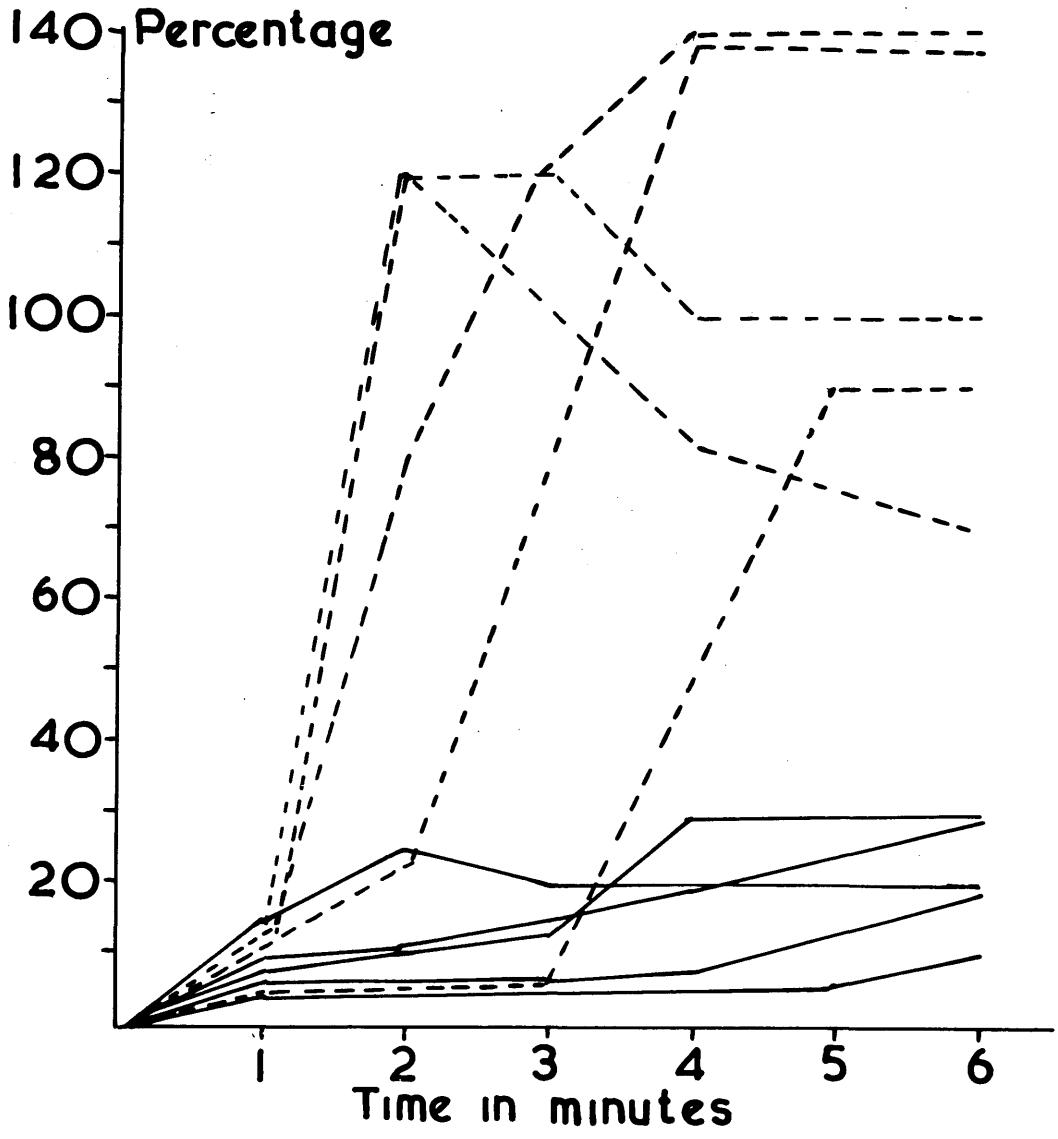
Figure (38)

Thromboplastin Generation Test - application in haemophilia.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

This figure shows the thromboplastin generation test on five haemophiliacs (continuous lines) and the corresponding normals for each test (discontinuous lines).


140
120
100
80
60
40
20
Percentage
1
2
3
4
5
6
Time in minutes

are usually normal in the mixtures. In comparison even 50% mixtures of normal and haemophilic plasma in the thromboplastin generation test are still markedly abnormal. (see figure 89).

It is a well recognized phenomenon that a haemophilic patient, transfused with blood or plasma, may continue to bleed despite correction of the whole blood clotting time and prothrombin consumption. On one occasion a child aged 6½ years and weighing 40 lb. required the extraction of two teeth. He was given one pint of fresh plasma and two-thirds of a pint of fresh whole blood before the extractions. His whole blood clotting time and prothrombin consumption index were normal, but he bled more than normal and his thromboplastin generation remained abnormal (fig. 90).

Christmas Disease. In figure 91 is shown the thromboplastin formation in five cases of Christmas disease. The shaded area shows the total range of thromboplastin formation using normal alumina plasma and serum samples which were tested in parallel with the samples from the subjects with Christmas disease. The curves show the results of replacing the normal serum by the patient's serum.

Functional platelet deficiency.

The concept of functional platelet deficiency was put

Figure (89)

Thromboplastin Generation Test - application in haemophilia.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

This figure shows the thromboplastin generation test with the normal serum and platelets constant and the adsorbed plasma varied. The adsorbed plasmas tested were normal, haemophilic and a mixture of equal parts of both.

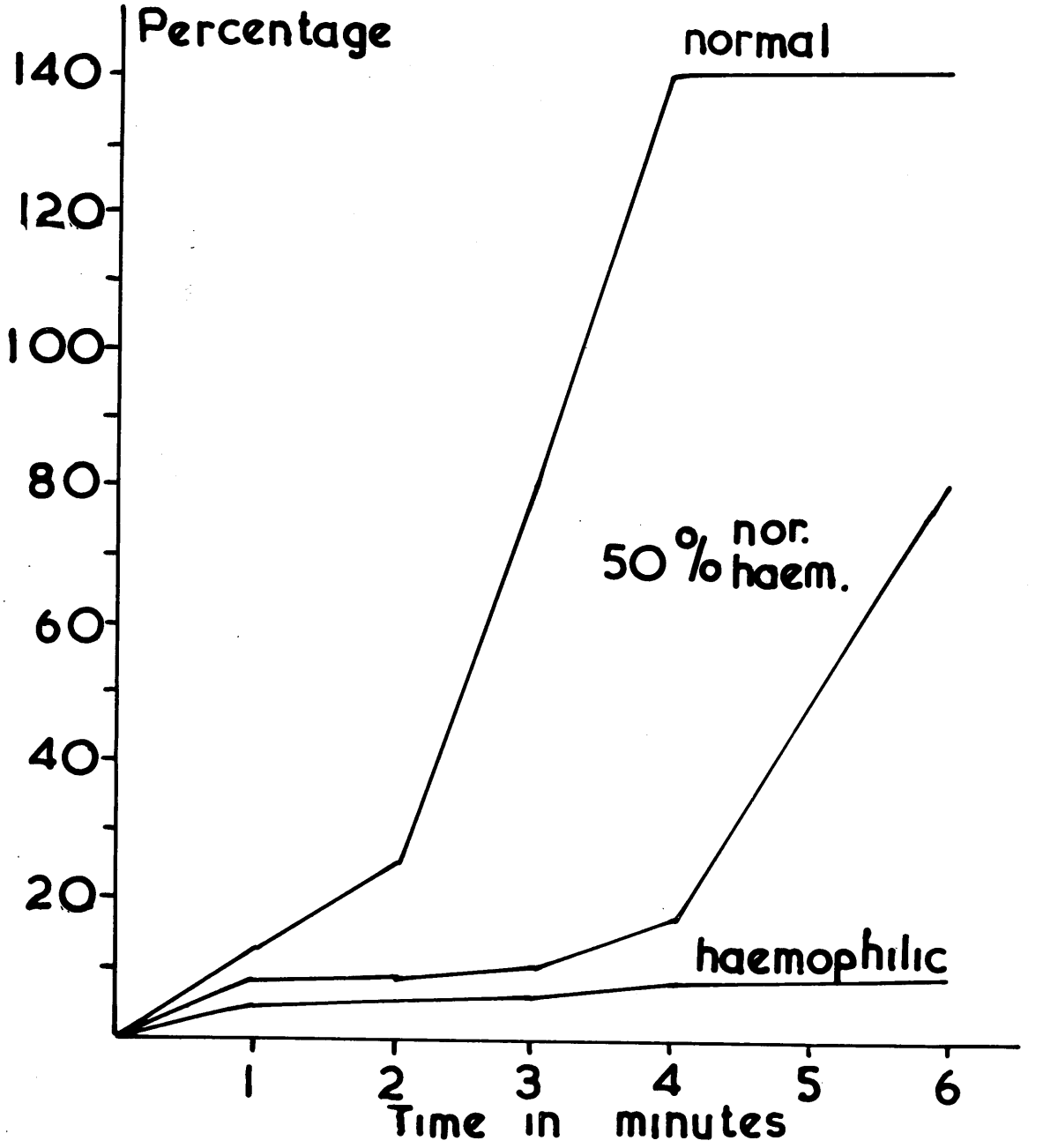

Percentage
140
120
100
80
60
40
20
normal
50% nor. haem.
haemophilic
1
2
3
4
5
6
Time in minutes

Figure (90)

Thromboplastin generation test - application in haemophilia.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

This figure shows the abnormal thromboplastin generation test in a haemophiliac (lower graph), who has received blood transfusion sufficient to restore other tests to normal. The defective thromboplastin formation in this patient is compared with the result from a normal.

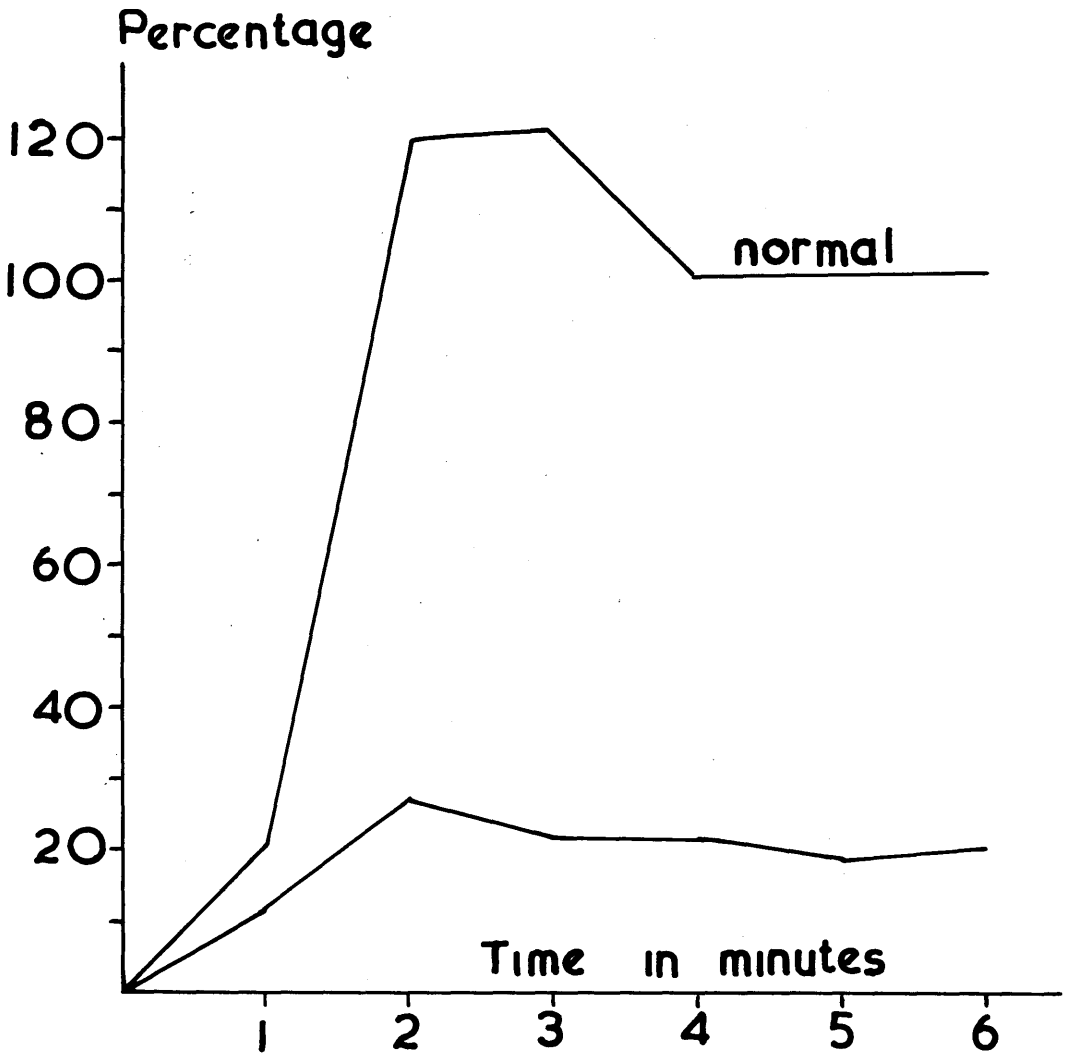

Percentage
120
100
80
60
40
20
normal
Time in minutes
1
2
3
4
5
6

Figure (91)

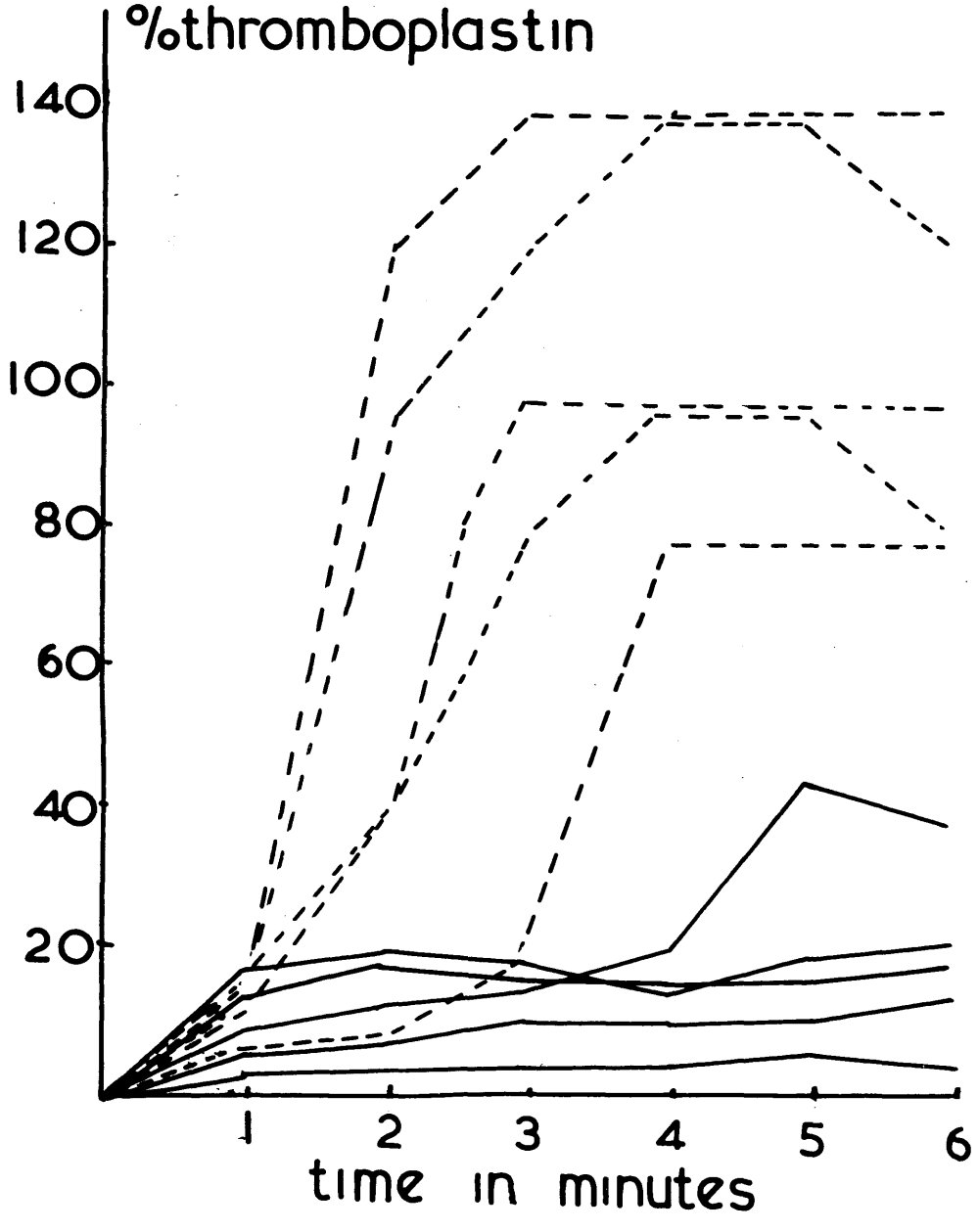
Figure (91)

Thromboplastin generation test - application in Christmas disease.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

This figure shows the thromboplastin generation test on five patients with Christmas disease (continuous lines) and the corresponding normals for each test (discontinuous lines).


%thromboplastin
140
120
100
80
60
40
20
1
2
3
4
5
6
time in minutes

forward by Glanzmann (1918). It is probable that some patients who were originally placed in this category would now be called cases of diffuse capillary defects or von Willebrand's disease. This type of functional platelet deficiency is sometimes called thromboasthenia. The name should be reserved for the few patients described who have functionally and morphologically abnormal platelets.

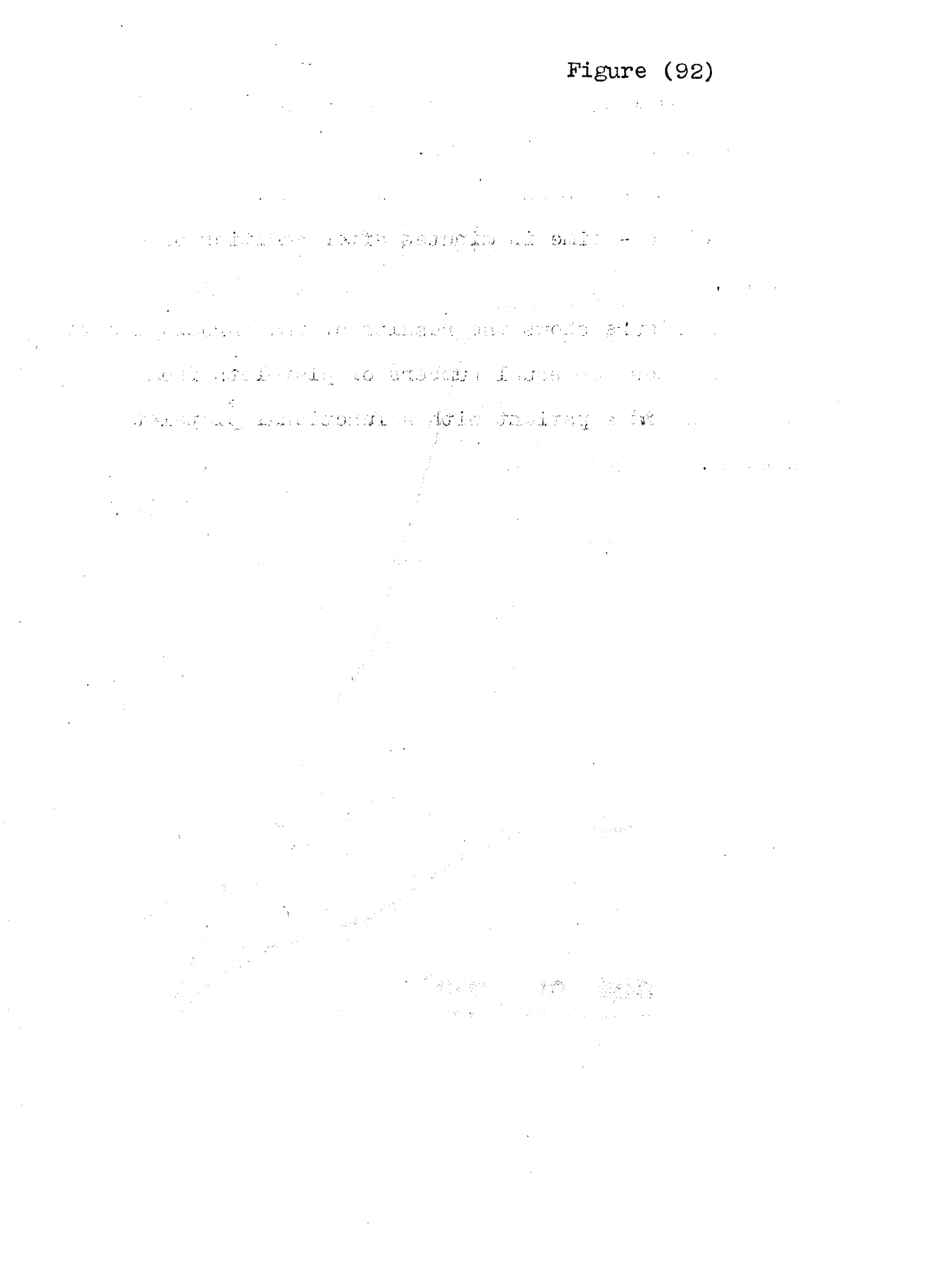
An example of this condition is described in Chapter 10. This patient's blood contained giant platelets almost as large as red cells. When compared in equal numbers with normal platelets, the patient's platelets behaved abnormally in the thromboplastin generation test, see figure 92. By comparison with dilutions of normal platelets, the patient's platelets were only about one tenth of the potency of the normal.

Thromboplastin inhibitors.

This subject is dealt with in much greater detail in Chapter 19 but the application of the thromboplastin generation test to the diagnosis of these will be described here.

This is an acquired coagulation defect characterised by defective prothrombin consumption:when the adsorbed plasma from the patient is interacted with normal serum or normal adsorbed plasma with patient's serum then there is defective thromboplastin formation in both of these

Figure (92)

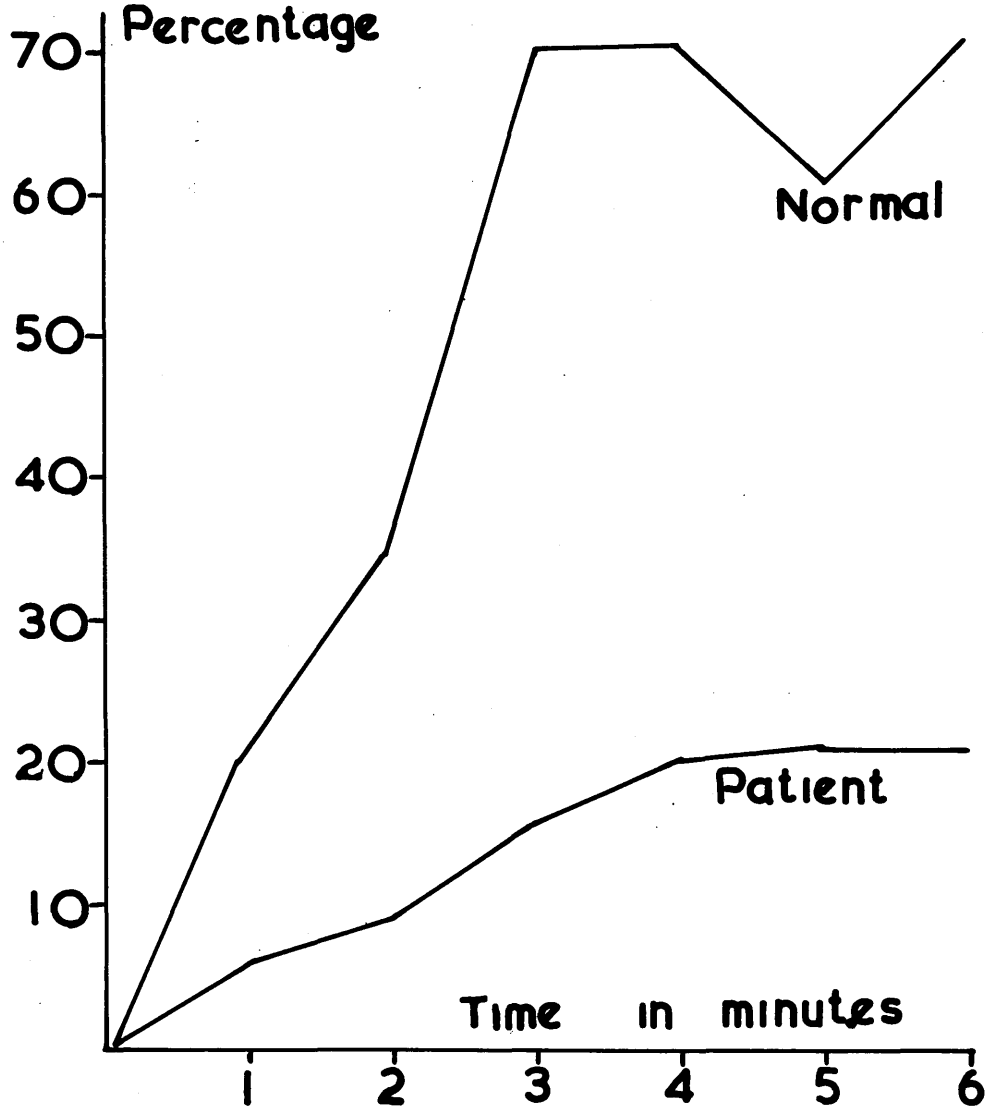


Thromboplastin Generation Test - application in functional platelet disorders.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

This figure shows the results of the thromboplastin generation test on equal numbers of platelets from the normal and a patient with a functional platelet disorder.


Percentage
70
60
50
40
30
20
10
Normal
Patient
Time in minutes
1
2
3
4
5
6

combinations of reagents. Furthermore when small concentrations of the patient's adsorbed plasma or of the patient's serum are added to the normal reagents capable of forming thromboplastin the formation is inhibited. The results of such an experiment are illustrated in figure 93 . In this experiment normal adsorbed plasma, platelets and normal serum were prepared and shown capable of inhibiting thromboplastin formation. In three tests final dilutions of 1 in 100, 1 in 250 and 1 in 500 of the patient's adsorbed plasma were added to the mixtures forming thromboplastin. It will be seen that at a concentration of 1 in 250 the patient's plasma inhibited the formation of blood thromboplastin. The patient's serum was similarly inhibitory.

The thromboplastin generation test is technically relatively simple, the final test system being essentially a series of one stage "prothrombin" times. The preparation of the platelet suspension is time consuming and the test has recently been modified by substitution of a lipoid prepared from brain. This lipoid is capable of replacing platelets but is not thromboplastic on its own. Throughout the work described in this thesis platelets were used in the test.

When applied to the investigation of appropriate problems, the test has great advantages over any previously described

techniques. Using this test, it is possible to differentiate between the abnormalities in which there is a reduction in the formation of blood thromboplastin. Moreover the test is a more sensitive indicator of abnormality than any previously described procedure.

This work was carried out in conjunction with Dr. Rosemary Biggs and has been published. (J. clin. Path. 1953, 6, 23). The relevant experimental data is described in the appendix pages 804-831.

Prothrombin Assay: Two-stage methods have been described which are believed to give true assays of prothrombin, the globulin fraction method (Douglas and Biggs 1953) and the area method (Biggs and Douglas 1953). These also are described in the appendix, page 570. The first method is dependent on the separation of prothrombin from antithrombin and its subsequent activation by brain thromboplastin and calcium. The second method is a two-stage technique where the generation of thrombin from the plasma is followed, until its complete destruction by antithrombin. The areas enclosed by the curves are then compared, the test plasma against a normal plasma.

Fibrinogen Assay: The method used in this work was a micro-Kjeldahl technique described in detail in the appendix

Figure (93)

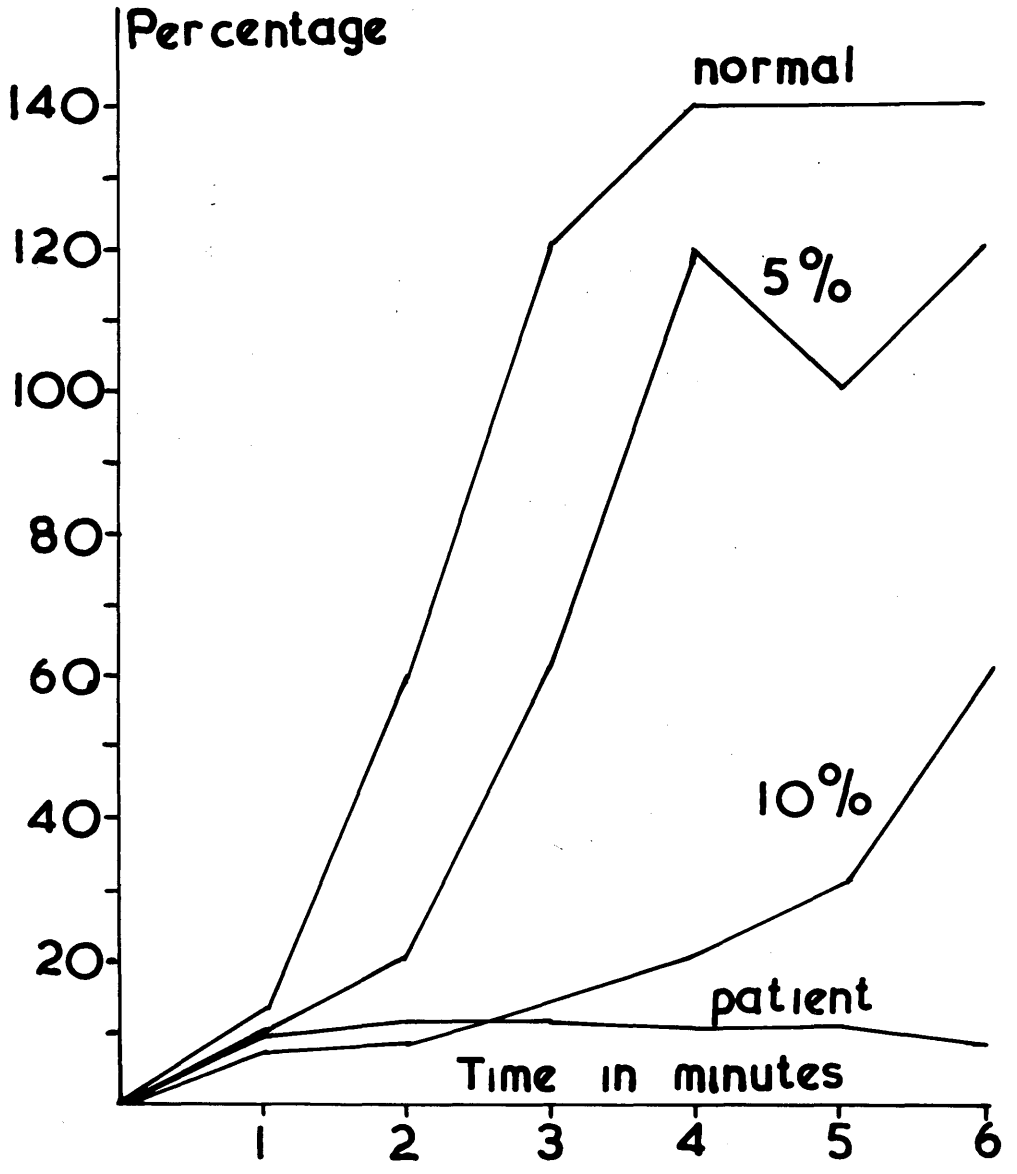
Figure (93)

Thromboplastin Generation Test - application in diagnosis of circulating thromboplastin inhibitors.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

This figure shows the inhibition of thromboplastin formation, by the addition to a thromboplastin generating system, of adsorbed plasma from a patient with a circulating anticoagulant. The curve labelled 5% gave a final dilution of $^1/_{500}$ of the inhibitor in the incubation mixture and the curve labelled 10% gave a final dilution of $^1/_{250}$ of the inhibitor in the incubation mixture.


Percentage
140
120
100
80
60
40
20
normal
5%
10%
patient
Time in minutes
1
2
3
4
5
6

page 580 . One ml. of plasma was diluted with twenty times its volume of citrate-saline and then the fibrinogen clotted with 1 ml. of thrombin. After allowing adequate time for the fibrin to form it was collected by rolling on to a glass rod. The fibrin while still on the glass rod was washed twice with saline. The fibrin was then removed from the glass rod, washed and transferred to the digestion tube of the micro-Kjeldahl apparatus.

Thrombin-Fibrinogen Reaction. This technique is also described in detail in the appendix. Dilutions in saline are made of a normal plasma and the test plasma. A preparation of thrombin is added to these and the clotting times recorded. The two common causes of disturbance of the thrombin-fibrinogen reaction are a deficiency of fibrinogen and the presence of heparin or allied compounds.

Measurement of Fibrinolytic Activity: This should be studied particularly where it is observed that the fibrin clot, formed in a specimen of blood collected from the patient, disappears on standing. A method of approximate assay is described in the appendix. Dilutions of plasma are made with saline and these clotted with a solution of thrombin or with calcium chloride. These are then incubated at 37°C, and the progress of lysis of the clots observed at

intervals. Active fibrinolysis may be found as an occasional complication of parenchymatous hepatic disease, prostatic carcinoma with metastases, pregnancy, surgical shock particularly after burn injuries, after pulmonary operations and less often in a variety of other diseases.

Capillary microscopy: This is technically a simple procedure to perform but requires considerable experience for correct interpretation. Using a strong light, the bed of the finger nail is illuminated and smeared with immersion oil. The capillaries are studied using the low power of the microscope. The examination is required in bleeding states without demonstrable coagulation defect. The conditions, which may be diagnosed by this technique are hereditary disturbances of the capillaries. One of these is diffuse and no abnormality can be determined on ordinary inspection of the skin. This condition is probably that described in the literature as von-Willebrand's disease or pseudohaemophilia. In contrast to this diffuse capillary defect there are more localised types, the condition called hereditary teleangiectasia and the rarer one of diffuse angiokeratoma. In some cases of idiopathic thrombocytopenia a structural capillary abnormality may also be seen.

Whole blood clotting time: Two techniques were used

throughout these investigations. The second method was introduced during the last two years of the work because cases were not infrequently seen away from my own hospital and laboratory and in consequence it was difficult to maintain temperature at 37° C.

Method (1). The details of this are given in the appendix page 565; it is a modication of the Lee and White (1913) technique and is described by Biggs and Macfarlane (1953). Four tubes of standard calibre are used, maintained at 37° C. and subsequently where necessary the prothrombin consumption of the specimens studied by Merskey's method. A wide bore needle must be used for the collection of the blood by clean venepuncture. No frothing is permissible during the collection. These rules are essential also for the prothrombin consumption technique. Methods using capillary glass tubing are of very limited value. If the whole blood clotting time is prolonged this is indicative of an abnormality of the coagulation system. Unfortunately there may be a serious haemorrhagic defect in the presence of a normal whole blood clotting time. This test may be prolonged in patients with haemophilia or Christmas disease or circulating anticoagulants or a deficiency of fibrinogen. It may also be prolonged as a consequence of heparin therapy. In the older descriptions of haemophilia

a prolonged whole blood clotting time was an essential of diagnosis. It is now appreciated that in this condition the coagulation time may be normal.

Method (2). (see page 565 of the appendix). Tubes of 10-11 mm. calibre were used and these were maintained at ward temperature, only being inclined once every five minutes.

Calcium Clotting Time. See appendix page 564. To 0.1 ml. of plasma is added 0.1 ml. of saline and 0.1 ml. of m/40 calcium chloride. The clotting time is recorded. In conditions where the whole blood clotting time is prolonged, the calcium clotting time is also likely to be longer than normal. It has the same limitations of interpretation as the whole blood clotting time; it may be normal in the presence of significant disturbance of the coagulation system. It is a valuable technique, however, for studying mixtures of plasma. The mutual correction of haemophilia and Christmas disease, for example, can be studied by this test. The presence of a circulating anticoagulant can be proved by the finding that the addition of a small amount of the test plasma will prolong the calcium clotting time of normal plasma.

Prothrombin Consumption Test. This test is dependent on the assay of prothrombin in serum after blood has stood

for an hour at 37° C. It has been fundamental to the progress of our knowledge of thromboplastin deficiencies, but has been rather superceded in the routine diagnosis of haemorrhagic states by the thromboplastin generation test. Nevertheless it remains as an important procedure for all interested in this field of research. Defective prothrombin consumption is a non-specific indication of a defect in the thromboplastin mechanism. This may be caused by haemophilia, Christmas disease, thrombocytopenia or the presence of circulating anticoagulants.

The technique used during the earlier years of these studies was that described by Merskey (1950) and in the later investigations the method described by Douglas and Biggs (1953) was employed. The method described by Merskey does not allow for changes in the antithrombin content between plasma and serum and gives too high a reading of the prothrombin content of serum. For routine diagnostic purposes, however, it is satisfactory. The method described by Douglas and Biggs (1953) gives a more accurate estimate of the prothrombin content but is rather more laborious.

Thrombin Generation Test. Again this is an important technique when engaged on research problems of blood clotting but it is not of prime importance in the routine diagnosis of haemorrhagic disease. The technique can be carried out

either on whole blood or on plasma. The pattern of thrombin generation is followed by transferring aliquots at intervals into fibrinogen. The methods are described in detail in the experimental appendix page 588 .

CURRENT CLASSIFICATION OF HAEMORRHAGIC DISEASE.

The platelet count, the bleeding time and the capillary resistance test will generally indicate whether the defect is in the platelet-capillary group. If these are all normal, the defect is sought in the coagulation mechanism itself. The conditions which this scheme of laboratory-investigation enable us to differentiate are shown in Table 17 .

TABLE 17

Current Classification of Haemorrhagic Disorders

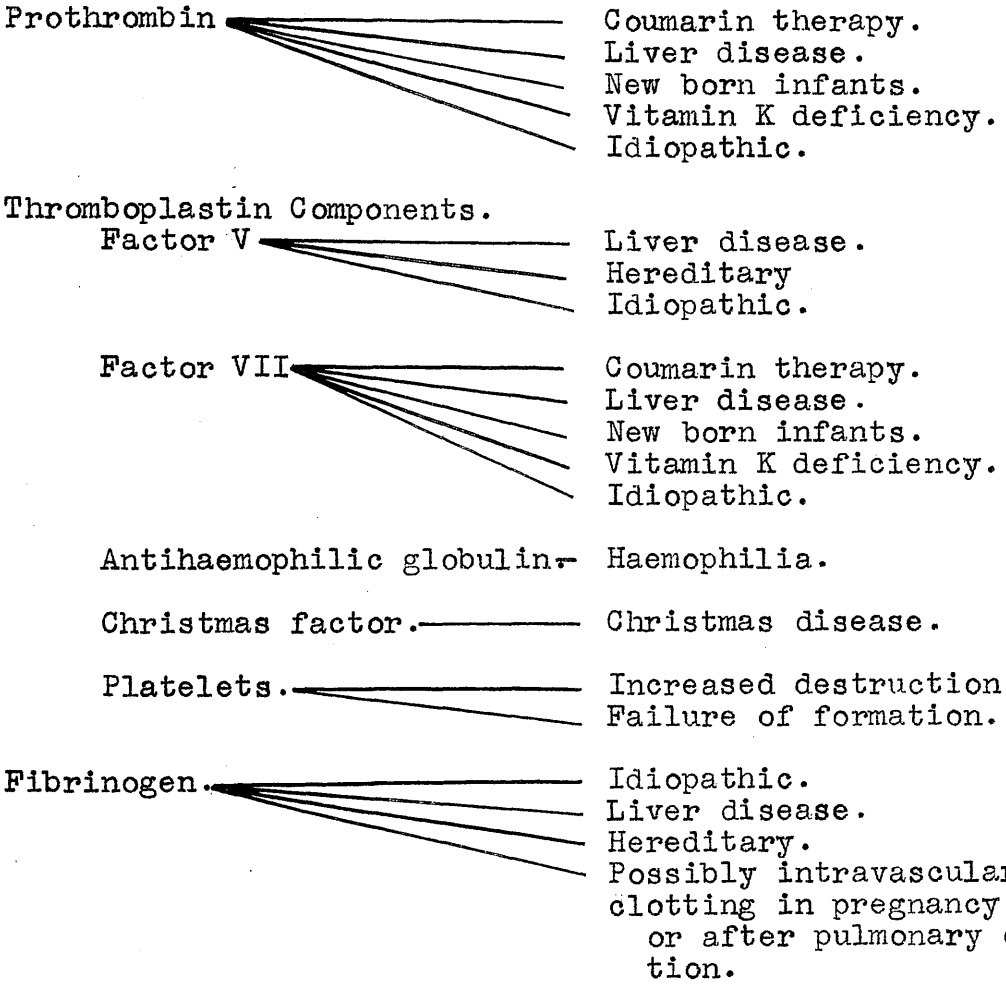
This Table classifies the conditions into those which have predominantly an abnormality of the platelets or capillaries and those where there is evidence of interference with the coagulation mechanism.

(a) Platelet-Capillary Disorders.

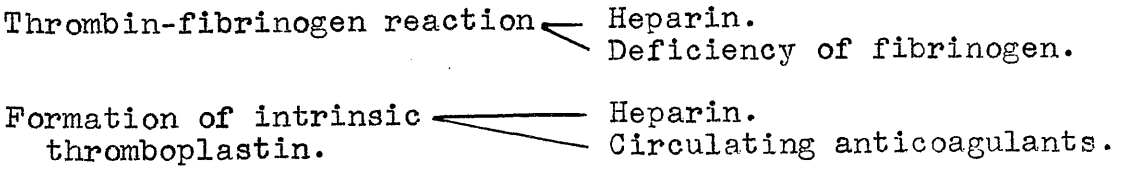
Quantitative Platelet Deficiency. (Thrombocytopenia)		Increased destruction.
		Failure of formation.
Qualitative Platelet Defect.		Rare condition with, at present, poorly defined characteristics.
Capillary Defects	Hereditary	Diffuse Teleangiectasia.
	Scurvy.	

(b) Disorders due to Interference with Coagulation.

(i) Deficiency of coagulation factors.



(ii) Interference with reactions within the coagulation mechanism.



(iii) Destruction of fibrin.

Active fibrinolysis. ——— Occasional complication of other conditions, e.g.
Parenchymatous hepatic disease.
Prostate carcinoma with metastases.
Pregnancy.
Surgical shock particularly after burn injuries or pulmonary operation.

SUMMARY

(1) Features of significance on clinical assessment of patients with haemorrhagic disease are discussed.

(2) The laboratory procedures employed in diagnosis and investigation are critically reviewed. These are divided into those, which are essential for diagnosis in all cases where a haemostatic defect is considered, and into a second category where the tests are occasionally required depending on the assessment after completion of the screening procedures. The value of certain confirmatory tests is discussed.

The application of the thromboplastin generation test to the diagnosis of disorders of blood thromboplastin formation is considered in detail.

(3) A current classification of haemorrhagic disorders is described together with the aetiology of the abnormalities.

REFERENCES

Biggs,R. and Douglas, A.S.(1953 a). The measurement of prothrombin in plasma. J. clin. Path. 6,15.

_____(1953 b). The thromboplastin generation test. J. clin. Path. 6, 23.

Biggs, R. and Macfarlane, R.G. (1953). Human blood coagulation and its disorders. Blackwell Scientific Publications, Oxford.

Douglas, A.S. and Biggs, R. (1953). The consumption of some components involved in physiological blood coagulation. Glasg. med. J. 34, 329.

Glanzmann, E. (1918). Hereditare hamorrhagische thromboasthenie. Jb. Kinderkeilk. 88, 113.

Ivy, A.C., Shapiro, P.R. and Milwick, P.(1935). The bleeding tendency in jaundice. Surg. Gyn. & Obst.60,781.

Lee, R.I. and White, P.D. (1913). A clinical study of the coagulation time of blood. Amer. J. med. Sci.145,495.

Lempert, H. (1935). A modified technique for the enumeration of blood platelets. Lancet 1, 151.

Merskey, C. (1950). The consumption of prothrombin during coagulation. The defect in haemophilia and thrombocytopenic purpura. J. clin. Path.3,130.

O'Brien, J.R. (1951). The bleeding time in normal and abnormal subjects. J. clin. Path. 4, 272.

Quick, A.J. (1935). The prothrombin in haemophilia and obstructive jaundice. J. biol. Chem. 109, 123.

Quick, A.J. (1938). The haemorrhagic diseases and the physiology of haemostasis. J. Amer. med.Ass,110,1658.

Part V. Platelet-Vascular Disorders

CHAPTER 10

PLATELET DISORDERS

CONTENTS.

CHAPTER 10

PLATELET DISORDERS

As a consequence of my interest in haemorrhagic disorders a considerable number of patients have been seen with thrombocytopenia. My studies in this aspect of the haemorrhagic diseases have yielded little which is new and therefore I will deal briefly with this section. An account of some of these studies has been incorporated in the experimental appendix with particular reference to platelet antibodies. Several months were spent on unsuccessful attempts to demonstrate these in idiopathic thrombocytopenia, while working in Dr. Dacie's department at the Postgraduate Medical School, Hammersmith Hospital, London.

The thrombocytopenic states have in common a decreased number of circulating blood platelets. This reduction in the number of circulating platelets may result from an increased destruction of blood platelets in the peripheral circulation or to a failure of delivery of platelets from the bone marrow. Bone marrow examination can be of considerable help in the differentiation of these two types of thrombocytopenia. In the patients where there is an increased peripheral destruction of platelets there is likely to be a normal or often increased number of megakaryocytes in the

bone marrow. Where there is failure of delivery of platelets from the bone marrow, it is likely that there will be a reduced number or absence of megakaryocytes. Though this in general is true in my experience it is often difficult to determine from a marrow smear or section, whether there is a normal number of megakaryocytes or otherwise. Sometimes the megakaryocytes are normal in number and appearance though the thrombocytopenia is non-regenerative. Drug thrombocytopenia can also be due either to increased peripheral destruction or failure of formation.

IDIOPATHIC THROMBOCYTOPENIA.

The patients with this condition can in general be divided into acute and chronic categories. The acute cases are numerically more frequent than the chronic.

ACUTE THROMBOCYTOPENIA.

The acute form was seen more often in childhood though occasionally adults were in this category. In the condition in childhood it is sometimes possible to obtain a history of an acute infection; in one child there was a possible relationship to a primary tuberculous infection. Bleeding usually occurred into the skin or from any of the mucous membranes particularly the nose and mouth and sometimes from other parts of the gastro intestinal tract, from the genito-

urinary tract or into the peritoneum. There were the usual accompanying phenomena of increased bleeding time, positive tourniquet test and impaired prothrombin utilization. The duration of the illness was frequently a few weeks, the majority of the cases being thrombocytopenic for 2-4 weeks, when the condition spontaneously recovered, never to return. A few remained thrombocytopenic and became cases of chronic thrombocytopenia.

CHRONIC THROMBOCYTOPENIA.

This was more common in adults than in children but a few cases of the chronic variety were seen in childhood. It has a course of months and years with alternating remissions and relapses. The characteristic complaint in these patients was of spontaneous bruising of the legs and arms and to a lesser extent of the body. Bleeding from mucous membranes was less common but occurred particularly from the nose and can come from the gastro-intestinal tract. Profuse menstrual loss was common. Women were more commonly affected than men and menorrhagia may well be the presenting complaint, the history of bruising being absent or minimal. In only a small number of patients was the spleen palpable. In the relapse period the platelet count was often found to be below 10,000 per cu. mm. but even when in remission the count was frequently below the lower limit of normal for the

method (180,000 per cu. mm.) - a figure of 100,000 to 120,000 being common.

PLATELET AGGLUTININS AND LYSINS.

Recent interest in this disease has centred on the demonstration of platelet agglutinins and lysins. One of the earliest indications that the syndrome of thrombocytopenic purpura might be due to a factor in the blood stream, which destroyed the platelets, came from the experimental work of Bedson; in 1922 he showed that the injection of guinea pig platelets into rabbits resulted in the development of a platelet antibody. The injection of this antiplatelet serum into guinea pigs caused thrombocytopenia and damage to the capillary endothelium of these animals. Evans and Duane (1949) observed that some patients with acquired haemolytic anaemia had persistent neutropenia and thrombocytopenia. They suggested that since acquired haemolytic anaemia is due to a demonstrable agglutinin, the thrombocytopenia and granulopenia might have a similar cause. They later demonstrated a thrombocyte agglutinating factor (Evans, Takahashi, Duane, Payne and Lin 1951).

Studies on the survival of transfused platelets appear to support the hypothesis that the platelets in idiopathic thrombocytopenia are destroyed abnormally rapidly. It has been shown that, in thrombocytopenia due to bone marrow

replacement or hypoplasia, the platelets survive for about two to four or five days (Stefanini, Chatterjea, Dameshek, Zammas and Santiago 1952, Stefanini, Dameshek and Adelson 1952, Stefanini and Dameshek 1953) but in cases of idiopathic thrombocytopenia the platelets often disappear in 30 mins. to 24 hours (Stefanini et al 1952, Spague, Harrington, Lange and Shapleigh, 1952).

Further and more convincing evidence of an antiplatelet factor in this disease came from the observations of Harrington and his colleagues. (Harrington, Minnich, Hollingsworth and Moor 1951). The transfusion of blood from patients with idiopathic thrombocytopenic purpura into normal individuals often caused a rapid fall in platelet count in the recipient, occasionally with actual purpura. This occurred both before and after splenectomy in the patients acting as donors, even when in these donors the platelet count had returned to normal following the splenectomy. Harrington et al (1953) found, subsequent to their earlier investigations, that blood from patients who had previously been transfused or who had been pregnant could cause a fall in the platelet count when given to normal recipients. The interpretation of these findings is rendered difficult on account of the observation by Stefanini and Chatterjea (1952) and of Wilson Eisemann and Chance (1952) that the transfusion

of normal blood or plasma to healthy recipients will cause a fall in the platelet count. Harrington et al (1953) have demonstrated platelet agglutinins in two thirds of their patients and Stefanini, Plitman, Dameshek, Chatterjea and Mednicoff (1953) in a quarter of their patients.

In my experience the demonstration of these antibodies has not been satisfactory. The results of the experimental work carried out on this at the Postgraduate Medical School in London are described in detail in the appendix, pages 832–852, but despite several months' full time work on the problem I was not satisfied that I could demonstrate these antibodies. The simplest technique used was a mixture of low spun normal plasma with high spun plasma from the patient. This was examined naked eye and under the microscope for agglutination. The anticoagulant was sequestrene which is said not to be anticomplementary, as is sodium citrate. The tests were tried under various conditions of temperature and pH. The effect of alteration of pH by the addition of HCl was tested. It was found that this gave false positives on account probably of some precipitation of protein to which the platelets adhered. On the principle that these antibodies might be incomplete and analogous to certain rhesus antibodies the effect of the addition of bovine albumin was tried. This again resulted in false positive results.

Stefanini et al 1953 and Fluckiger, Härsig and Koller (1953) applied the principle of the red cell Coombs' test (Coombs , Mourant and Race 1945) to platelets. I have tried this but the difficulties of washing platelets even with the help of a refrigerated centrifuge are such that the suspension of platelets to which the Coombs' serum was to be applied, was often showing agglutination to a variable degree, before use in the test. Serum tended to give more false positives even than the plasma. The adsorption of the sera with alumina resulted in fewer false results than were obtained from the untreated serum. A technique employing the principle of complement fixation was tried (see page 836 of the appendix). This gave negative results. Recently a technique has been described using tanned red cells. The principle of the method is that the tanned red cells become coated with the platelet antigen by exposing them to a suspension of platelets which have been alternately frozen and thawed. When suspended in a potent antiplatelet serum such cells undergo agglutination. I have not tried this method but in general I think that there is more hope from this than from techniques where one is dependent on the agglutination of red cells, rather than of platelets, as the end point.

TREATMENT OF IDIOPATHIC THROMBOCYTOPENIC PURPURA.

The patients described in the appendix illustrate some of the problems of management in this condition. In former times emergency splenectomy was not infrequently required in this condition, but the introduction of A.C.T.H. and cortisone has reduced the need for this. Splenectomy is generally not indicated in acute thrombocytopenia, but if the course is longer than four to six months then it will have to be considered. There is ever present with the thrombocytopenia the risk of cerebral haemorrhage and this is an important consideration in the management.

Splenectomy is indicated in the chronic variety of the disease. In rare examples of the acute variety it may be required, where cortisone has failed to meet the need of the case. The very variable result of splenectomy is illustrated by the case reports. In some patients there was a considerable rise in the platelet count following splenectomy, but by some months later they were markedly thrombocytopenic again, though often clinically asymptomatic. In general the patients are better following the splenectomy than they were before even though no sustained rise in platelet count has occurred. In others subjected to splenectomy there was a satisfactory rise and maintained rise in the platelet count.

ROLE OF CORTISONE AND A.C.T.H.

The cases seen provide confirmatory evidence that cortisone and A.C.T.H. have no appreciable action in correcting thrombocytopenia, but that they have an effect in reducing the bleeding time and a definite place in the management of the acute thrombocytopenic incident. They should be used prior to splenectomy but discontinued immediately before the operation. Cortisone can be restarted if haemorrhage recurs, but the deleterious effect on wound healing has been observed and if possible the hormones should not be used after the operation.

NEONATAL THROMBOCYTOPENIC PURPURA.

Occasionally an infant is born with thrombocytopenic purpura to a mother who has or has had idiopathic thrombocytopenic purpura. This phenomenon probably represents the best available evidence of the existence of platelet antibodies in idiopathic thrombocytopenic purpura. The infants would appear to be thrombocytopenic on account of transmission of an antiplatelet factor across the placenta from the maternal to the foetal circulation. The thrombocytopenia in the infants is transient.

In most of the reported cases the mother has had thrombocytopenic purpura (Sanford, Leslie and Crane 1936, Davidson

1937, Waters 1946, Talmadge and Berman 1947, Stroebel, Campbell and Hagedorn 1949, Epstein, Lozner, Cobbey and Davidson 1950, Litchfield, Sternberg and Zweifler 1950, Robson and Walker 1951), Harrington et al (1953) have investigated eight infants with neonatal purpura born to three mothers each of whom had undergone splenectomy for thrombocytopenic purpura.

Very occasionally the mother has never shown either thrombocytopenia or purpura and has never been given a transfusion. Occasionally a Rhesus incompatibility accompanies the thrombocytopenia.

Three examples of this condition have been seen. Only one of these was investigated completely but all three were tested for the presence of platelet agglutinins with inconclusive results. In two of the patients there was thrombocytopenic purpura while in the third there was no thrombocytopenia in the mother suggesting a platelet group incompatibility between mother and foetus. One patient is described below the other two in the appendix.

MRS. A. A. Age 21.

The patient was attending an antenatal clinic in her first pregnancy. Two months before delivery the clinic doctor noticed petechial spots on the patient's arms after taking her blood pressure. She was delivered without undue

bleeding but the child was observed to have some bruising of the legs and feet 3 days after birth. She was first seen by me one month after the delivery of the child complaining of petechiae spots which had been present since parturition. She was admitted to hospital and treated with A.C.T.H. and subsequently splenectomy with satisfactory clinical response but remained thrombocytopenic. In a second pregnancy she continued to be thrombocytopenic and was delivered of a thrombocytopenic and purpuric infant. The counts on the mother and the child were: Platelet count in thousands

		Mother	Child
Day after delivery		10	10
Puerperium	2	16	14
	4	23	67
	7	31	50
	10	56	45
	24	50	138

No platelet antibodies were demonstrable in the mother or child.

The problem sometimes arises as to whether splenectomy should be carried out in the thrombocytopenic mother after initiation of pregnancy. In general this does not seem to influence the incidence of thrombocytopenia in the child. If chronic thrombocytopenic purpura is diagnosed before the

fifth month it is advisable to go ahead with splenectomy but if not until after the fifth month then it is probably advisable to postpone it until two months after completion of the pregnancy. A.C.T.H. is well tolerated should there be any bleeding during pregnancy.

THROMBOCYTOPENIA INDUCED BY DRUGS.

It is now appreciated that drug thrombocytopenia may be of two types. The one is due to damage to the marrow by the drug and is a consequence of the failure to deliver platelets into the circulation. The withdrawal of the offending drug may or may not be followed by slow recovery of the thrombocytopenia.

Gold, Arsenic P.A.S., Streptomycin, Butazolidine and Nitrogen Mustard.

In the first category of drug thrombocytopenias examples have been seen as follows:-

Gold - in the treatment of rheumatoid arthritis.

Arsenic - organic arsenicals in syphilis.

P.A.S. and Streptomycin in the management of pulmonary tuberculosis.

Butazolidine in rheumatoid arthritis.

Temporarily after nitrogen mustard.

(see appendix page 852)

The second type of drug thrombocytopenia is exemplified

by sedormid or quinidine thrombocytopenia and is due to an entirely different mechanism where peripheral destruction of the platelets is the essential feature. In this type of purpura withdrawal of the drug is followed by rapid return of the platelet count to normal. The most likely explanation of these findings would appear to be that the drug unites with platelets and, acting as a hapten confers antigenic properties upon them. Thrombocytopenia in this type of drug purpura is therefore thought to be the result of lysis of the platelet-drug antigen by antibody and complement, the antigen being formed whenever the drug is taken. In addition in this group of drug purpuras there is probably an independent capillary lesion. Platelets and capillaries seem to have some antigenic similarity and it would seem reasonable to suppose that the capillary damage in sedormid purpura is due to the action of the antibody which causes platelet lysis the drug having combined with the endothelial cells to form a further similar antigen. This capillary effect can be demonstrated by skin testing with the drug when a purpuric area results at the region of application of the test. In this group of patients the agglutination of the patient's platelets "in vitro" in the presence of the drug can be demonstrated in the plasma subsequent to recovery from the thrombocytopenic incident.

This type of purpura has been very thoroughly investigated by Ackroyd (1955) in relation to Sedormid purpura.

Quinine.

In this second category only one case has been seen - a case of quinine thrombocytopenia. I made only preliminary assessments on this patient and the case was subsequently investigated in much greater detail and reported on by Bolton and Young (1953). (see appendix page 853)

Dextran.

An interesting patient was seen who developed marked thrombocytopenia following dextran infusion. This was transient recovering in 48 hours. The exact mechanism was not determined but the following was an account of the case.

Haemorrhage following administration of dextran.

A boy aged 10 with Type II nephritis was given 3 pints of dextran. He began to bleed from the nose and blood collected by venepuncture failed to clot. He was seen by me 3 hours later. Specimens collected revealed that he was markedly thrombocytopenic - 6000 per cu. mm., bleeding time was 17 minutes and he had a moderately positive Hess test. There was a plasma and serum defect of thromboplastin generation. Reassessment 72 hours later showed that all these abnormalities had been corrected.

12/1/55.

	1	2	3	4	5	6
Normal ads. plasma. Normal serum.	68	28	11	9	9	10
Normal ads. plasma. Patient serum.	72	60	41	29	20	19
Patient ads. plasma. Normal serum.	70	45	29	20	17	16
Patient ads. plasma. Patient serum.	47	49	44	40	37	37

Platelet count 6,000 per cu. mm.
Bleeding time - 17'
Hess test - mild positive.

15/1/55.

Normal ads. plasma. Normal serum.	8	8	9	9	9	9
Patient ads. plasma. Patient serum.	20	9	8	8	8	9

Platelet count 446,000 per cu. mm.
Bleeding time - 4'
Hess test - negative

Thrombin-fibrinogen reaction - Normal 18"
Patient 19"

Intravenous infusion of dextran has been accepted as a satisfactory plasma volume expander and it has been used more recently in the treatment of nephritis with oedema. It is used as 6% dextran in saline and is available commercially.

The first report of abnormal haemorrhage after the use

of this material was by Carbone et al (1954). Their patient suffered from epistaxis and melaena. James et al (1954) reported epistaxis and haematuria and Jaenicke and Waterhouse (1955) also reported on abnormal haemorrhage.

Ricketts (1952) showed by "in vitro" experiments that a precipitate of dextran and fibrinogen was formed using about 10 per cent of the available fibrinogen. When dextran is sulphated the resultant dextran sulphate possesses anticoagulant properties similar to heparin (Walton 1951, 1952). There is no definite evidence that the abnormal haemorrhage following dextran infusions is a manifestation of the sulphation of the dextran.

Scott (1955) incriminates dextran as responsible for precipitating coagulation failure in certain obstetrical emergencies. He suggests that in this condition there is dilution of fibrinogen by the dextran and a diminution of the remaining concentration by formation of an insoluble complex with fibrinogen.

Carbone et al (1954) and Jaenicke and Waterhouse (1955) report prolongation of the bleeding time in those patients with a haemorrhagic tendency following dextran infusion. Adelson (1955) found that 40% of normal humans develop prolonged bleeding time after the infusion of 1000 ml. of commercial dextran and believed that the dextran effect was on

the platelets.

THROMBOCYTOPENIA FOLLOWING RADIATION.

One patient has been seen in whom thrombocytopenia developed after radiation given to the chest for the treatment of asthma. This was a married woman of 38 years of age, who died as a consequence.

THROMBOCYTOPENIA FOLLOWING BURNING.

A 10 year old girl developed thrombocytopenia three days after an extensive burn of the legs, one arm and trunk. The mechanism of production of this again was obscure but it may have a similar mechanism to the haemolysis which follows burns, - damage to the blood elements as they pass through the area of burning.

THROMBOCYTOPENIA IN PERNICIOUS ANAEMIA, AND ITS RESPONSE TO VITAMIN B_{12} THERAPY.

Two patients were studied. Both of these were very severe cases of Addisonian Pernicious Anaemia with red cell counts below one million per cu. mm. In figure 94 is shown the mean result from these two patients. The rise in the platelet count began the day after the start of the rise in the reticulocytes and proceeded very rapidly thereafter rising to normal levels within three days, and being at these levels one week after starting B_{12} therapy. This illustrates the

Figure (94)

Figure (94)

Platelet response to vitamin B_{12} therapy in pernicious anaemia.

Ordinate - Red cell count in millions per cubic mm.

Platelet count in hundred thousands per cubic mm.

Percentage reticulocytes.

Percentage haemoglobin.

Abscissa - Time in days after starting vitamin B_{12} therapy.

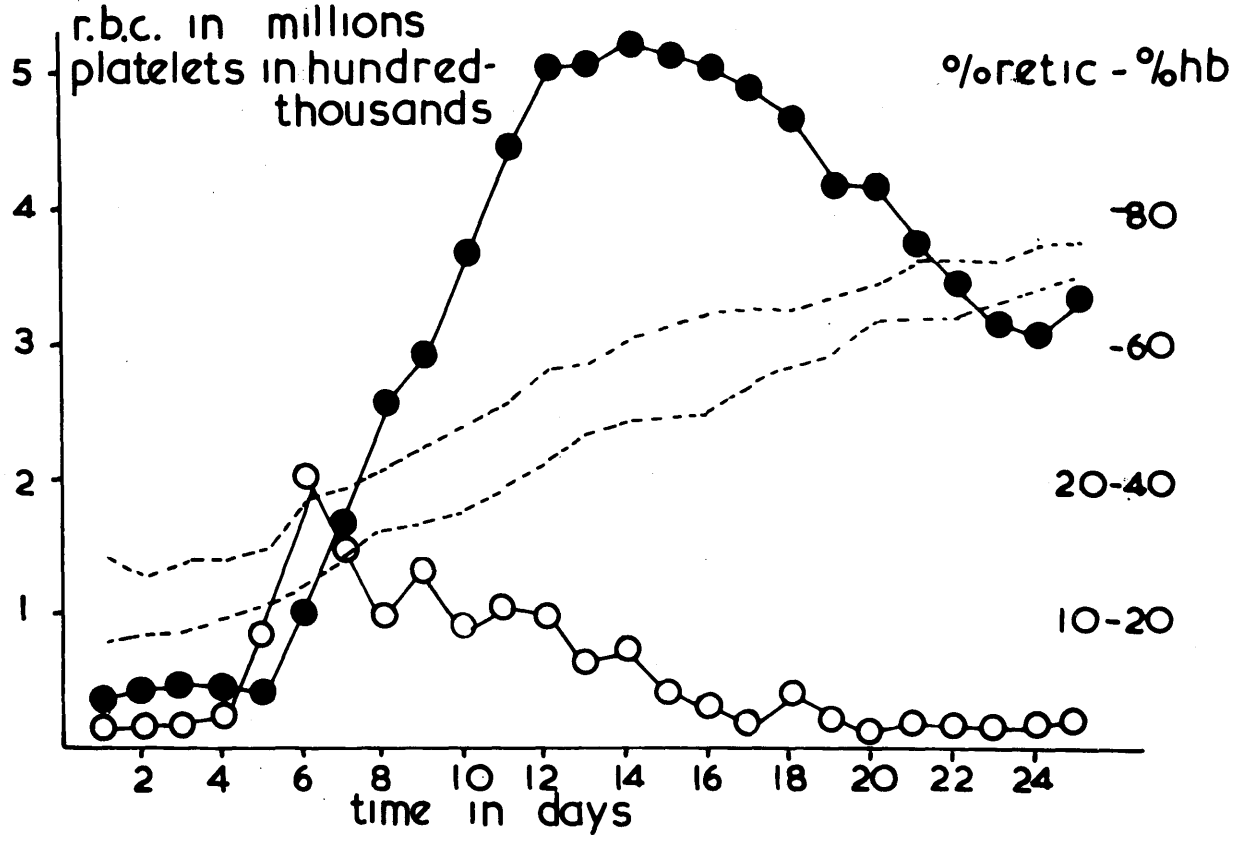
Mean of the results on two patients.

●——● platelet count.

O——O percentage reticulocytes.

------ upper - percentage haemoglobin.

------ lower - red cell count.


r.b.c. in millions
platelets in hundred-thousands
%retic - %hb
5
4
3
2
1
80
60
20-40
10-20
2
4
6
8
10
12
14
16
18
20
22
24
time in days

enormous potential for producing platelets, possessed by the marrow. The rise in platelets continued past the normal level so that there is a temporary thrombocytosis; thereafter it falls to normal levels (see Figure 94). This improvement in haemostatic efficiency can be shown on thromboplastin generation as in figure 95 . Platelets are prepared under identical conditions from the same volume of blood at intervals subsequent to the administration of vitamin B_{12}. The improved thromboplastin generation in virtue of the rise in the platelet count is illustrated.

THROMBOCYTOPENIA IN FOLIC ACID DEFICIENT MEGALOBLASTIC ANAEMIA - IN STEAHORRHOEA.
- response to therapy.

One patient was studied who was suffering from a megaloblastic anaemia, which responded to folic acid. There was an accompanying thrombocytopenia which responded in the same way to folic acid as did the platelet deficiency to vitamin B_{12} in pernicious anaemia (see Fig. 96). The details of this patient are given in the appendix page 866-7.

"HYPERSPLENIC" THROMBOCYTOPENIA.

This is a variety of thrombocytopenia often accompanied by anaemia and leucopenia occurring as a complication of splenomegaly in the so called hypersplenic syndrome. The patient described in the appendix page 873 had these features

Figure (95)

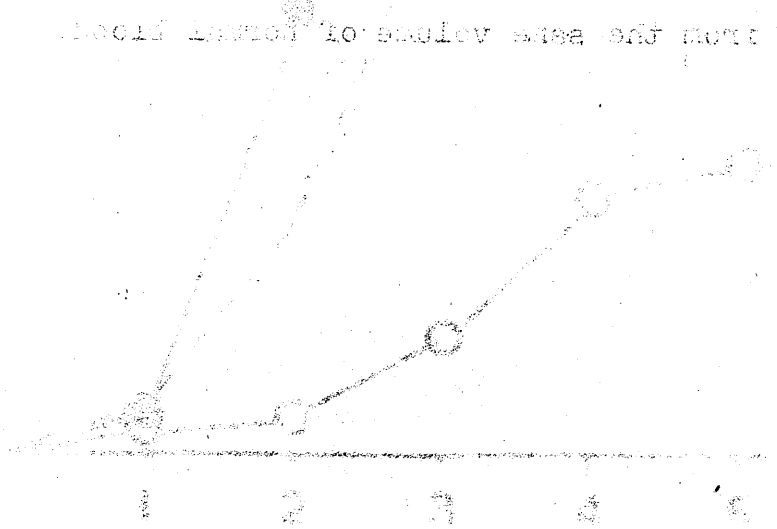


Figure (95)

Platelet response to vitamin B_{12} therapy in pernicious anaemia.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

●——● Thromboplastin formation from platelets collected from a standard volume of blood after response to vitamin B_{12}

O——O Thromboplastin formation from platelets collected from the same volume of blood before starting vitamin B_{12}

----- Thromboplastin formation from platelets collected from the same volume of normal blood.

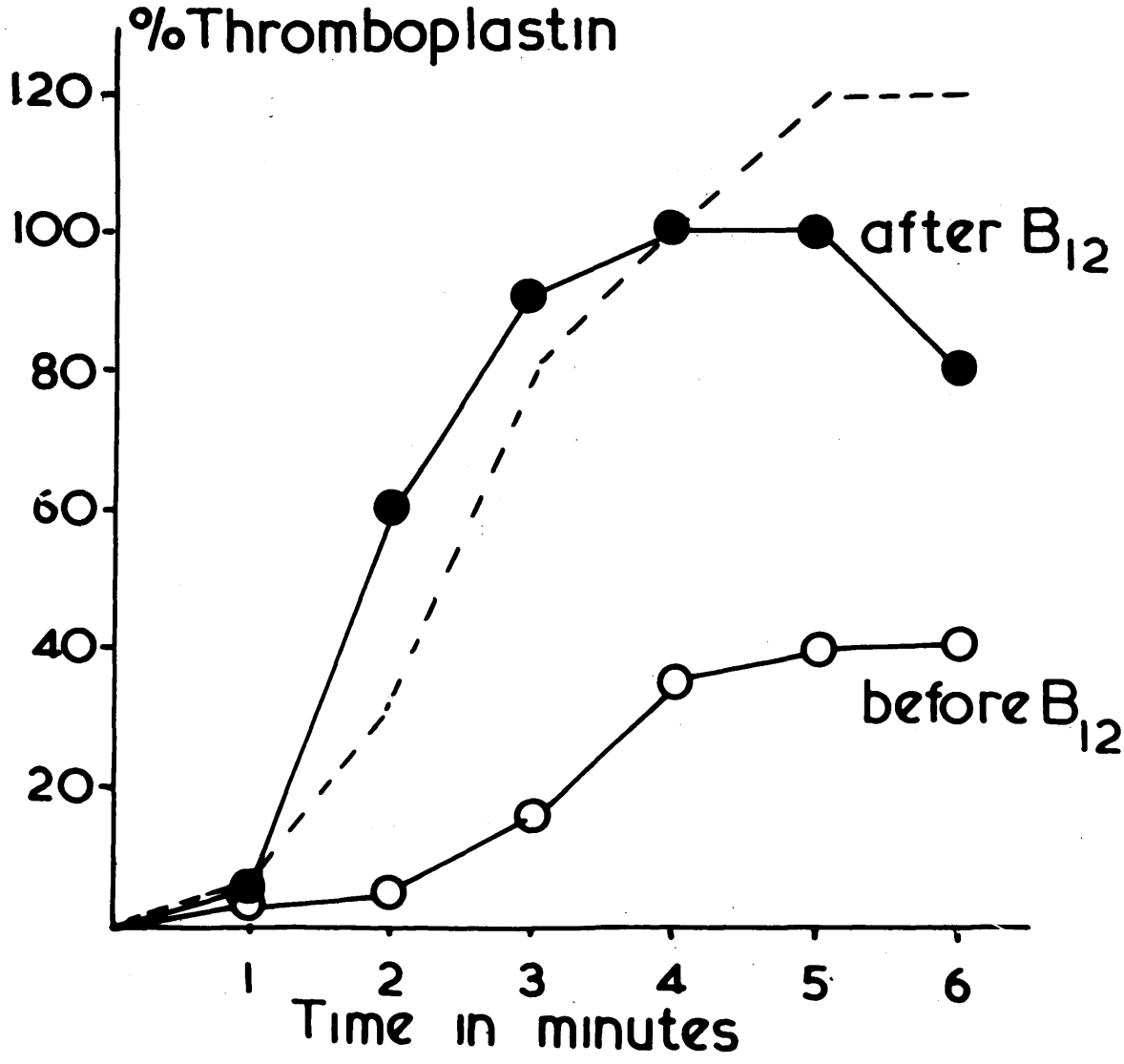

%Thromboplastin
120
100
80
60
40
20
after B_{12}
before B_{12}
1
2
3
4
5
6
Time in minutes

Figure (96)

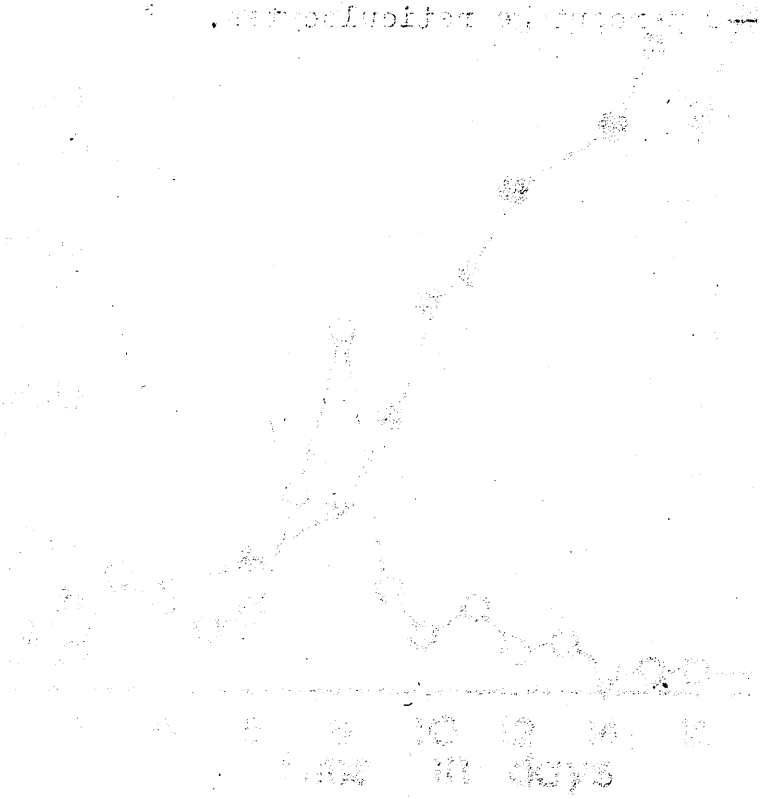


Figure (96)

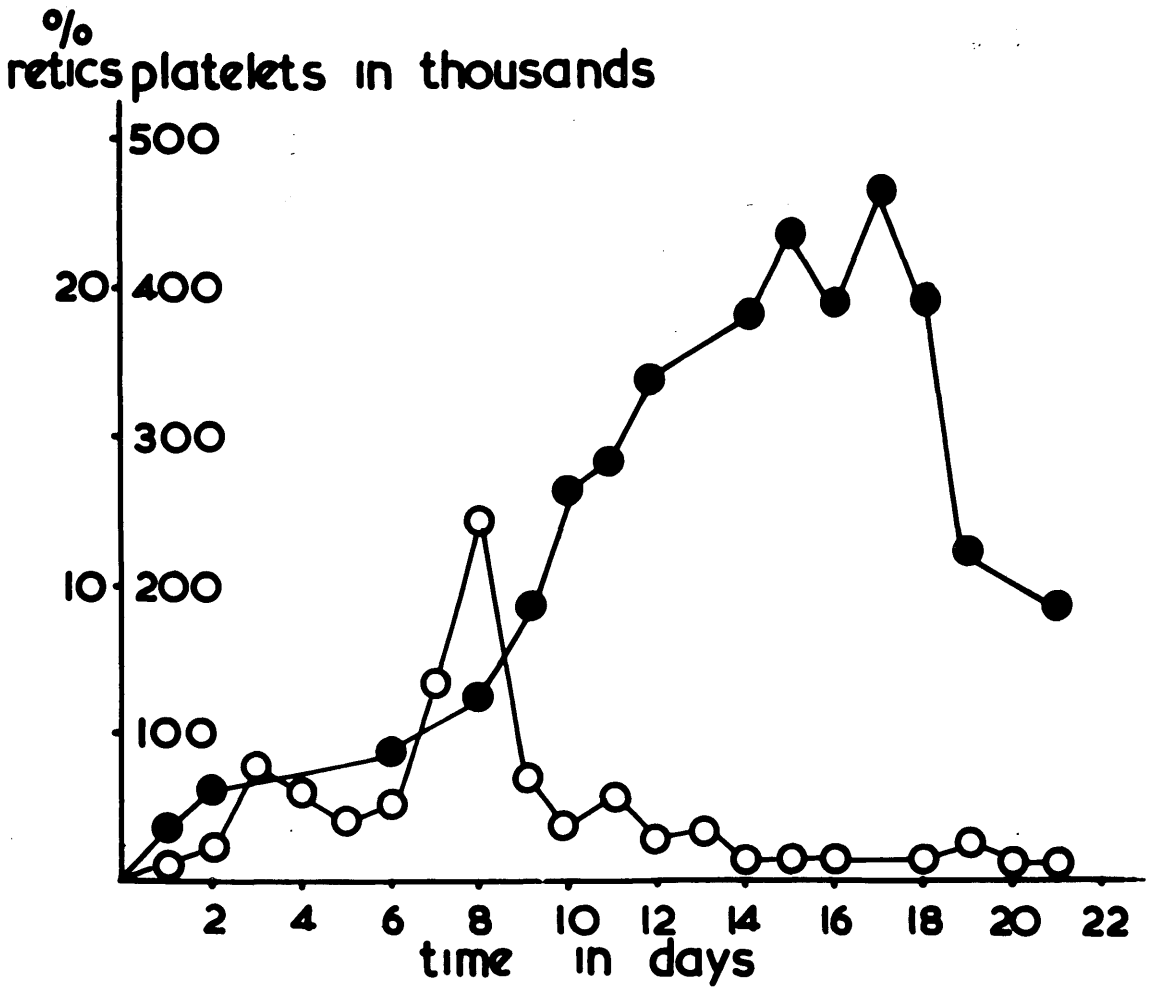
Platelet response to folic acid therapy in folic acid deficient megaloblastic anaemia.

Ordinate - percentage reticulocytes platelet count in thousands per cu . mm.

Abscissa - time in days after starting folic acid therapy.

●——● platelet count.

O——O percentage reticulocytes.


%
retics
platelets in thousands
500
20
400
300
10
200
100
2
4
6
8
10
12
14
16
18
20
22
time in days

as a complication of cirrhosis of the liver with congestive splenomegaly. This patient had leucopenia and anaemia in addition to the thrombocytopenia. Splenectomy was carried out but the patient died eleven days after the operation.

THROMBOCYTOPENIA IN LIVER DISEASE.

Some patients with parenchymatous liver disease have been found to have thrombocytopenia. This is discussed in more detail in the section on liver disease.

THROMBOCYTOPENIA AS THE PRESENTING FEATURE IN LEUKAEMIA.

A male patient (W. McM.) aged 37 years was seen on account of troublesome haemorrhage after tooth extraction which was followed some weeks later by recurrent epistaxis. There was no previous history of any haemorrhagic tendency. He was found to be markedly thrombocytopenic and was treated with cortisone. This reduced the bleeding time so long as it was being administered, but had no effect on the platelet count. Peripheral blood film and sternal marrow biopsy showed no abnormality. After several episodes of bleeding a splenectomy was performed, but without appreciable change in the platelet count. He continued in indifferent health with recurrent epistaxis and progressive anaemia. Sternal marrow biopsy - two years after the first incident of haemorrhage - revealed the features of an acute leukaemia.

Thrombocytopenia in leukaemia is of course a well known and frequently observed phenomenon. This patient, described above, is mentioned because the disease presented as thrombocytopenia without any other criteria, which might have suggested leukaemia. The bone marrow at the onset of the illness was normal.

THROMBOCYTOPENIA IN DIFFUSE LUPUS ERYTHEMATOSUS.

In two patients with diffuse L.E., who also had a haemolytic type of anaemia, there was thrombocytopenia. Since these patients have a propensity for developing antibodies, a quest was made for platelet agglutinins or lysins. Again the results were inconclusive. (see appendix page 869)

CYCLICAL THROMBOCYTOPENIA.

Two examples of this have been seen, one of which was investigated in considerable detail. The thrombocytopenic episodes coincided with menstruation and were accompanied by periods of anaemia and agranulocytosis. The marrow at those times showed aplasia. (see appendix page 870)

OTHER NON-REGENERATIVE THROMBOCYTOPENIA.

These have been seen frequently as a complication of acute leukaemia and less often in marrow failure in aplastic anaemia (including one case of Fanconi type).

THROMBOCYTOPENIA IN ASSOCIATION WITH TUBERCULOSIS

Thrombocytopenia has been seen in association with one case of primary infection in childhood and in two adults where the phenomenon could not reasonably be attributed to chemotherapeutic agents.

QUALITATIVE PLATELET DEFECT

One patient was seen who was believed to have a qualitative abnormality of his platelets. This patient had a splenectomy for a haemolytic anaemia. The blood film subsequent to splenectomy showed very large and morphologically abnormal platelets. They were also present in excessive numbers. He was started on tromexan on account of a pulmonary embolism but started to bleed at a level on the one-stage test, which would not normally have caused bleeding. When equal numbers of this patient's platelets and normal platelets were compared in the thromboplastin generation technique it was found that they were very defective in their ability to form thromboplastin. (Figure 92). The platelet suspensions were prepared from equal volumes of blood and suspended in the same volume of saline. There were twice as many platelets in the patient's platelet suspension as the normal but the ability to form blood thromboplastin, despite this, was less from the patient's platelets. In a subsequent observation, the two suspensions were of equal potency but the

patient's platelets were ten times more numerous than the control.

SUMMARY

Since my investigations of thrombocytopenia have contributed little which is new in a positive sense, this subject has been dealt with, relatively briefly.

(1) Attempts to demonstrate platelet antibodies in idiopathic thrombocytopenic purpura have been unsatisfactory.

(2) Patients with neonatal thrombocytopenia are described.

(3) Non-regenerative drug thrombocytopenias due to Gold, Arsenic, P.A.S. with Streptomycin, Butazolidine, and Nitrogen Mustard are described.

(4) One patient with "platelet-drug-antibody" thrombocytopenia due to Quinine is described. The mechanism is the same as that described by Ackroyd in relation to Sedormid thrombocytopenia.

(5) Temporary thrombocytopenia following dextran infusion is described. This was accompanied by unexplained plasma and serum defects on thromboplastin generation.

(6) Fatal thrombocytopenia following radiotherapy to the chest for asthma, is reported.

(7) Temporary thrombocytopenia after severe burn injuries has been observed.

(8) The response of the platelet count to B_{12} therapy in pernicious anaemia, was studied. The rapidity and extent of the rise in the platelet count once started is of interest. The count rose from 40,000 to 500,000/cu. mm. in six days. The response in the platelet count started about the same time as the reticulocyte count started to rise.

(9) A similar response was observed to folic acid in a folic acid deficient megaloblastic anaemia.

(10) Thrombocytopenia is described as part of the "hypersplenic" syndrome, as a complication of liver disease and accompanying the haemolytic anaemia in diffuse lupus erythematosus.

(11) Investigations of cases of cyclical thrombocytopenia revealed that in at least one of these there was a corresponding cyclical marrow aplasia.

(12) One patient who presented with features acceptable as chronic thrombocytopenic purpura, two years later developed acute leukaemia.

(13) One patient is described who had a functional platelet deficiency on thromboplastin generation.

REFERENCES

Ackroyd, J.F. (1955). Platelet agglutinins and lysins in the pathogeneous of thrombocytopenic purpura with a note on platelet groups.
Brit. med. Bull. 11, 28.

Adelson, E. (1955). The mechanism of the haemostatic defect caused by intravenous dextran.
Clinical Research Proceedings 2, 103.

Bedson, S.P. (1922). Blood platelet anti-serum, its specificity and role in experimental production of purpura.
J. Path. & Bact. 25, 94.

Carbone, J.V., Furth, F.W., Scott, R. Jr., Crosby, W.H. (1954). An haemostatic defect associated with dextran infusion.
Proc. Soc. Exp. Biol. & Med., 85, 101.

Coombs, R.R.A., Mourant, A.E. and Race, R.R. (1945). A new test for the detection of weak and "incomplete" Rh. agglutinins.
Brit. J. exp. Path. 26, 255.

Davidson, L.T. (1937). Congenital thrombopenia.
Amer. J. Dis. Child. 54, 1324.

Epstein, R.D., Lozner, E.L., Cobbey, T.S. and Davidson, C.S. (1950). Congenital thrombocytopenic purpura haemorrhagua in pregnancy and in the newborn.
Amer. J. Med. 9, 44.

Evans, R.S. and Duane, R.T. (1949). Acquired haemolytic anaemia. I. The relation of erythrocyte antibody production to activity of the disease. II. The significance of thrombocytopenia and leukopenia.
Blood 4, 1196.

Evans, R.S., Takahashi, K., Duane, R.T., Payne, R. and Lin, C.K. (1951). Primary thrombocytopenic purpura and acquired haemolytic anaemia; evidence for a common etiology.
Arch. int. Med. 87, 48.

Flückiger, P., Hassig, A. and Koller, F. (1953). Ueber den Thrombocytin - Coombs test.
Schweiz. Med. Wshnschr. 83, 1035.

Harrington, W.J., Minnich, V., Hollingsworth, J.W. and Moore, C.V. (1951). Demonstration of a thrombocytopenic factor in the blood of patients with thrombocytopenic purpura.
J. Lab. clin. Med. 38, 1.

Harrington, W.J., Sprague, C.C., Minnich, V., Moore, C.V., Ahlvin, R.C. and Dubach, R. (1953) Immunologic mechanisms in idiopathic and neonatal thrombocytopenic purpura.
Ann. int. Med. 38, 433.

Jaenicke, J.R., Waterhouse, C. (1955). Metabolic and haemodynamic changes induced by prolonged administration of dextran.
Circulation 11, 1.

James, J., Gardello, G., Metcoff, J. (1954). Effects of infusion of hyperoncotic dextran in children with the nephrotic syndrome.
J. clin. Invest. 33, 1346.

Litchfield, H.R., Sternberg, S.D. and Zweifler, B.M. (1950). Congenital thrombocytopenic purpura of the newborn infant. Report of a case.
J. Pediat. 37, 94.

Ricketts, C.R. (1952) Interaction of dextran and fibrinogen.
Nature 169, 760.

Robson, H.N. and Walker, C.H.M. (1951). Congenital and neonatal thrombocytopenic purpura.
Arch. Dis. Childh. 26, 175.

Sanford, H.N., Leslie, E.I. and Crane, M.M. (1936). Congenital thrombocytopenia.
Amer. J. Dis. Child. 51, 1114.

Scott, J.S. (1955). Blood coagulation failure in obstetrics. Effects of dextran and plasma.
Brit. med. J. 2, 290.

Sprague, C.C., Harrington, W.J., Lange, R.D. and Shapleigh, J.B. (1952). Platelet transfusions and pathogenesis of idiopathic thrombocytopenic purpura.
J. Amer. med. Ass. 150, 1193.

Stefanini, M. and Chatterjea (1952). Studies on platelets IV. A thrombocytopenic factor in normal human blood, plasma or serum. Proc. Soc. Exp. Biol. & Med. 79, 623.

Stefanini, M., Chatterjea, J.B., Dameshek, W., Zannos, L. and Santiago, E.P. (1952). Studies on platelets II. The effect of transfusion of platelet-rich polycythaemic blood on the platelets and haemostatic function in "idiopathic" and "secondary" thrombocytopenic purpura. Blood, 7, 53.

Stefanini, M. and Dameshek, W. (1953). Collection, preservation and transfusion of platelets. New Engl. J. Med. 248, 797.

Stefanini, M., Dameshek, W. and Adelson, E. (1952). Studies on platelets VII. Shortened "platelet survival time" and development of platelet agglutinins following multiple platelet transfusions. Proc. Soc. Exp. Biol. and Med. 80, 230.

Stefanini, M., Plitman, G.I., Dameshek, W., Chatterjea, J.B. and Mednicoff, J.B. (1953). Studies on platelets XI. Antigenicity of platelets and evidence for platelet groups and types in man. J. lab. clin. Med. 42, 723.

Stroebel, C.F., Campbell, D.C. and Hagedorn, A.B. (1949). The problem of essential thrombocytopenic purpura. Med. clin. N. Amer. 33, 1027.

Talmadge, J. and Berman, B. (1947). Congenital thrombocytopenic purpura. J. Pediat. 30, 691.

Walton, K., (1951). Experiments with dextran sulphate as an anticoagulant. Proc. R. Soc. Med., 44, 563.

Walton, K., (1952). The biological behaviour of a new synthetic anticoagulant (dextran sulphate), possessing heparin-like properties. Brit. J. Pharmacol. 7, 370.

Waters, H.W. (1946) Neonatal thrombocytopenic purpura. Amer. J. Obstet. Gynaec. 51, 708.

Wilson, S.J., Eisemann, G. and Chance, J.H. (1952). Effect of the "thrombocytopenic factor" of idiopathic thrombocytopenic purpura on platelet levels as measured by direct and indirect methods.
J. lab. clin. Med. 40, 498.

CHAPTER II

V A S C U L A R D E F E C T S

CONTENTS.

CHAPTER 11

VASCULAR DEFECTS

Physiological haemostasis is dependent on normal vascular function and an efficient coagulation mechanism. The relative importance of each of these in the normal control of haemorrhage from injury is difficult to estimate. The present account deals with those diseases where there is evidence of failure in the vascular wall rather than in the coagulation mechanism itself. As investigation of these disorders has added little, which is new the subject is described only briefly.

HEREDITARY HAEMORRHAGIC TELANGIECTASIA.

This is a vascular abnormality characterised by the presence of visible dilatations of capillaries and arterioles producing characteristic telangiectatic areas; there is gross dilatation of the capillaries in some parts whereas other parts are apparently normal. The abnormal areas are most commonly found on the tongue, nose and face. Less common sites of localisation are the finger tips, toes, ears, trunk and conjunctivae. At autopsy on one patient extensive teleangiectatic areas were seen in the large bowel.

The condition is transmitted as a simple dominant by both sexes. An example of a family tree of one of the families

seen is shown in figure 97.

The telangiectatic areas are found as bright "red point" lesions or sometimes as "spider type" lesions. Epistaxis is the most common form of bleeding, followed by haemoptysis, melaena and haematuria. Severe hypochromic anaemia often accompanies the condition (see appendix page 876). The condition generally becomes more troublesome as the patient grows older. This may be due to the decreasing elasticity and contractility of the blood vessel wall.

Most cases present skin lesions and when these are sought the diagnosis is not difficult. Occasionally, however, the skin lesions are minimal or absent and under these circumstances the diagnosis is more difficult.

Investigation of the coagulation mechanism in these patients failed to reveal any abnormality.

Treatment is by protection of the telangiectatic areas from injury and direct pressure can be applied where possible to the areas of haemorrhage. Electrocoagulation and radium can be used locally for example in the nose, but repeated application of these methods is liable to result in perforation of the septum. In the cases reported here I have not seen serious bleeding from the gastro-intestinal or renal tracts but such has been reported. Occasionally surgery may be required. Nephrectomy and resection of the bowel have

Figure (97)

Figure (97)

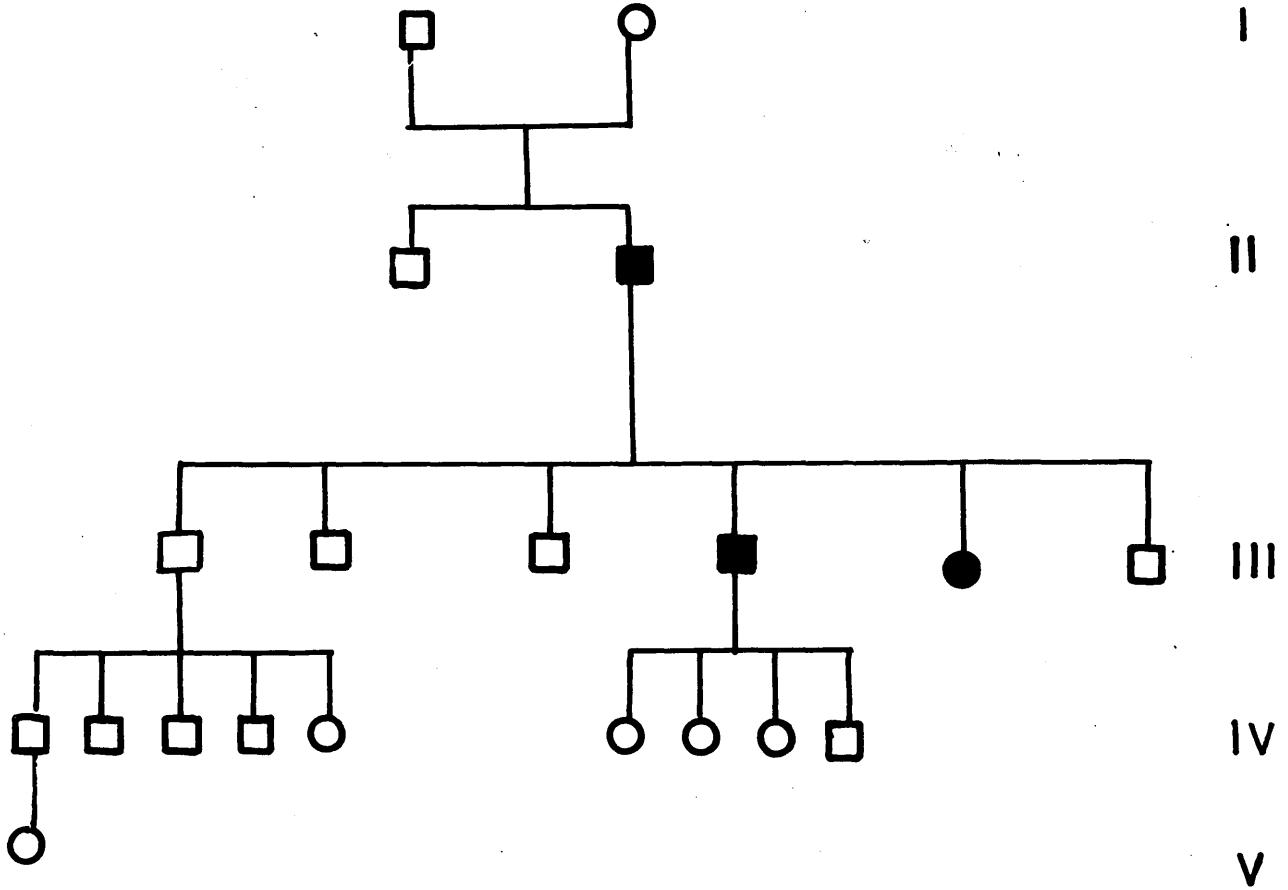
Family tree in hereditary telangiectasia.

□ unaffected male.

■ affected male.

O unaffected female.

● affected female.


I
II
III
IV
V

been required in some of the cases reported in the literature.

SPIDER NAEVI IN LIVER DISEASE.

These in appearance are not unlike the teleangiectatic areas in hereditary teleangiectasia though the central area in the hepatic telangiectasis is not so prominent as in those in the hereditary condition. The distribution is also different as the telangiectasis in liver disease does not occur, so far as I have seen, on the tongue or lips or fingers or inside the nose. The spider naevi of hepatic disease are said always to occur on the upper part of the trunk, face and arms. This is not so, as I have seen patients with hepatic disease who have these lesions over other parts of the body. They do not present a problem of haemostatic control. Abnormal bleeding may of course occur in liver disease but the telangiectatic areas are not the sites of haemorrhage.

DIFFUSE VASCULAR DEFECTS.

(von Willebrand's disease; Vascular pseudohaemophilia). This is a haemorrhagic diathesis, where a grossly prolonged bleeding time is the essential demonstrable abnormality. The term "diffuse vascular defect" is preferred because von Willebrand's disease probably includes many different entities and pseudohaemophilia has been applied to almost every bleeding disease of undefined aetiology.

Two cases of this condition have been seen and the details of these are given in the appendix.

Capillary Microscopy

The fundamental abnormality in vascular pseudohaemophilia is not clearly understood - Macfarlane (1941) attributed the abnormal bleeding in this disorder to a capillary defect, after demonstrating by direct visualization that the nail bed capillaries in patients with pseudohaemophilia appeared bizarre and distorted and failed to constrict normally after puncture. These findings have not been confirmed in all cases (Buchanan and Leavell 1956). The capillaries of the two cases seen by me showed distortion, the tips of many of the loops being bulbous and coiled. Normally the arterial limb of the loop has a calibre much narrower than the venous limb of the loop. In this disease the relationship between the arterial and venous sides of the loop is often lost, the afferent limb being the same calibre as the efferent. Whether this represents the fundamental abnormality is not known.

Coagulation Mechanism

Buchanan and Leavell (1956) in a review of the literature and an account of their own cases, recorded that 12 patients had defective prothrombin consumption and in other 12 it was normal. The nature of the coagulation abnormality in these

patients has not been established by the more modern techniques. Bernard and others (1953) offered evidence that the defective prothrombin consumption in pseudohaemophilia represented a platelet defect, since they were able to correct the prothrombin consumption deficiency in plasma of one of these patients by the addition of isolated normal platelets. They were not able to correct the defective prothrombin consumption in deplateletized normal plasma by addition of the platelets or plasma from a patient with pseudohaemophilia, although this was corrected by the addition of normal platelets. In chapter 15 patients are described who have a prolonged bleeding time in association with A.H.G. deficiency. It is possible that some of the reported cases of pseudohaemophilia with defective prothrombin consumption may be similar.

Neither of the two cases seen by me showed any deficiency of prothrombin consumption. There was no demonstrable abnormality in the coagulation system. If anything the patients' thromboplastin mechanism was even more efficient than the normal, in virtue of associated thrombocytosis.

As another explanation of the abnormal bleeding in this disease a qualitative platelet defect has been postulated. Upon agglutination and lysis the platelets are thought to release a vasoconstrictor substance. This latter platelet function might reconcile the opposing ideas of a vascular

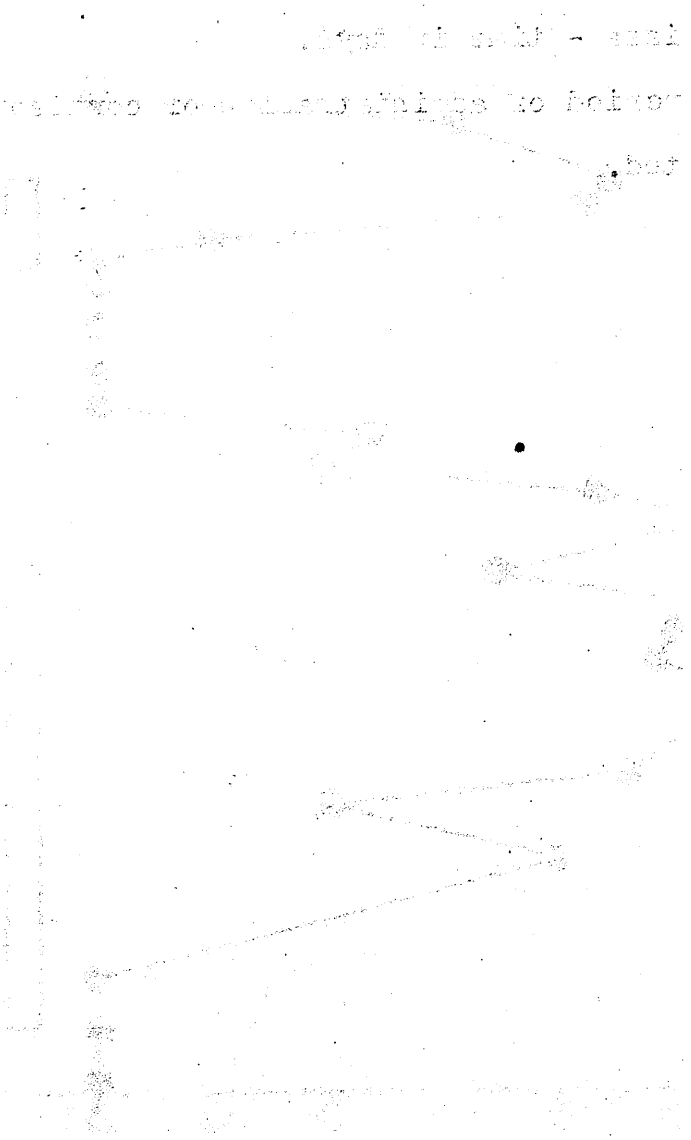
versus a platelet defect in pseudohaemophilia. Bigelow (1954), however, has reported normal serotonin activity in the plasma of two patients believed to have pseudohaemophilia.

The studies on this condition are therefore somewhat conflicting and suggest that not all patients with pseudo-haemophilia have the same defect in haemostasis. Whether the patients in this group represent a disease with a common aetiology, or a syndrome with a common defect in haemostasis or an even more heterogeneous group, remains to be determined.

In the patients recorded the hereditary pattern of the condition is difficult to establish. There is some evidence that the inheritance is as a dominant sex-linked character since children of either sex from an affected parent may show the abnormality. In the cases described here, one had a negative family history, while in the other it was indefinite - possibly a maternal uncle had the condition.

Both the patients suffered from epistaxis and from less frequent incidents of gastro-intestinal bleeding. One of them experienced troublesome bleeding from the gums. The patients have a tendency to bruise, but they do not develop the massive haematomata seen in the haemophilic patient. Dental extraction or other minor operative procedures may result in serious bleeding but again this is not so troublesome as in the patient with a coagulation defect.

Figure (98)

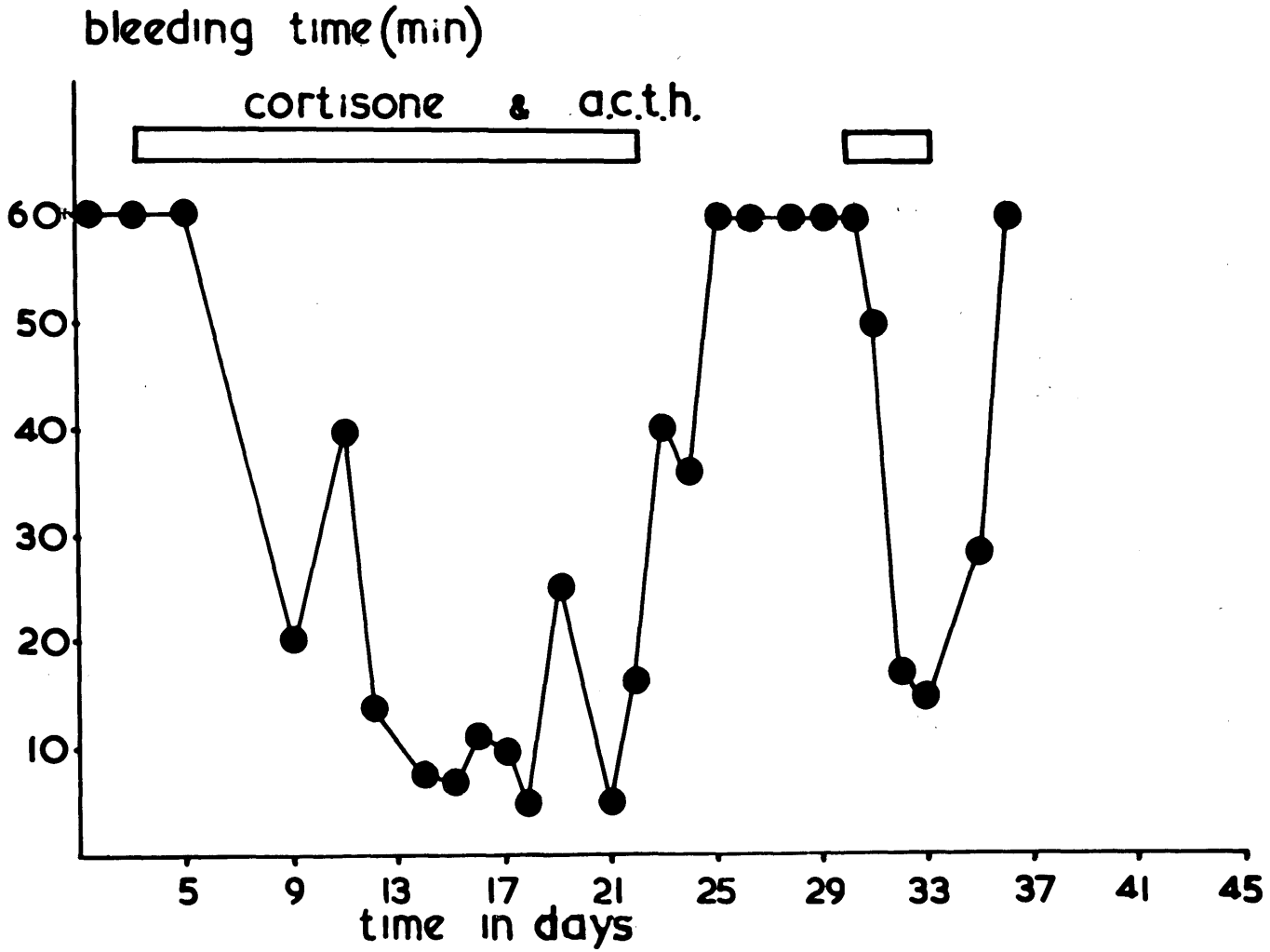


Effect of cortisone on the bleeding time in diffuse capillary defect.

Ordinate - bleeding time in minutes.

Abscissa - time in days.

The period of administration of cortisone is indicated.


bleeding time (min)
cortisone & a.c.t.h.
60
50
40
30
20
10
5
9
13
17
21
25
29
33
37
41
45
time in days

Treatment: The management of haemorrhage in these patients should consist in the first place of the application of local pressure where this is possible. In the appendix (page 879) and figure 98 is shown the effect of Cortisone on the bleeding time in one patient. It would appear on this single observation to have had considerable effect on the bleeding time and would be worthy of trial in prolonged bleeding in this condition, or to cover an operative procedure such as tooth extraction.

MIBELLI'S SYNDROME (DIFFUSE ANGIOKERATOMA).

This is a very rare vascular anomaly and the two patients described here have been reported by Brown and Milne 1952. The patients were half brothers born of the same mother but having different fathers. The condition is a congenital vascular lesion which has the appearance of a purpura-like skin rash of a fixed type. The lesions however are not purpuric but can be emptied by firm maintained pressure with a glass slide.

Case (1). Age 30. The red rash had been observed from the age of 7 years at which time it had appeared with extraordinary rapidity and had gradually become more obvious and extensive during the next 23 years. The rash had started on the lower abdomen and inguinal regions extending on to the upper parts of the thighs, later spreading to involve more

of the thighs and most of the trunk. Injury to some of the spots during childhood had resulted in troublesome haemorrhage, but there had not been a serious tendency to abnormal bleeding. A feature of the patient's condition had been a burning paraesthesia in the fingers and toes. There had been a recent cerebral incident with vertigo, vomiting and numbness on the left side of the body, shortly before the patient was first seen.

On examination he was a mentally defective man of very poor physical development. In addition to the skin manifestations there was a palpable spleen, and a hypochromic, iron deficiency type of anaemia. The neurological lesion was in the pons involving the sensory nucleus of V and adjacent area. The skin lesion was the most striking feature of this case. On the skin of the proximal part of the thighs, on the buttocks, on the penis and scrotum and on the ulnar aspects of the forearms, there were numerous small red or bluish red spots. These were most numerous and prominent on the lower part of the trunk and over the costal margin. They were also particularly numerous over certain prominences such as the vertebral column in the lumbar region and the iliac crests. There were a few small spots on the inner aspect of the lips. Capillary microscopy of the skin revealed that each spot was a small sac distended with blood. Puncture of a blood sac

resulted in immediate extravasation of the contained blood and collapse of the vesicle. There was no observed tendency to continuation of bleeding from the puncture. This suggests that the communicating vessels must be minute and that the circulation through the vesicle must be slow.

Case 2 (Aged 29 years). This was a younger half brother of Case 1 by the same mother. He had been known to have a skin rash from the age of 13 years - this like his brother having appeared rapidly over a few days. He also began to have paraesthesiae of the hands and feet at the same time. The skin lesion was similar in distribution to that in his brother but the lesions were not nearly so numerous.

This condition differs from the better known hereditary haemorrhagic telangiectasia. The character of the lesions is rather different. In hereditary telangiectasis the lesions are more stellate and the distribution is different occurring on face, lips and ears, hands and feet and on the mucosae of the nose and mouth. The distribution in diffuse angiokeratoma is the trunk and proximal parts of the limbs. In most of the reported cases of angiokeratoma the lesions have appeared in childhood whereas in hereditary teleangiectasis the vascular areas do not appear until later years.

Coagulation mechanism:- This was assessed in full detail only in Case 1 during a subsequent admission to hospital for

a further cerebral incident four years after the first. This incident was clinically very similar to the first attack and presumably represented a further vascular incident from the same vessel in the region of pons. The coagulation mechanism was normal. The details are given in the appendix page 881.

EHLERS-DANLOS SYNDROME.

This is a developmental mesenchymal dysplasia believed to be transmitted as a simple dominant. The defect is said to be in the elastic tissue and there is in consequence a tendency to haemorrhage on account of the defective elastic lamina of the vessel. Cases have been reported of profuse bleeding from trivial injuries, tonsillectomy or tooth extraction. Large haematomas may follow light blows, or even stretching the skin for demonstration (Ronchise 1949). The other manifestations of the condition are (a) hyperelasticity of the skin which may be raised in large folds, (b) hyperextensibility of the joints readily demonstrable at the metacarpal phalangeal joints, (c) the response of the skin to trauma is characteristic; a slight bump or glancing blow may result in a gaping wound or skin flap. On venepuncture the skin may split when the needle is inserted through the skin. A small cut, laceration or incision may

become wider and deeper as a result of the skin edges pulling away. Because of the difficulties in proximating the edges of wounds, lacerations are slow to heal. The resulting scars, a prominent feature of the syndrome, are wrinkled and criss-crossed or bulging and ballooned and are located mostly on the bony prominces, such as the forehead, knees, elbows and tibial areas.

Other occasional features of the condition are the spheroid or cyst-like subcutaneous tumours, wide spaced eyes, wide bridge of the nose, notched lower incisors, mental retardation and lipomatosis.

Three cases of this condition have been seen and the coagulation mechanism investigated with negative results. The first patient was a mentally retarded youth of 17 years of age. He was admitted with massive haematoma formation in the left upper thigh with evidence of complete arterial obstruction to the left leg. At operation it was found that this was due to a rupture of the left femoral artery and at subsequent autopsy the defective elastica of the artery was demonstrated on histological section. The second and third patients had many of the features mentioned above but only in one of these cases was there any evidence of haemorrhage - this following tooth extraction.

The coagulation mechanism in these patients was fully

investigated and no abnormality found. (see page 882 of the appendix).

SCURVY.

In Glasgow, in the Royal Infirmary and other hospitals serving the poorer parts of the city, Scurvy is a not uncommon disorder and an account of only a few of the cases seen is given in the appendix. These patients come often from the model lodging house dwellers, who have been living on a "tea and bread" dietary regime. Two cases have been seen amongst patients placed on a peptic ulcer regime. The haemorrhagic areas are generally to be seen in the legs - in the thighs, below the knees in the calves and on the dorsum of the feet. The characteristic perifollicular haemorrhages are most commonly to be found on the thighs and on the calves. "Croquet-loop" hairs are less often seen.

The only positive feature on assessment of the haemostatic function is the well known positive capillary resistance test. Thrombocytopenia is sometimes reported to occur but I have not observed this. The full details of the investigation of the coagulation mechanism are described in the appendix (Page 884). The bleeding tendency is believed to be due to failure of synthesis of the cement substance of the capillary wall.

HAEMORRHAGE IN POLYCYTHAEMIA.

This has been seen on two occasions. It is possibly surprising that polycythaemia should be associated with a haemorrhagic tendency in view of the thrombocythaemia, which so frequently accompanies the condition. The two examples of this, which have been seen, occurred after tooth extraction; the first was secondary polycythaemia in a patient with cyanotic congenital heart disease (a patent interventricular septal defect); the second was a patient with polycythaemia vera. Full details of these patients are given in the experimental appendix (page 888). The detailed investigation of the coagulation mechanism failed to reveal any significant abnormality. Examination of the capillaries by microscopy showed gross abnormality, the capillary loops in the finger beds being markedly distended with blood. It seems reasonable to suggest that this distension of the capillary loops forms the basis of the haemostatic abnormality. The distension presumably is the consequence of the greatly increased blood volume.

SENILE PURPURA.

This is a common form of vascular defect in the elderly and is generally seen on the dorsum of the hand. The areas are usually ecchymotic. It is said that the condition arises through loss of subcutaneous fat in ageing patients. In

addition to the dorsum of the hand, the legs, feet and forearms often show the ecchymotic areas. There are frequently accompanying trophic changes in the overlying skin. Three patients with this condition were examined and the only demonstrable change in the haemostatic mechanism was a slightly positive Hess test.

PURPURA FACTITIA:

This consists of self inflicted purpuric and bruised areas. Careful inspection of the areas may give some indication of the size and shape of the agent. Sometimes suction with the mouth is used by the patient to induce purpura and then the areas appear within reach of the mouth. Only one patient with this has been seen - the bruising had been induced on the deltoid region of the arms and on the breasts and upper abdomen.

BRUISING IN HYPERTENSION.

Patients have been frequently referred for investigation on account of bruising, where the only abnormality on physical or laboratory investigation was the presence of hypertension. Haematomata and ecchymosis are often present in the lower limbs.

PURPURA AS A MANIFESTATION OF HAEMOPHILIA.

One haemophiliac has been seen who was admitted with profuse generalised purpura - this lasted 2-3 days and then faded without recurrence. No haemostatic abnormality was discovered other than the deficiency of A.H.G. The platelet count was normal and the platelets formed thromboplastin normally. This has only been seen once by me in haemophilia and must be a most unusual phenomenon.

POSSIBLE VASCULAR DEFECT IN LEUKAEMIA.

Two patients have been studied with chronic myeloid leukaemia and massive spontaneous bruising. In one of these a haematoma in the forearm had caused a median nerve lesion. Detailed assessment of the coagulation mechanism was normal and it would seem reasonable to presume that the lesions were vascular due to infiltration of the vessel wall with leukaemic cells.

OTHER CAUSES OF PURPURA OR BRUISING IN PATIENTS INVESTIGATED.

Two patients have been investigated on account of purpura where the diagnosis was subacute bacterial endocarditis. Extensive purpuric eruptions have been seen in cases of meningococcal septicaemia. Bruising has been studied in two cases of Cushing's disease (page 880 - appendix) but no abnormality demonstrated in the coagulation mechanism.

ACUTE AND CHRONIC VASCULAR (ANAPHYLACTOID) PURPURA

Acute - Acute anaphylactoid purpura is essentially a disease of childhood and my experience of these patients is limited. In the appendix there are described some examples of such cases. There is generally a history of an upper respiratory infection or sore throat two or three weeks before the onset of this disease. The purpura appears suddenly and consists of large and often confluent haemorrhagic macules. The purpura is frequently the first manifestation of the disease.

Joint Symptoms

This varies in degree but the joints may become greatly swollen and limited in the range of movement.

Gastro-intestinal Symptoms

These are variable; in some only minor abdominal cramps occur, while in others the pain may be much more severe and accompanied by blood in the stool.

Renal Symptoms

Gross haematuria is sometimes seen. This generally clears quickly but progressive renal damage may ensue. (see Case *pg 894*). Albuminuria and eventual renal failure follow.

Chronic - The case described on page 892 of the appendix is a typical example of this condition. The disease is found more frequently in adults where there may be an accompanying chronic bacterial infection.

Assessment of the haemostatic mechanism in these patients failed to reveal any abnormality other than a moderately positive Hess test. The details are given in the appendix - pages 892 - 895.

SUMMARY

(1) Certain vascular defects have been investigated. The only contribution of my experiments on these patients is to establish by the recently developed and more sensitive methods, that there is no disturbance of the blood coagulation mechanism.

(2) The conditions investigated included hereditary haemorrhagic telangiectasia, diffuse generalised vascular defects (pseudohaemophilia, von Willbrand's disease), diffuse angiokeratoma, Ehlers-Danlos Syndrome and scurvy.

(3) Abnormal haemorrhage was observed as an occasional manifestation of polycythaemia. The coagulation mechanism is normal and it is likely that this also is a vascular defect.

(4) The blood coagulation mechanism was also normal in senile purpura, the bruising of hypertension, of Cushing disease and in acute and chronic anaphylactoid purpura.

(5) Massive spontaneous bruising in chronic myeloid leukaemia was investigated and the coagulation found to be normal. The defect is likely to be vascular due to damage to the vessel walls from leukaemic infiltration.

REFERENCES

Bernard, J., Beaumont, J.L., and Charreyron, M.C. (1953).
Etude de la fonction thromboplastique des plaquettes dans deux cas de dystrophie thrombocytaire hemorragipare congenitale. Rev. d'hemat. 8, 20.

Bigelow, F.S. (1954).
Serotin activity in blood.
J. Lab. clin. Med. 43, 759.

Buchanan, J.C. and Leavell, B.S. (1956).
Pseudohaemophilia: Report of 13 new cases and statistical review of previously reported cases.
Ann. int. Med. 44, 241.

Brown, A., Milne, J.A. (1952).
Diffuse angiokeratoma. Report of two cases with diffuse skin changes, one with neurological symptoms and splenomegaly.
Glasg. med. J. 33, 361.

Macfarlane, R.G. (1941).
Critical review: The D mechanism of haemostasis.
Quart. J. Med. 33, 1.

Ronchise, F. (1949).
Dermofragility.
Rhode Island med. J. 32, 80.

Part VI. Disorders of Coagulation

CHAPTER 12

DISORDERS DUE TO INTERFERENCE WITH COAGULATION

Prothrombin deficiency - report of a case.

Test for factor V.

Test for factor VII.

Two-stage prothrombin test.

Effect of vitamin K.

In vivo survival of prothrombin.

Factor V deficiency.

Factor VII deficiency.

A.H.G. and C.F. deficiencies.

Platelet deficiency.

Fibrinogen deficiency.

(1) in cirrhosis of liver.

(2) constitutional

(3) unexplained aetiology.

(4) in pregnancy.

CHAPTER 12

DISORDERS DUE TO INTERFERENCE WITH COAGULATION

Apart from disturbances of the coagulation system induced by the therapeutic action of anticoagulant drugs, the occurrence of severe clotting abnormalities in the everyday clinical practice of medicine is uncommon. Certain of these disorders, however, are more common than the others which are rarities. Amongst the more common are the "prothrombin- factor VII" deficiencies such as in the newborn infant and avitaminosis-K. Of the constitutional disorders of blood clotting haemophilia is the most common with Christmas disease next in order of incidence. These will be dealt with in Chapters 13, 15 & 16. The other rarer conditions will be described in this chapter. The order of description will follow that set out in the Classification of Haemorrhagic Disorders described in Chapter 9 and Table 17.

PROTHROMBIN: The dual deficiency of prothrombin and factor VII such as is found in coumarin drug therapy, salicylate therapy, newborn infants and vitamin K deficiency are described in Chapter 7 & 13. These are relatively common whereas isolated deficiency of prothrombin itself is very rare, the case to be described being the only one as yet described in the literature.

CASE REPORT. G.W. was a man aged 26 years. The patient had been well until 15 months before investigation. He then developed haematuria, investigation of the renal tract revealing no cause. Nine months later large spontaneous bruises of the right calf occurred, on both arms and in the neck. There was a history of epistaxis and bleeding from the gums and also of transient pains in the knees. As a child the patient underwent tonsillectomy, and two years before the onset of his present illness a tooth was removed with no excessive bleeding. There was no history of abnormal bleeding in his relatives. At the time of onset of the bleeding the patient was working in a brewery. Soon afterwards he went to a rubber factory, where he was employed in cutting out from sheets of rubber.

The abnormality in this patient was acquired in adult life and could not easily be attributed to his occupation; nor did he admit to taking any form of medicine.

Clinical examination was negative.

LABORATORY INVESTIGATIONS - Haemoglobin was 13.7 g. %, the white-cell count 5,100 per cu. mm. The blood film appeared normal.

Plasma proteins were 6.6g/100ml (albumen 3.8g; globulin 2.4g; fibrinogen 360 mg). The thymol turbidity test was

negative, as also was the colloidal gold test. The sucrose tolerance curve was normal. The bilirubin level was 0.3 mg.% The sedimentation rate was raised.

Fat excretion was normal, with an intake of fat of 280g. per day and an excretion of fat of 7.2g. (2.6%). There was therefore no evidence of any malabsorption of fat. The bleeding time (Ivy's technique) was 5 minutes (normal $2\frac{1}{2}$-7 minutes). A tourniquet test was negative. A platelet count gave 645,000-1,121,000/cu.mm.

Investigation of Coagulation Defect (see pages 897-910 of appendix). The whole blood clotting time, determined by the modified method of Lee and White (1913) was $13\frac{1}{2}$-20 minutes (normal 5-10); the one-stage prothrombin time was 18-22 seconds (normal 14 seconds). The antithrombin measured by Astrup and Darling's (1942) method was 179 units (normal plasma 193 units). The reaction of the plasma to thrombin was normal.

Test for Factor V - The one-stage clotting time of the patient's plasma was not shortened by the addition of normal plasma treated with $Al(OH)_3$.

Tests for Factor VII. The one-stage clotting time of the patient's plasma was not shortened by the addition of normal serum, which contains an excess of factor VII.

Additions of the patient's plasma shortened the clotting time of plasma from a patient under treatment with tromexan (which lacks factor VII) as well as did similar additions of normal plasma. These results have been described in Chapter 7 and are given also in the appendix page 902 .

From these it can be concluded that the patient's plasma lacked neither of the known accelerators of blood coagulation.

Two-stage Prothrombin Test. The curves illustrating thrombin formation in normal and in the patient's plasma, using the two-stage method are shown in Figure 99. From these curves using the principles described by Biggs and Douglas(1953)it can be calculated that the patient's plasma contains about 11% of the normal amount of prothrombin.

The Effect of Vitamin K Vitamin K was given to the patient in three different ways, with an interval of three days between each trial. 100 mg. of a water soluble analogue, "synkavit", was given intravenously; 1000 mg. of vitamin K_1 was given orally and 500 mg. of vitamin K_1 was given intravenously by the method of Davidson and MacDonald (1943). In none of these trials did the vitamin K cause a rise in prothrombin tested by the two-stage method. The technique of Davidson and MacDonald for administration of vitamin K_1 is described in Chapter 23.

Figure (99)

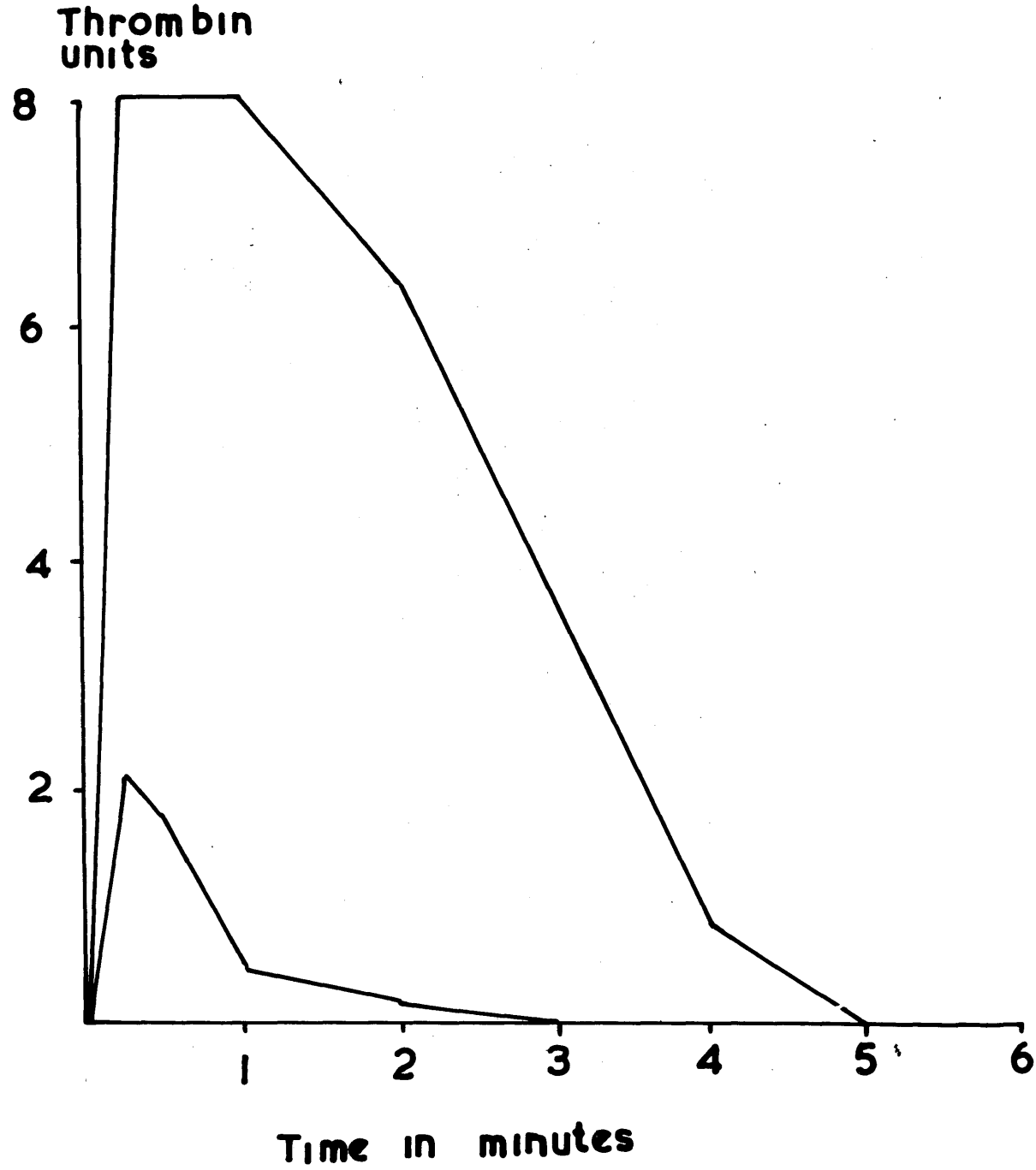
Figure (99)

Two-stage area prothrombin test on the normal and the patient with prothrombin deficiency.

Ordinate - Thrombin units.

Abscissa - time in minutes after addition of calcium.

The smaller curve is the result from the prothrombin deficient patient and the larger curve that from the normal.


Thrombin units
8
6
4
2
1
2
3
4
5
6
Time in minutes

The effect of intravenous administration of normal plasma. Fresh citrated plasma (860 ml.) was given to the patient. The plasma was given within two hours of its collection from three donors. On a second occasion 500 ml. of stored citrated plasma was given to the patient. On each occasion the rise in prothrombin as tested by the two-stage method was slight and the effect transient (Fig.100). The ineffectiveness of transfusion suggests either that the normal rate of prothrombin utilization was very high or that it was unusually fast in this patient.

The patient is apparently the first to be described in whom a coagulation defect can be attributed to an uncomplicated deficiency of prothrombin. The unusual features of the case are the relatively slight alteration in the one-stage "prothrombin" time and the lengthened whole blood clotting time. The slight lengthening of the one-stage clotting time suggests that the one-stage test is relatively more sensitive to changes in factors V and VII than it is to prothrombin. The lengthened whole-blood clotting time may be related to a very low level of thrombin generated by the blood thromboplastin system when prothrombin is grossly deficient. (Figure 101 shows the thrombin generation from whole blood - from a normal and the patient).

Figure (100)

(Figure 100)

Prothrombin survival following infusion of plasma to the prothrombin deficient patient.

Ordinate - percentage prothrombin.

Abscissa - time in hours after administration of plasma.

The results of two infusions are shown. The details are given in the text.

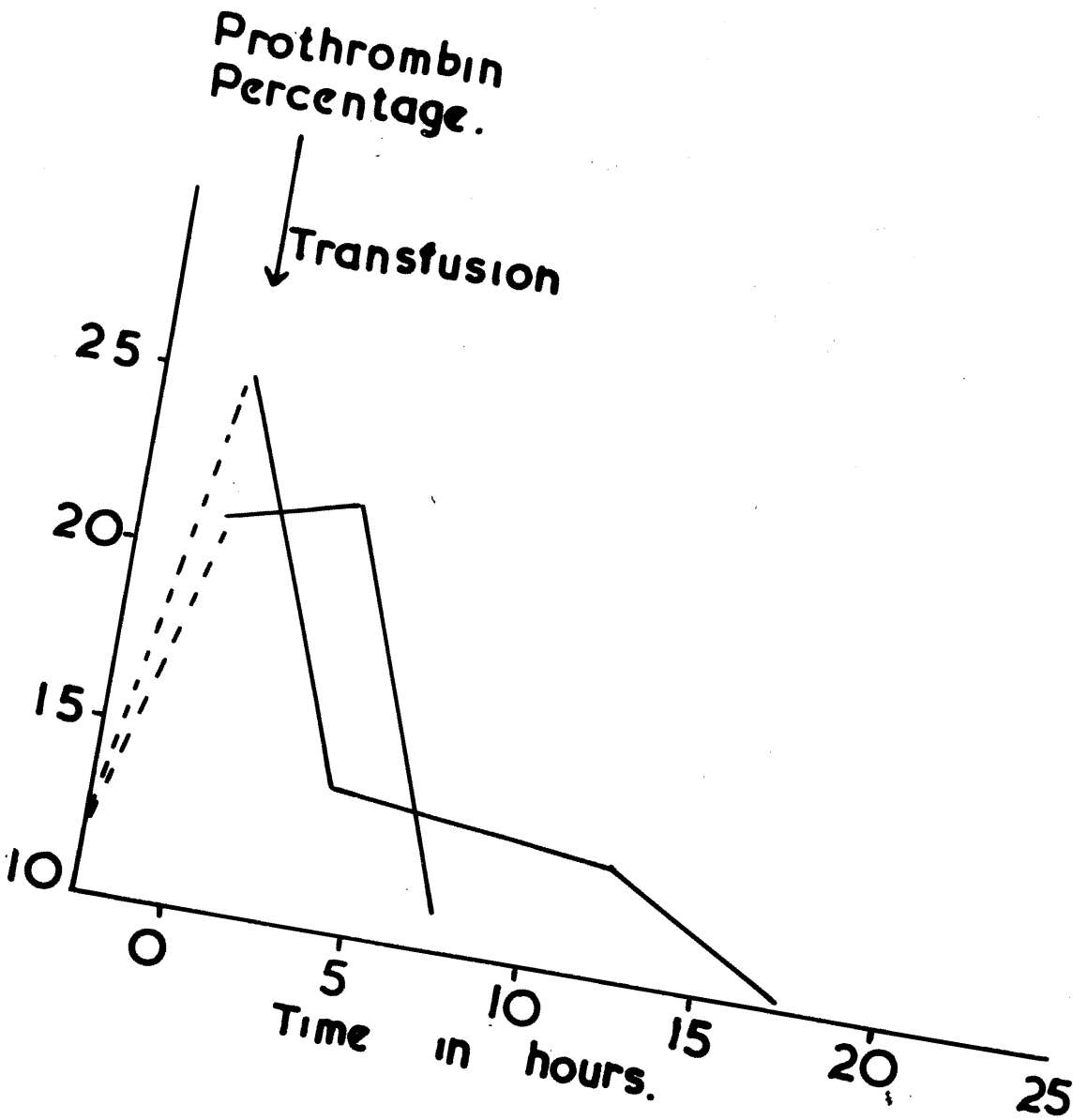

Prothrombin
Percentage.
Transfusion
25
20
15
10
0
5
10
15
20
25
Time in hours.

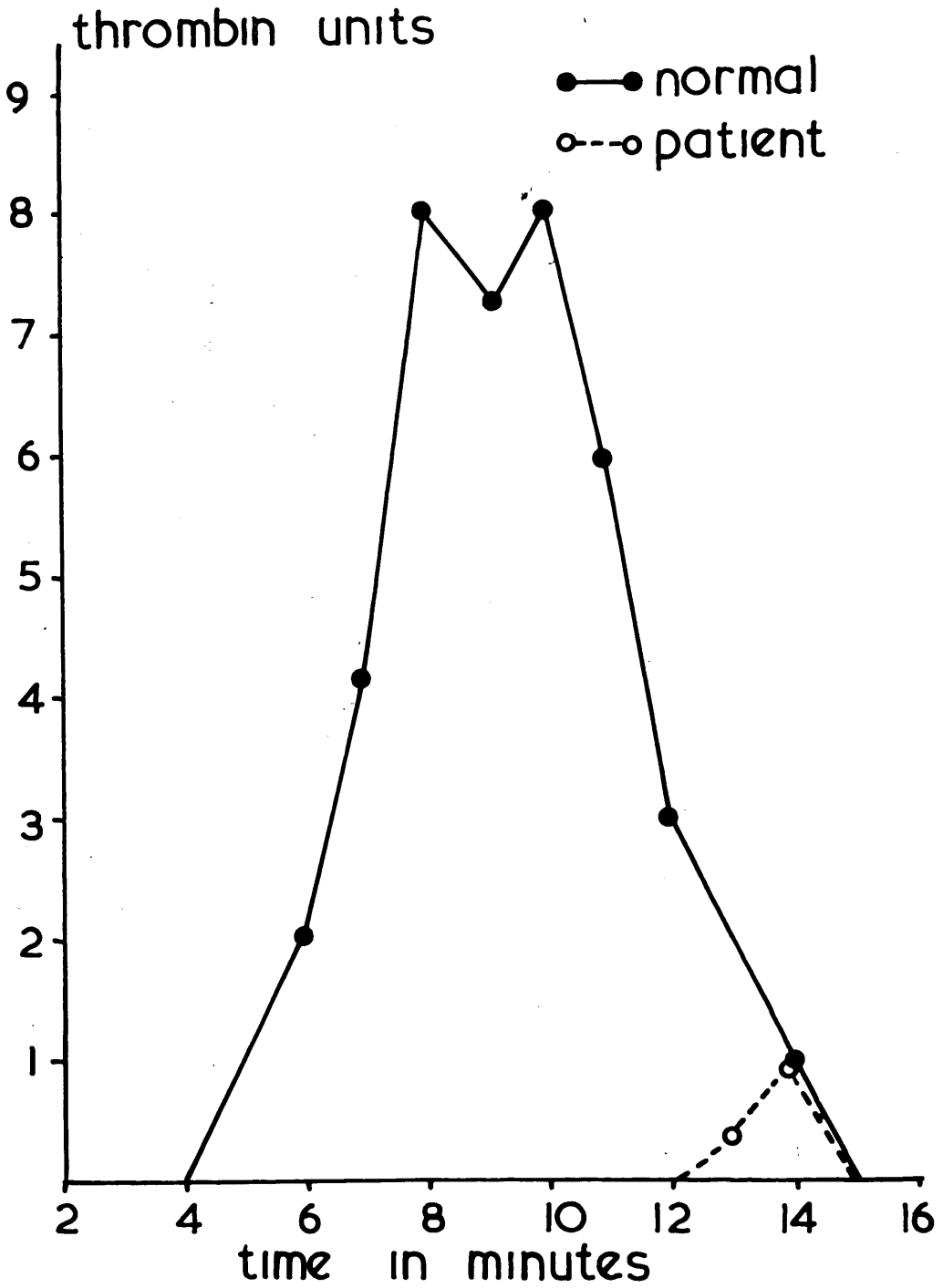
Figure (101)

Figure (101)

Thrombin generation from normal blood and from blood of the prothrombin deficient patient.

Ordinate - Thrombin units.

Abscissa - Time in minutes after venepuncture.


thrombin units
normal
patient
9
8
7
6
5
4
3
2
1
2
4
6
8
10
12
14
16
time in minutes

This is the only patient in my experience to have a normal factor VII content in the presence of gross prothrombin deficiency. As will be described in Chapter 13 there is generally significant depression of factor VII before prothrombin becomes deficient. The only instances, which have not followed this rule, were in parenchymatous liver disease where, though there was some depression of factor VII the prothrombin content was relatively lower in comparison with the other prothrombin - factor VII deficiencies.

THROMBOPLASTIN COMPONENTS FACTOR V.

Deficiency of factor V and of fibrinogen are rare disturbances of blood coagulation. I have not seen a case of constitutional factor V deficiency. I have seen it as a complication of liver disease where other coagulation components have also been deficient. The term parahaemophilia was introduced by Owren who described the first case. The reported cases of constitutional absence of factor V have recently been reviewed by Owen and Cooper (1955). The laboratory assessment of these patients revealed a prolonged one-stage prothrombin time which was shorted by the addition of one-tenth part of adsorbed plasma. Plasma so treated contains no prothrombin and no factor VII and the shortening can be attributed to the addition of factor V. This deficiency can be confirmed by the failure to correct the one-stage

"prothrombin" time of the patient's plasma by the addition of stored oxalated plasma which is deficient in factor V. Prothrombin concentration in these patients was normal but the conversion in the presence of brain thromboplastin abnormally slow. Factor VII deficiency can be excluded by the ability of the patient's plasma to correct coumarin plasma. Forty-six cases (involving 13 families) of factor V deficiency have been reported in the literature. A review of these cases suggests that an incompletely dominant gene is the most likely method of transmission. Inheritance is direct from one generation to the next, both sexes being affected.

The two patients seen by me who had significant factor V deficiency were, a woman on anticoagulant therapy with the coumarin drugs who developed congestive failure and hepatomegaly and a man, with cirrhosis of the liver who developed a haemorrhagic state. Both of these are described in the appendix (pages 911 ; ref 341).

FACTOR VII. Combined prothrombin-factor VII deficiency will be described in Chapters 13 & 14 .

Isolated factor VII deficiency as an established disturbance of blood coagulation has not been observed by me though again this is reported. In the early days of coumarin therapy as explained in Chapter 7 there is marked depression of factor VII before there is significant fall in prothrombin

but I refer at present to an established isolated factor VII deficiency irrespective of coumarin drug administration or vitamin K deficiency. Such cases of factor VII deficiency are reported by Alexander et al (1951), Jenkins (1954) and Hicks (1955). These have a prolonged one-stage clotting time corrected by the addition of serum but not of adsorbed plasma. The evidence available on inheritance is as a simple dominant either sex being affected.

ANTIHAEMOPHILIC GLOBULIN:CHRISTMAS FACTOR.

Haemophilia and Christmas disease are given consideration in Chapter 15 where a survey of the condition in the three million population of the West of Scotland is reported. In these cases seen in Scotland I have adopted standard criteria of investigation throughout the series.

PLATELETS. Thrombocytopenia has been discussed in detail under the platelet-capillary group of disorders. Such deficiency of platelets will of course be associated with corresponding disturbance of thromboplastin formation.

FIBRINOGEN. Deficiency of fibrinogen is amongst the rarest of the coagulation disturbances. From the work of Pinniger and Prunty (1946) and Stefanini and Petrillo (1949) it appears that clotting efficiency changes significantly when the fibrinogen concentration is reduced to 60mg/100ml.

Exceptionally, however, patients whose clotting defect has been attributed to fibrinogen deficiency have been found to have fibrinogen levels greater than 100 mg/100 ml. (Allibone and Barr 1943, Heinild 1944).

I have observed three patients in whom significant deficiency of fibrinogen was responsible for disturbance of clotting efficiency. In only one of these was the fibrinogen level markedly below the level of 100 mg./100 ml. The cases were all first suspected of fibrinogen deficiency on observation of the poor fibrin clot on recalcification of the plasma. The patients are described in detail in the appendix but the relevant features can be summarized as follows:-

(1) FIBRINOGEN DEFICIENCY IN CIRRHOSIS OF THE LIVER

T.W., aged 75. This patient presented with a subarachnoid haemorrhage. On examination he was found to have multiple haematomata and clean venepuncture resulted in widespread ecchymosis. The subarachnoid haemorrhage was thought to be the outcome of defective haemostasis. On recalcification of the plasma the fibrin formed was a poorly formed thready clot which rapidly lysed. Fibrinogen assay revealed only 46mg/100ml. This patient had at autopsy very advanced cirrhosis of the liver. There were other aspects of his clotting defect in that there was deficiency of prothrombin,

factors V and VII, very active fibrinolysis, a disturbed thrombin-fibrinogen reaction in consequence of the low fibrinogen level, and thrombocytopenia.

(2) CONSTITUTIONAL FIBRINOPENIA.

H.McI., aged 36 . This patient had for many years suffered from haematemesis and melaena of unexplained aetiology. No lesion had ever been found in the upper gastro-intestinal tract. On recalcification of the plasma poor fibrin formation was observed. Fibrinogen readings on consecutive days were in mg./100ml. 105, 101, 88. One litre of fresh plasma was given. Immediately after the infusion the level was 122 mg. but readings at 4 hours and 8 hours after completion of the infusion recorded values of 105mg/100ml. The "in vivo" life of the fibrinogen was therefore very short in this particular patient. Hardisty and Pinniger (1956) found a loss of half the concentration of administered fibrinogen over 24 hours.

(3) UNEXPLAINED FIBRINOGEN DEFICIENCY.

M.S., aged 16. This was a girl with an unexplained pyrexia associated with a haemorrhagic diathesis - spontaneous haematomata and bleeding from needle punctures. She also had a haemolytic anaemia. The clot observed on the whole blood clotting time test was very poor. The clot

observed on recalcification of the plasma was also poor. The fibrinogen content was estimated at 75-125 mg/100ml. The patient was in addition thrombocytopenic. The results on this patient are described in detail in the appendix page 917 . The exact pathology was never established.

In these three patients it is believed that fibrinogen deficiency was contributing to the coagulation abnormality. In two of them the level of fibrinogen was higher than is generally accepted as a critical level for haemorrhage. In two of the patients there was an associated thrombocytopenia. The association of fibrinogen deficiency with thrombocytopenia suggests the possibility of intravascular clotting from release of thromboplastin into the circulation. It is unlikely that this could be the explanation in the first case but the nature of Case 3 was never determined.

(4) FIBRINOGEN DEFICIENCY IN PREGNANCY.

There has recently been considerable interest in a newly recognised disturbance of blood coagulation in pregnancy. The exact nature of the clotting disturbance in this condition has never been investigated but two accepted features are a deficiency or absence of fibrinogen in the presence of a very active fibrinolytic mechanism. This haemorrhagic state, arising in some obstetrical emergencies, has been

recognised for many years but has recently received renewed attention, possibly as a consequence of the work of Schneider. This condition is most commonly seen in association with accidental antepartum haemorrhage. It has been reported to occur also in amniotic fluid embolism, in association with retention of a macerated foetus in a Rh immunized mother, in missed abortion and in hydatidiform mole. There have been many theories to account for this depletion of fibrinogen; some believe that it is due to excessive utilization as a consequence of intravascular clotting or of the formation of retroplacental clot or of fibrin deposition in the uterine wall; others believe that the depletion is the consequence of abnormally active proteolytic systems in the blood causing fibrinolysis or possibly fibrinogenolysis.

The theory which has gained most support is that of Schneider who believes that tissue thromboplastin is liberated into the circulation as a consequence of the disruptive process in the uterus and thereby intravascular clotting is initiated. The resultant fibrin forms multiple tiny emboli in the pulmonary and other capillary beds. It has been postulated that the patient may die because of the fibrin in the pulmonary arterial tree or that there may be a depletion of fibrinogen with resultant disturbance of blood clotting and a haemorrhagic diathesis. It has been suggested that

the fibrin thrombi in the kidney in renal cortical necrosis accompanying severe accidental haemorrhage may be a manifestation of this hypothetical liberation of thromboplastin into the circulation.

The other theory as to the nature of this coagulation defect stresses the active proteolytic system in the blood of these patients. This is demonstrated when a reasonable amount of clot has been formed but quickly lysed when maintained in vitro at 37° C. This proteolytic activity is readily demonstrable upon fibrin, but may be non-specific and capable of proteolysis of fibrinogen and other clotting factors. Tissue is a known activator of the fibrinolytic system of plasma and it can be postulated that the activity is also a consequence of release of thromboplastin into the circulation. Alternatively this activity could be merely the body's attempt to remove the fibrin which has been formed intravascularly.

Similar disturbances of blood coagulation have been reported in conditions other than pregnancy, including lung operations, multiple gunshot wounds, prostatic disease and pancreatic tumours. In the lung operations and gunshot wounds release of thromboplastin into the circulation can again be postulated; in prostatic disease and pancreatic tumours release of fibrinolytic enzymes may be responsible.

Only one example of afibrinogenaemia of pregnancy has been seen and this is described in the appendix page 915. Apart from the deficiency of fibrinogen the only other depletion was of antihaemophilic globulin. Since this is used up during blood clotting, it may be that this again is a manifestation of intravascular coagulation.

REFERENCES

Alexander, B., Goldstein, R., Landwehr, G. and Cook, C.D. (1951).
Congenital S.P.C.A. deficiency: a hitherto unrecognized coagulation defect with haemorrhages rectified by serum and serum fractions.
J. clin. Invest. 30, 596.

Allibone, E.C. and Barr, H.S. (1943).
Fibrinogen deficiency as a factor in haemorrhagic disease.
Arch. Dis. Child. 18, 146.

Astrup, T. and Darling, S. (1942).
Measurement and properties of antithrombin.
Acta Physiol. Scand. 4, 293.

Biggs, R. and Douglas, A.S. (1953).
The measurement of prothrombin in plasma.
J. clin. Path. 6, 15.

Davidson, C.S. and MacDonald, H. (1943).
Effect of vitamin K_1 oxide on hypoprothrombinaemia induced by dicumarol.
New Engl. J. Med. 229, 353.

Hardisty, R.M. and Pinniger, J.L. (1956).
Congenital afibrinogenaemia: further observations on the blood coagulation mechanism.
Brit. J. Haemat. 2, 139.

Heinild, S. (1944).
On familiar constitutional fibrinopenia.
Acta Med. Scand. 118, 479.

Hicks, N.D. (1955).
A coagulation disorder due to a factor VII-like defect.
Med. J. Aust. 2, 331.

Jenkins, J.S. (1954).
Haemorrhagic diathesis due to a deficiency of factor VII.
J. clin. Path. 7, 29.

Owen, C.A. and Cooper, T. (1955).
Parahaemophilia.
Arch. intern. Med. 95, 194.

Pinniger, J.L. and Prunty, F.T.G. (1946).
Some observations on the blood-clotting mechanism. The role of fibrinogen and platelets with reference to a case of congenital afibrinogenaemia.
Brit. J. exp. Path. 27, 200.

Stefanini, M. and Petrillo, E. (1949).
The relative importance of plasmatic and vascular factors of haemostasis in the pathogenesis of the haemorrhagic diathesis of liver dysfunction.
Acta med. Scand. 134, 139.

CHAPTER 13

H Y P O P R O T H R O M B I N A E M I A

CONTENTS.

CHAPTER 13

HYPOPROTHROMBINAEMIA

When the features of the defect in coumarin drug therapy had been established it became clear that a reassessment of the other common "hypoprothrombinaemias" was needed.

The salient features of the abnormality following coumarin drug therapy were the deficiencies of factor VII and to a lesser extent prothrombin and the reduced capacity of the serum to form blood thromboplastin. The other "hypoprothrombinaemias" which were investigated were:

(a) in the newborn.
(b) in salicylate therapy.
(c) in steatorrhea.
(d) nutritional vitamin K deficiency.
(e) obstructive jaundice, (this is described in the next chapter together with the changes in parenchymatous liver disease).

PROTHROMBIN: This was estimated either by the area method or the globulin fraction technique (see pages 570, 571 of the appendix).

FACTOR VII: This was measured by the method described by Biggs and Macfarlane (1953).

The method employs tromexan, dindevan or other coumarin plasma collected during the first few days of therapy - when the one-stage clotting time is prolonged due to deficiency

of factor VII, before there is significant fall in the concentration of the prothrombin. By making mixtures of the normal and the unknown with tromexan plasma and using the graph shown in Figure 102 an assay of factor VII content can be made as in the following example.

Example: Tromexan plasma = 44"
1/10 normal plasma.
in tromexan plasma = 21"
1/10 test plasma
in tromexan plasma = 38"

Reading from graph:

44" = 3%
21" = 14.2%
38" = 3.8%

Therefore percentage factor VII in test plasma

$$= \frac{3.8 - 3}{14.2 - 3}$$

$$= \frac{0.8}{11.2} = 7\%$$

The results of this assay procedure were checked from time to time, by making a dilution curve of a normal plasma in the individual tromexan or dindevan plasma being used in the particular assay (see figure 103).

FACTOR V. This was estimated by the methods previously used in the thesis and described in detail in the appendix (pages 573 , 574).

Figure (102)

Figure (102)

Factor VII assay curve.

Ordinate - one-stage clotting times in seconds.

Abscissa - percentage factor VII as a reciprocal.

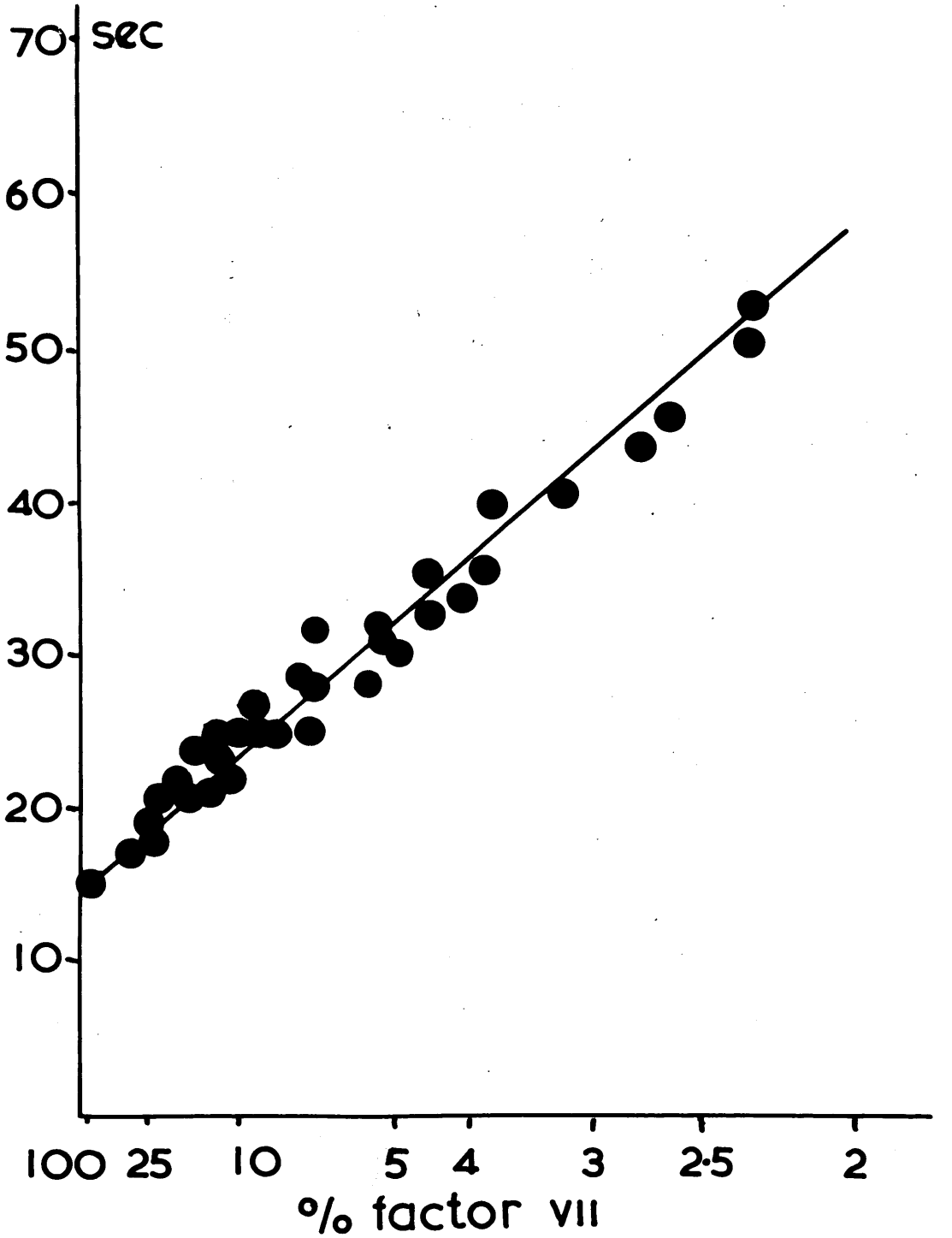

70
sec
60
50
40
30
20
10
100
25
10
5
4
3
2·5
2
% factor VII

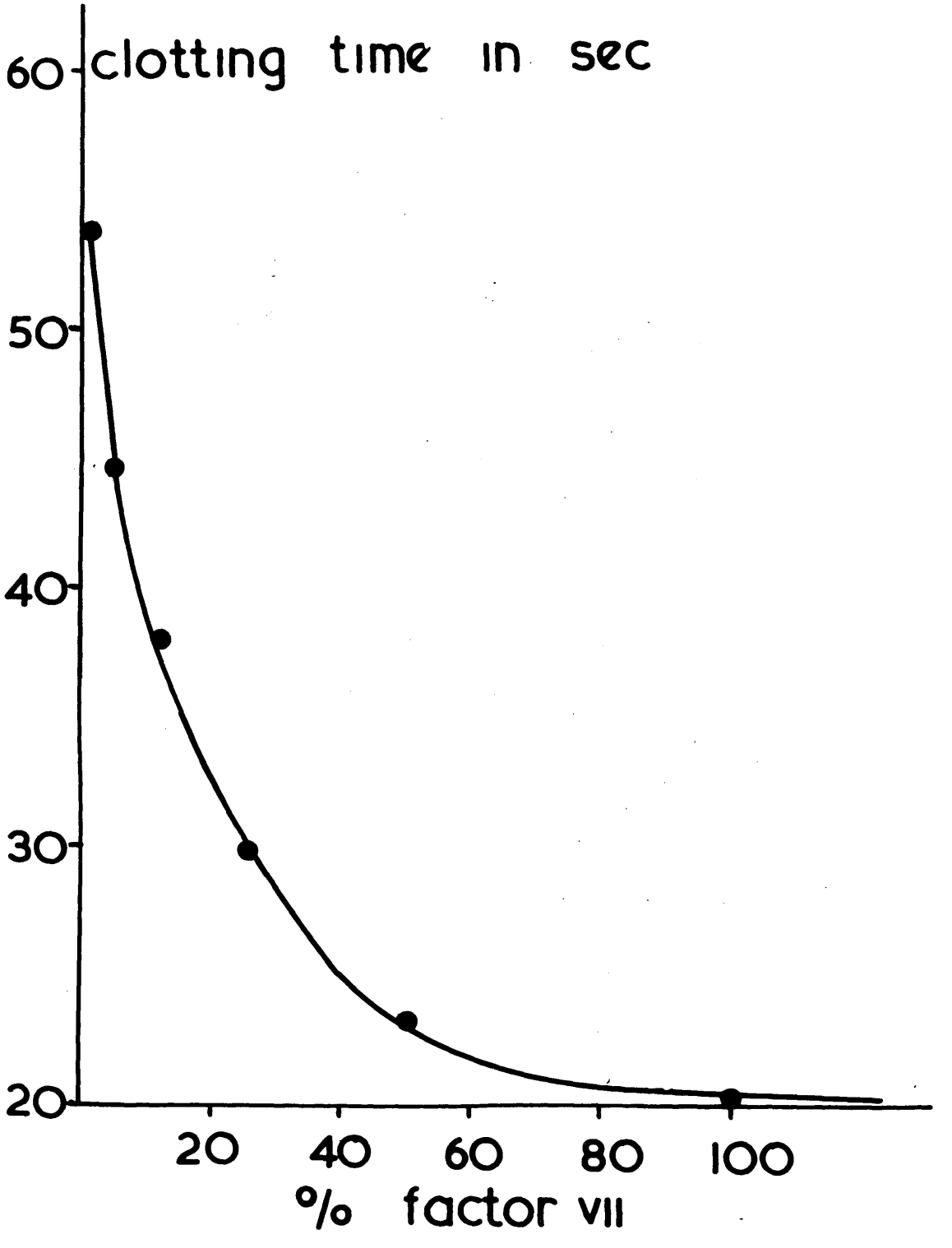
Figure (103)

Figure (103)

Factor VII assay curve.

Ordinate - one-stage clotting times in seconds.

Abscissa - percentage factor VII.


clotting time in sec
60
50
40
30
20
20
40
60
80
100
% factor VII

NEWBORN.

Healthy infants were examined. Specimens of cord blood were collected shortly after birth, into a syringe by venepuncture of the umbilical vein. Specimens of blood collected by dripping from the cut end of the cord almost invariably clot, probably due to admixture of tissue thromboplastin from the cord. At 48-72 hours after birth a further specimen was obtained by venepuncture of the antecubital veins, and on occasion of the external and internal jugulars. It is inevitable that more specimens were collected than could be included in this investigation, many having to be excluded on account of clotting. 1.8 ml. were collected into 0.2 ml. of 3.8 sodium citrate. This gave just sufficient plasma to be able to do Quick's one-stage test, the area two-stage test and the factor VII content of each specimen. To half of the infants 10 mg. synkavit were administered by intramuscular injection at the time of withdrawal of the cord blood.

Results.

Factor VII and Prothrombin.

42 infants were examined. To 21 of these 10 mg. of vitamin K were given as described above. The mean of the results is shown in Fig. 104 and Table 18.

Figure (104)

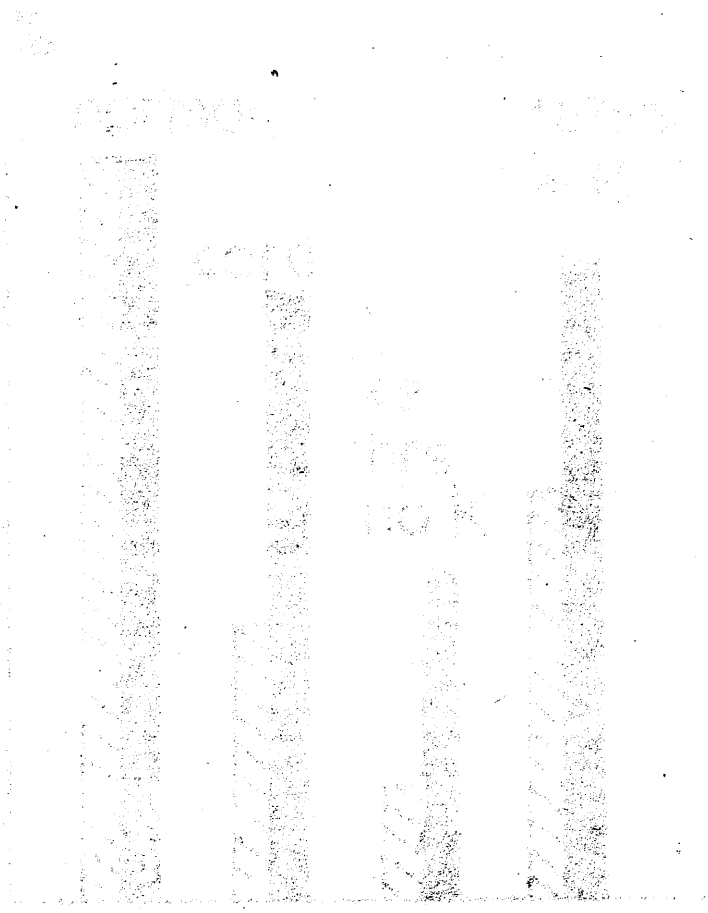


Figure (104)

Prothrombin and factor VII concentrations in cord blood and in blood collected 48 hours after birth with and without administration of vitamin K.

Ordinate = percentage prothrombin and factor VII.

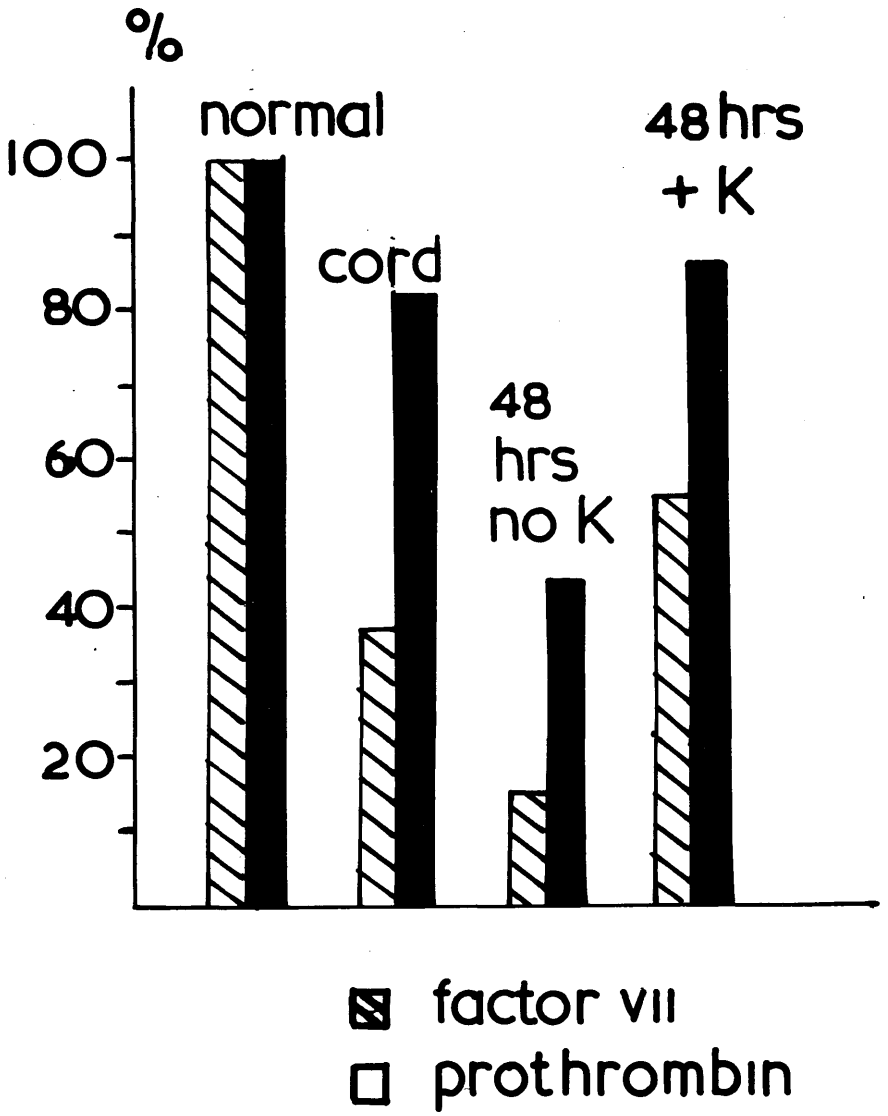

%
normal
48 hrs
+ K
100
cord
80
48
hrs
no K
60
40
20
factor VII
prothrombin

TABLE 18

	Normal	Cord	3rd day No K.	3rd day With K.
Factor VII	100%	38%	15%	54%
Prothrombin	100%	82%	44%	85%
Quick's one-stage test	100%	49%	12%	42%

There is a reduced level of both factor VII and prothrombin in the cord blood. These levels fall considerably further at the third day. If vitamin K is given it fails to produce normal content of these factors on the third day but it maintains levels at least as good as in the cord blood. In Figs. 105 and 106 the mean of the observations on the two stage test are shown. The antithrombin content of the infant's blood was found to be similar to that of normal plasma. This was an essential prerequisite to the application of the two-stage method. (see page 571 of the appendix).

Factor V.

The factor V was estimated by the two methods described above.

(a) Four specimens of 3rd day infant blood were examined.

Figure (105)

Figure (105)

Two-stage area prothrombin test in normal blood, cord blood and blood collected 48 hours after birth without previous administration of vitamin K.

Ordinate - thrombin units.

Abscissa - time in minutes after addition of calcium.

Mean of 21 observations.

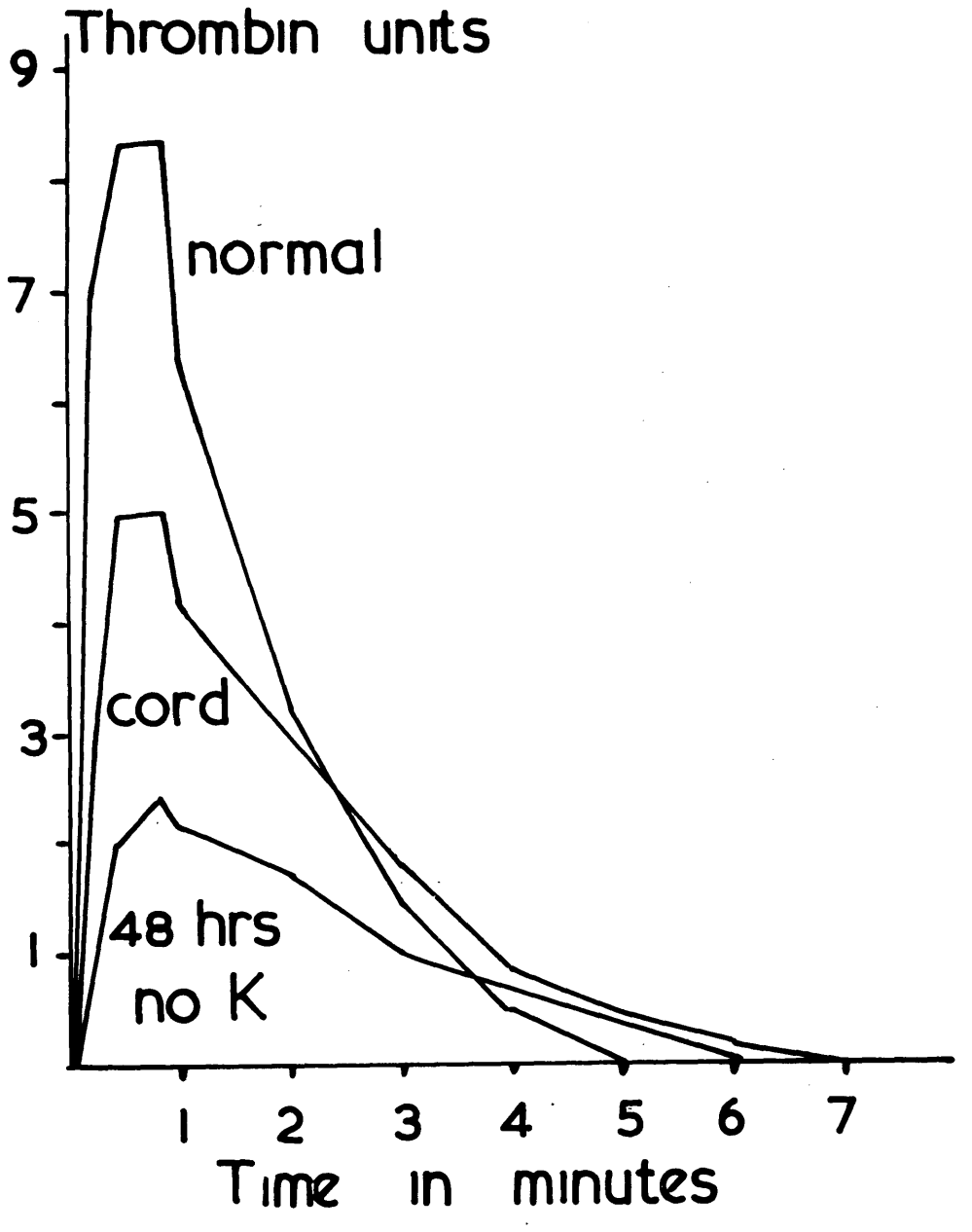

Thrombin units
9
7
5
3
1
normal
cord
48 hrs
no K
1
2
3
4
5
6
7
Time in minutes

Figure (106)

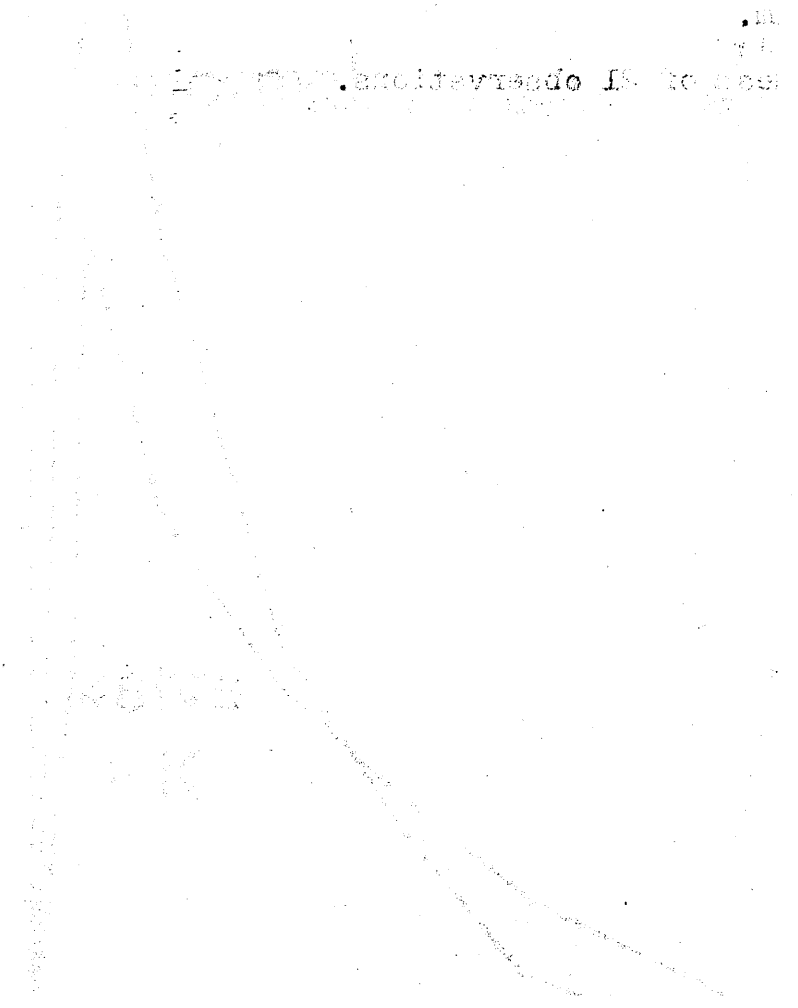


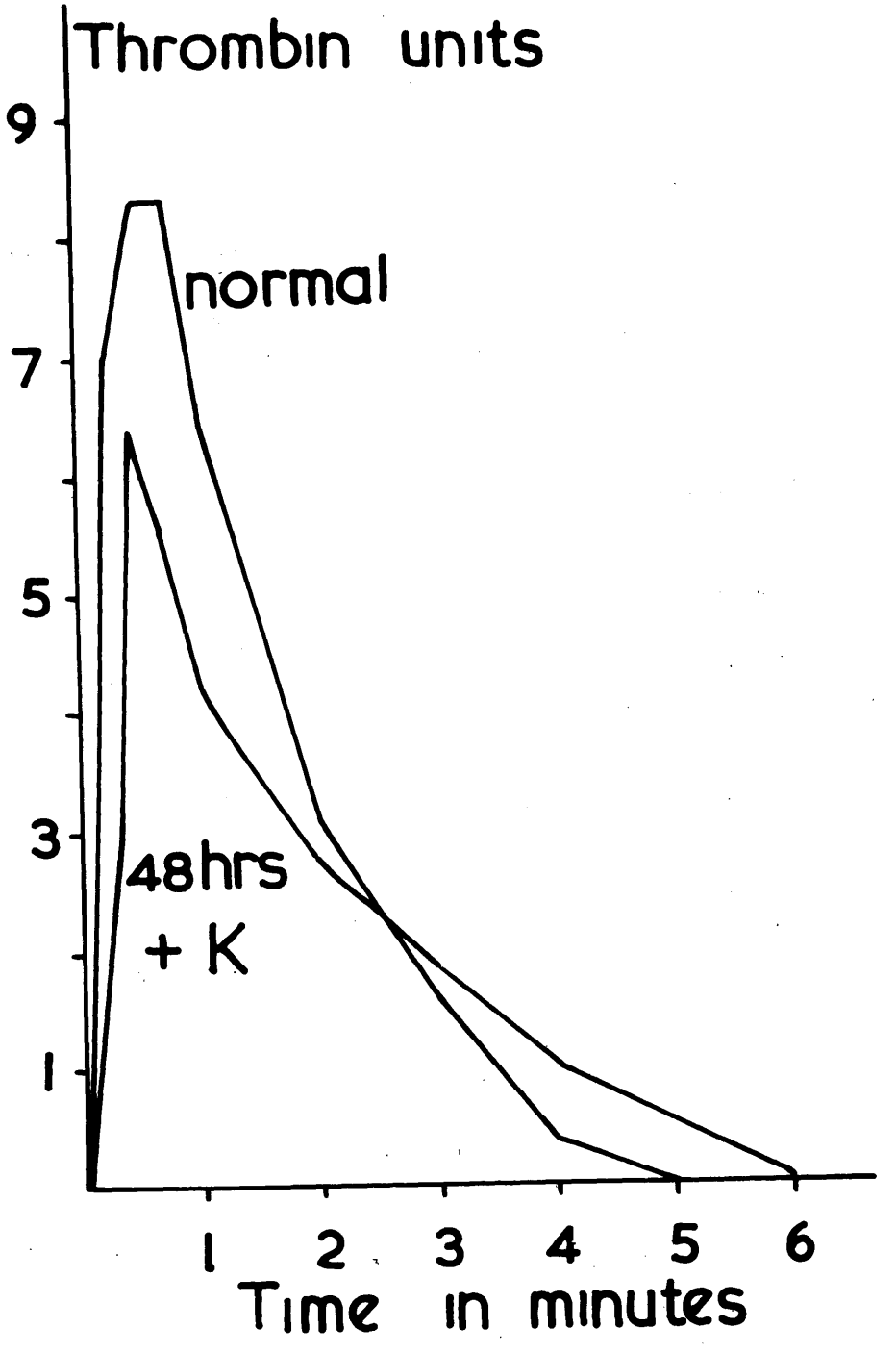
Figure (106)

Two-stage area prothrombin test in normal blood and blood collected 48 hours after birth with previous administration of vitamin K.

Ordinate - Thrombin units.

Abscissa - time in minutes after addition of calcium.

Mean of 21 observations.


Thrombin units
9
7
5
3
1
normal
48hrs
+ K
1
2
3
4
5
6
Time in minutes

The mean activation of the prothrombin under the influence of factor V from these specimens is as good as that of the mean of the normals (Fig. 107).

(b) The correction of stored oxalated plasma is shown in Table 19 .

TABLE 19

Stored plasma	+ ½ normal plasma	+ ½ infant plasma
21.8"	15.7"	16.7"

(Mean of 7 observations)

From these results it may be concluded that the "hypoprothrombinaemia" of the newborn is due to a deficiency of both factor VII and prothrombin. It is probable that the deficiency in factor VII is the cause of the prolonged one-stage clotting time, since the degree of prothrombin deficiency observed is not sufficient to be likely to cause lengthening of the one-stage time (Biggs and Douglas, 1953). The degree of deficiency of these factors is much greater on the third day than it is in the cord bloods. The administration of water soluble vitamin K to the infant prevents the fall in factor VII and prothrombin, but does not restore these factors to normal levels.

Figure (107)

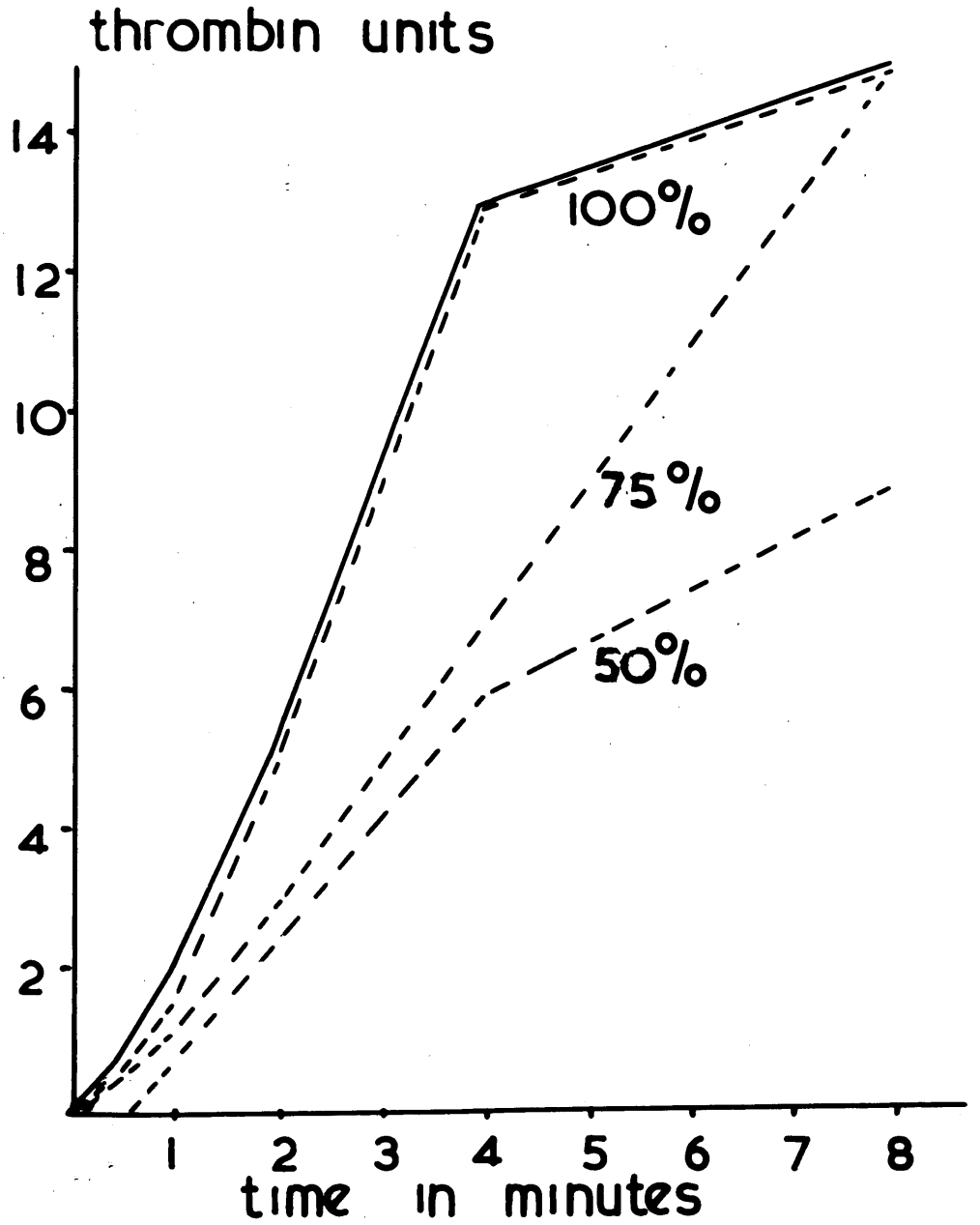
Figure (107)

Factor V estimation in the newborn.

Ordinate - thrombin units.

Abscissa - time in minutes after addition of calcium.

This figure shows the activation of prothrombin by the factor V from the infant blood by a continuous line (mean of four 3rd day infant bloods) as compared with that from the normal (discontinuous lines).


thrombin units
14
12
10
8
6
4
2
100%
75%
50%
1
2
3
4
5
6
7
8
time in minutes

Loeliger and Koller (1952) have also investigated the factor VII and prothrombin content of cord blood and concluded that both of these factors were deficient. They record much lower figures than were found in this investigation (24% factor VII and 28% prothrombin in a series of 33 cord bloods). The techniques employed by Loeliger and Koller are very different from those used in this investigation.

From the limited number of observations made in this study it is probable that there is no great deficiency of factor V in the newborn. This confirms the findings of Stefanini (1951) who found "the labile-factor activity only slightly depleted, whereas plasma prothrombin activity was much reduced during the third day after birth". Stefanini was using a technique involving correction of the prolonged one-stage clotting time of stored oxalated plasma.

There has been some doubt thrown on the value of vitamin K in prevention and management of haemorrhagic disease of the newborn. Hay, Hudson and Rodgers (1951) found that vitamin K administered to the mothers did not reduce the incidence of this disease. Sandford et al (1942) found that vitamin K administered to the baby, or to the mother before birth, raised the prothrombin level but did not affect the frequency of haemorrhagic manifestations. It was not the object of

this investigation to study the complete coagulation mechanism in the newborn, nor to establish the relationship of vitamin K to the incidence and management of haemorrhagic disease of the newborn. It may well be that the coagulation defect is more extensive than deficiency of factor VII and prothrombin. Biggs and Macfarlane (1953) have shown that the thrombin-fibrinogen reaction is abnormal in the newborn. Below it is shown that the serum from an infant with haemorrhagic disease of the newborn was defective in its ability to form intrinsic thromboplastin. Haemorrhage in the newborn may be due to abnormalities other than those associated with a prolongation of the one-stage clotting time - for example, thrombocytopenia of the newborn is a well recognised entity.

This investigation presents reasonable evidence that the administration of vitamin K to the newborn infant arrests the usual fall in both factor VII and prothrombin. Even if deficiency of factor VII and prothrombin proves ultimately to be only a contributory factor in haemorrhagic disease of the newborn, it is of importance to know that vitamin K does control the level of these substances.

Assessment of the coagulation defect in a case of haemorrhagic disease of the newborn.

The investigations described above were carried out in the Radcliffe Infirmary, Oxford, and Churchill Hospital,

Oxford, in 1952 and 1953. In 1954 the case to be described was investigated from the Royal Hospital for Sick Children, Yorkhill, Glasgow. The details of the case and of the results of the investigations were as follows:-

Baby C.: This newborn infant had haemorrhagic disease of the newborn. The child's mother had a severe epistaxis at the onset of labour; the cause of this was unknown though she was said not to be hypertensive. The child was born with numerous bruises on face, neck and limbs. These increased during the following 36 hours and the baby developed some oozing from the umbilicus but no bleeding from the bowel or other site.

Bleeding time was greatly prolonged but platelets were abundant.

The results of the investigation on this infant's blood were as follows:-

One-stage clotting time - Patient $2\frac{1}{2}$' $3\frac{1}{2}$' Control 16" 17"

Recalcification time - Patient 8' 10' Control $1\frac{1}{2}$' $1\frac{3}{4}$'

Thromboplastin generation test.

	(1)	(2)	(3)	(4)	(5)	(6)
Normal adsorbed plasma Normal serum Platelets	10	8	8	8	8	8

Thromboplastin generation test (Contd.)

	(1)	(2)	(3)	(4)	(5)	(6)
Patient adsorbed plasma Normal serum Platelets	8	9	8	8	8	8
Normal adsorbed plasma Patient serum Platelets	20	20	22	17	20	15

Prothrombin assay

by globulin fraction method

(1 ml. of plasma + 0.2 ml. normal serum)

Normal 16" Patient 76"

= 20%

To test ability to correct Christmas disease serum on thromboplastin generation.

Normal adsorbed plasma

Platelets	(1)	(2)	(3)	(4)	(5)	(6)
Serum - 0.2 Christmas Serum plus 0.1 normal serum	15	11	10	11	11	12
0.1 patient serum	60	16	16	14	15	10
0.1 saline	30	30	18	18	21	19

Comment - Inability to correct Christmas disease serum (see figure 108).

Subsequent study.

One-stage clotting time - Infant 10' + Normal 17"
+ 1/10 serum = 60"
+ 1/10 adsorbed normal plasma = 8'

Figure (108)

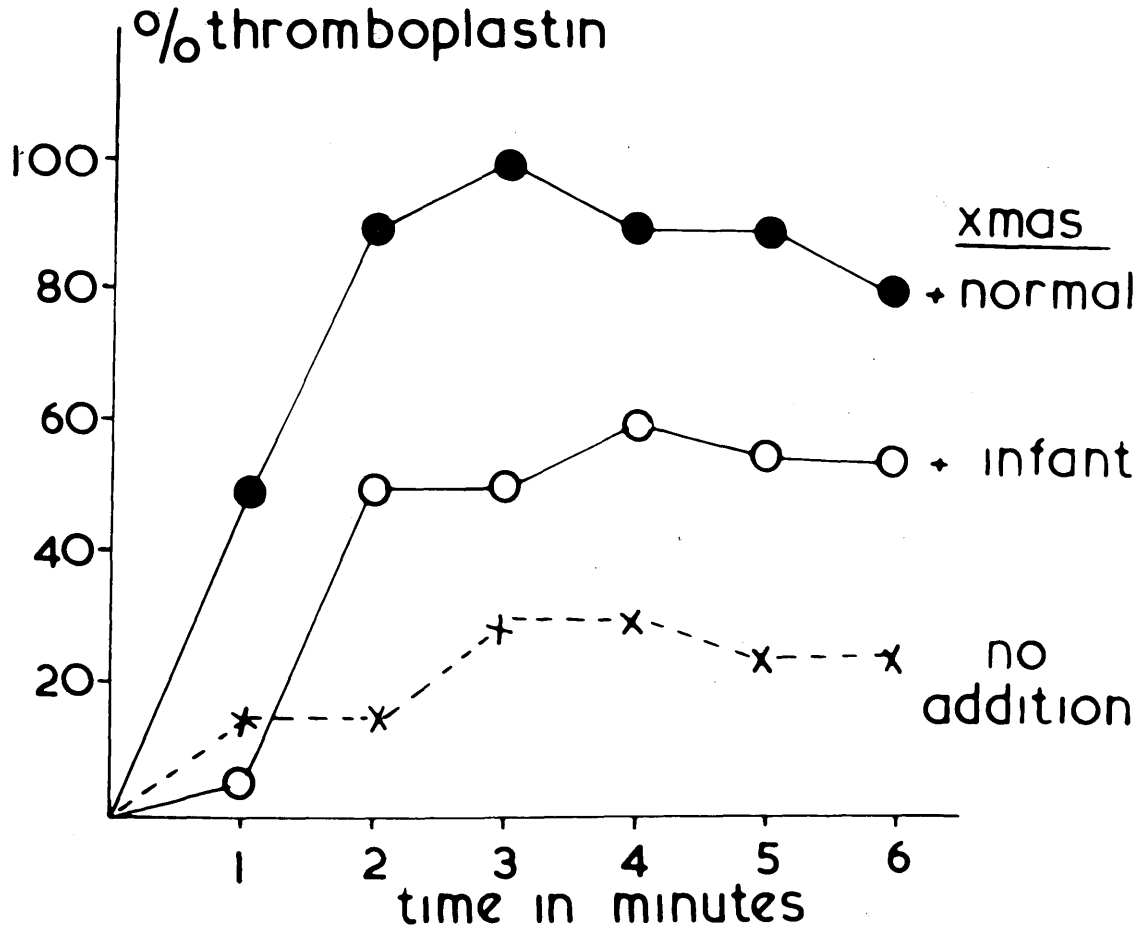
Figure (108)

The inability of serum in haemorrhagic disease of the newborn to correct the thromboplastin defect in Christmas disease serum.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

This figure demonstrates the impaired ability of the infant's serum to correct the thromboplastin defect in Christmas disease serum using the thromboplastin generation test.


% thromboplastin
100
80
60
40
20
1
2
3
4
5
6
time in minutes
xmas
normal
infant
no addition

The features of this case are the very prolonged one-stage clotting time with deficiencies of factor VII and prothrombin but not of factor V. The recalcification time was greatly prolonged and the serum showed marked inability to form thromboplastin and to correct the defect in Christmas disease serum (see figure 108).

In Salicylate Therapy.

The chemical similarity between salicylate and dicumarol and the discovery that salicylic acid was the primary degradation product of dicumarol suggested to Link (1943) that dicumarol might owe its effectiveness 'in vitro,' to conversion to salicylic acid. In experimental animals on a low vitamin K diet salicylates were found to reduce the prothrombin level (Link et al 1943). In human beings, 'hypoprothrombinaemia' has been found without restriction of vitamin K. The reductions reported have generally been less than 50 per cent of normal (Seegers 1951).

It is of interest to note that Jaques and Lepp (1947) found that intravenous administration of salicylates to rabbits caused no alteration in prothrombin time, while oral administration resulted in a prolongation. After the oral administration of sulfasuxidine the oral administration of sodium salicylate did not affect the prothrombin time. They suggest that salicylates may be converted to a coumarin substance with

prothrombinopenic properties by bacterial action in the intestinal tract. Tarnoky and Steingold (1951) have shown that P.A.S. (P-aminosalicylic acid) caused a definite lowering of prothrombin levels as determined by a one-stage technique on diluted plasma. This effect was partly reversed by streptomycin. A similar explanation to that of Jaques and Lepp (1947) could operate.

All the investigations of 'in vivo' salicylate effects were carried out by one-stage techniques. The use of whole plasma was found by most workers (e.g. Link 1943) to be relatively insensitive to the degree of change produced by salicylate so that 12.5 per cent plasma has been more extensively studied.

SALICYLATE THERAPY

Results.

Factor VII and Prothrombin.

The patients studied were on P.A.S. (with streptomycin) for the treatment of pulmonary tuberculosis or on sodium salicylate for rheumatoid arthritis or acute rheumatism. The mean results where both factor VII and prothrombin were estimated are shown in figure 109 and Table 20 . The details are given in the appendix pages 947-959.

Figure (109)

Figure (109)

Prothrombin and factor VII concentrations in salicylate therapy and steatorrhoea.

Ordinate - percentage prothrombin and factor VII.

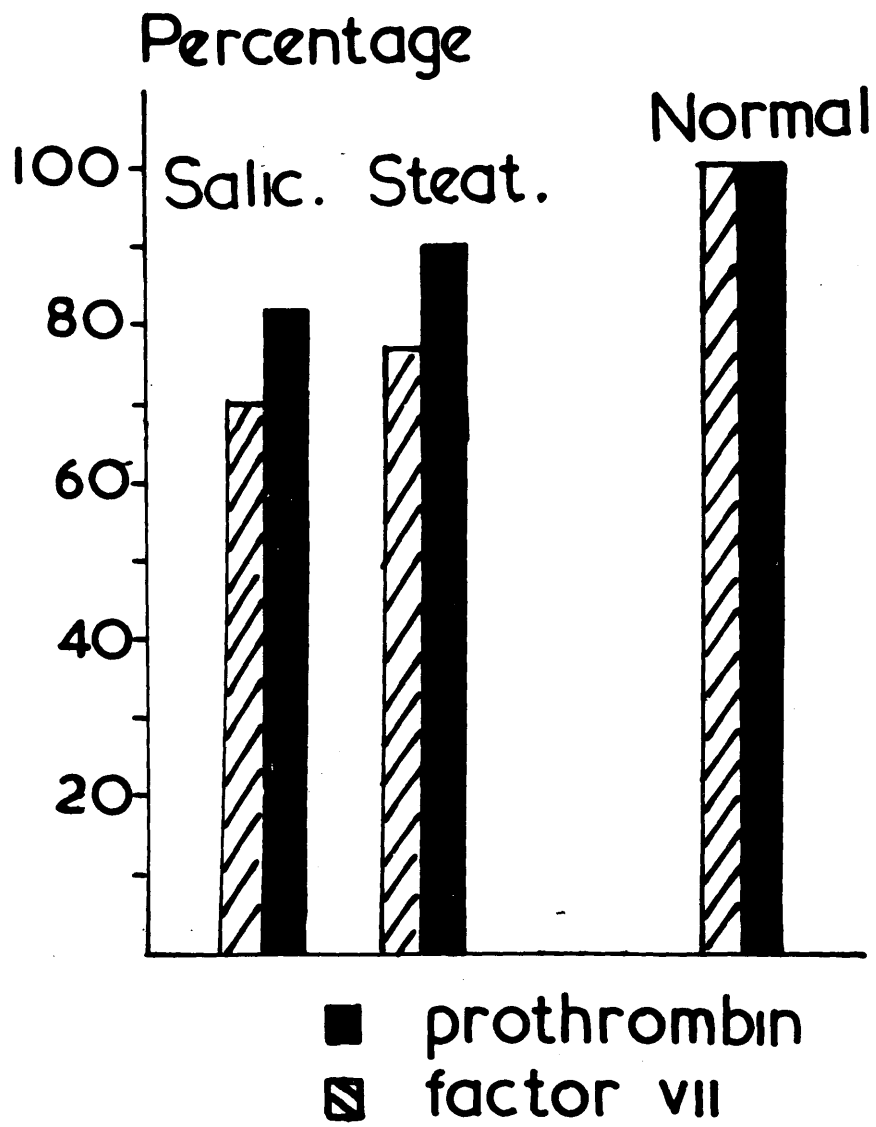

Percentage
100
80
60
40
20
Salic.
Steat.
Normal
prothrombin
factor VII

TABLE 20

		Normal	Salicylate
30 observations	Factor VII	100%	70%
	Prothrombin	100%	81%
	Quick's one-stage test	100%	89%

The area method of prothrombin assay could be applied as it was shown that the antithrombin content of the normal and the salicylate cases was similar. In a few results the corresponding blood salicylate level was estimated.

Case of salicylate overdosage

This was a girl of 15 years treated by her practitioner with 180 gr. of sodium salicylate daily for 5 days at the end of which time she was admitted to hospital with symptoms of salicylism. There were deficiencies of prothrombin and factor VII and the progress of these on stopping salicylates are shown in figure 110 .

Defective serum reaction in the thromboplastin generation technique

This was studied in a patient with acute rheumatism during the third week of treatment with full doses of sodium salicylate. The results were as follows:-

Figure (110)

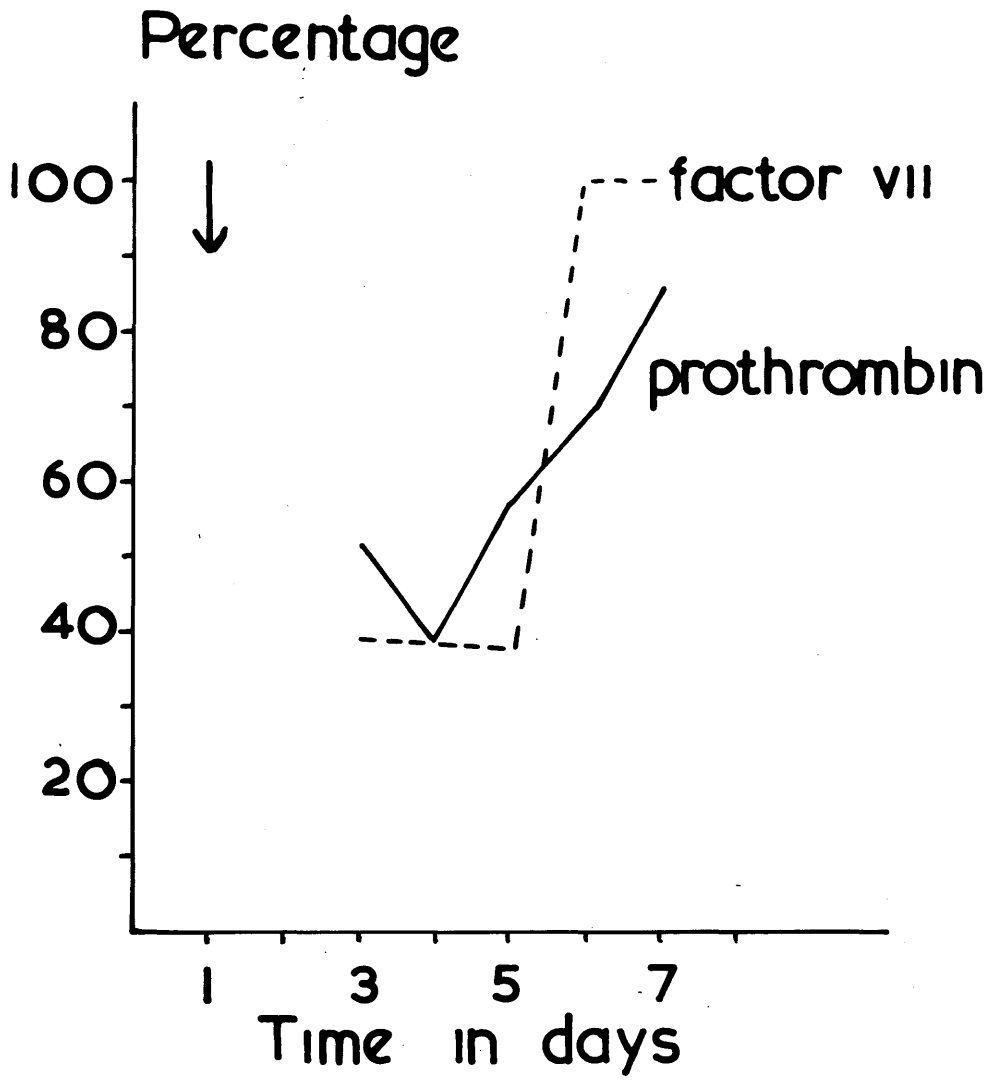
Figure (110)

Recovery of prothrombin and factor VII subsequent to cessation of salicylate overdosage.

Ordinate - percentage prothrombin and factor VII.

Abscissa - time in days.

Arrow indicates date of withdrawal of salicylate.


Percentage
100
80
60
40
20
factor VII
prothrombin
1
3
5
7
Time in days

	(1)	(2)	(3)	(4)	(5)	(6)
Normal ads. plasma Normal serum	42	30	12	9	9	9
Patient ads. plasma Normal serum	45	32	12	9	9	9
Normal ads. plasma Patient serum	50	34	17	14	13	14

Normal 15"
Patient 22"
Dindevan 21"

Mixture of Patient and Dindevan = 21"

Comment - in addition to the deficiency of prothrombin and factor VII there is a serum thromboplastin defect. The salicylate defect has the same features as that produced by the coumarin drugs.

Steatorrhoea.

Results.

Using the same techniques the blood of patients with idiopathic steatorrhoea was examined. Many of these were adult cases of steatorrhoea under out-patient surveillance for that condition. These patients were under the care of Professor L. J. Witts and Dr. J. Badenoch of the Nuffield Department of Medicine at the Radcliffe Infirmary, Oxford. All were proved cases of malabsorption, having had fat balance examinations as in-patients on previous admissions. None

were receiving any supplements of vitamin K. It will be seen that here also there was depression both of factor VII and prothrombin.(Table 21) and(Fig. 109). The extent of the depression of these factors was not great and this explains why Quick's one-stage clotting time was so often within normal limits. In a further series of these cases (see appendix page 964) the prothrombin only was estimated.

TABLE 21

		Normal	Steatorrhoea
12 observations	Factor VII	100%	77%
	Prothrombin	100%	90%
	Quick's one-stage test	100%	95%

Haemorrhagic states from "hypoprothrombinaemia" in the malabsorption syndrome have been reported, but the nature of this "hypoprothrombinaemia" has not been investigated previously. In a series of thirty-six cases of primary steatorrhoea described by Aldersberg and Schein (1947), of six deaths haemorrhage was the cause in two. There is a certain uniformity in the clinical picture. The onset occurs with dramatic suddenness. Haemarthrosis, severe haematemesis, epistaxis, haematuria and melaena are common presenting symptoms. Large subcutaneous haematomata usually on the back or other sites of pressure have been described. Intractable haemorrhage occurs from skin wounds and the gums

may bleed after slight trauma (Kark et al 1940).

Two patients with steatorrhoea and a very severe haemorrhagic state have been seen. In one of these, this complication was fatal.

R.C. - male, aged 20 years, December 1950. This youth had been diagnosed as a case of steatorrhoea since the age of 13 years when the condition presented with diarrhoea. He was first seen in Professor Davis' wards at the age of 17 years complaining of pains in the lower limbs particularly around the knees and ankles. Fat balance studies revealed defective fat absorption - the figure being 90% absorption. Radiologically the bones showed osteoporosis and rachitic changes and he had a flat glucose tolerance curve. Radiological examination of the small bowel showed a characteristic deficiency pattern. Subsequent to this admission he had a further one, on account of an acute psychosis. Three days before his death he attended as an outpatient complaining of pains in the legs. At that time his haemoglobin was 85%. The day before his admission and death he developed epistaxis and was admitted 'in extremis' with a Hb of 16%. His one-stage 'prothrombin' time was over 10 minutes and his whole blood coagulation time over one hour. He died within two hours of admission despite blood transfusion and intravenous synkavit.

A.E. (aged 57), June 1956. This patient was admitted under Dr. Wright's care in the Royal Infirmary, Glasgow. The immediate cause of admission was an extensive spontaneous haematoma of the left arm. She had previously been in hospital on two occasions - five years and two years before the present admission, and had been diagnosed as a case of steatorrhoea, the defective fat absorption having been demonstrated. These admissions had been on account of anaemia, tetany and a disseminated neurological lesion.

Blood withdrawn on the recent admission and examined gave the following findings:-

Whole blood coagulation time (method (2)).	3 hours +
One-stage test	- $4\frac{1}{2}$'
+ 1/10 nor. serum	- 35"
+ 1/10 ads. nor. plasma	- $3\frac{3}{4}$'

Prothrombin consumption test method of Douglas and Biggs 1953 - single specimen examined at one hour:

- one hour specimen — 100% prothrombin.

On the thromboplastin generation technique the serum was unable to correct the defect in Christmas disease serum - see figure III . The plasma was unable to correct the defective prothrombin consumption of Christmas disease plasma (see figure II2). Intravenous administration of vitamin K_1 (50 mg.) resulted in correction of the defect in 24 hours.

Figure (III.)

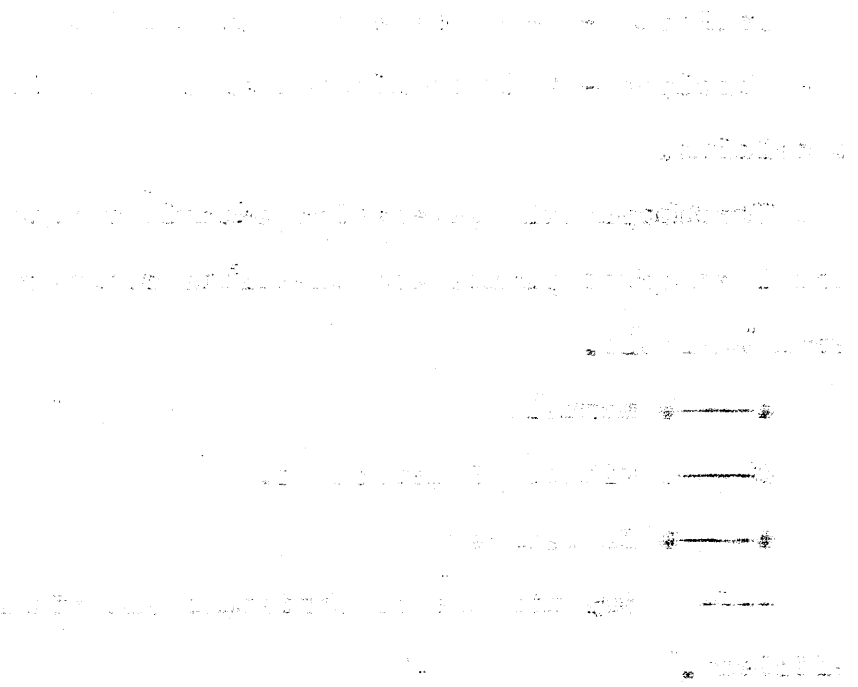


Figure (111)

Failure of serum in vitamin K deficiency to correct the thromboplastin defect in Christmas disease serum.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation technique with normal adsorbed plasma and platelets constant - serum variable.

●——● normal.

O——O vitamin K deficient.

●——● Christmas.

---- 50% mixture of Christmas and vitamin K deficient.

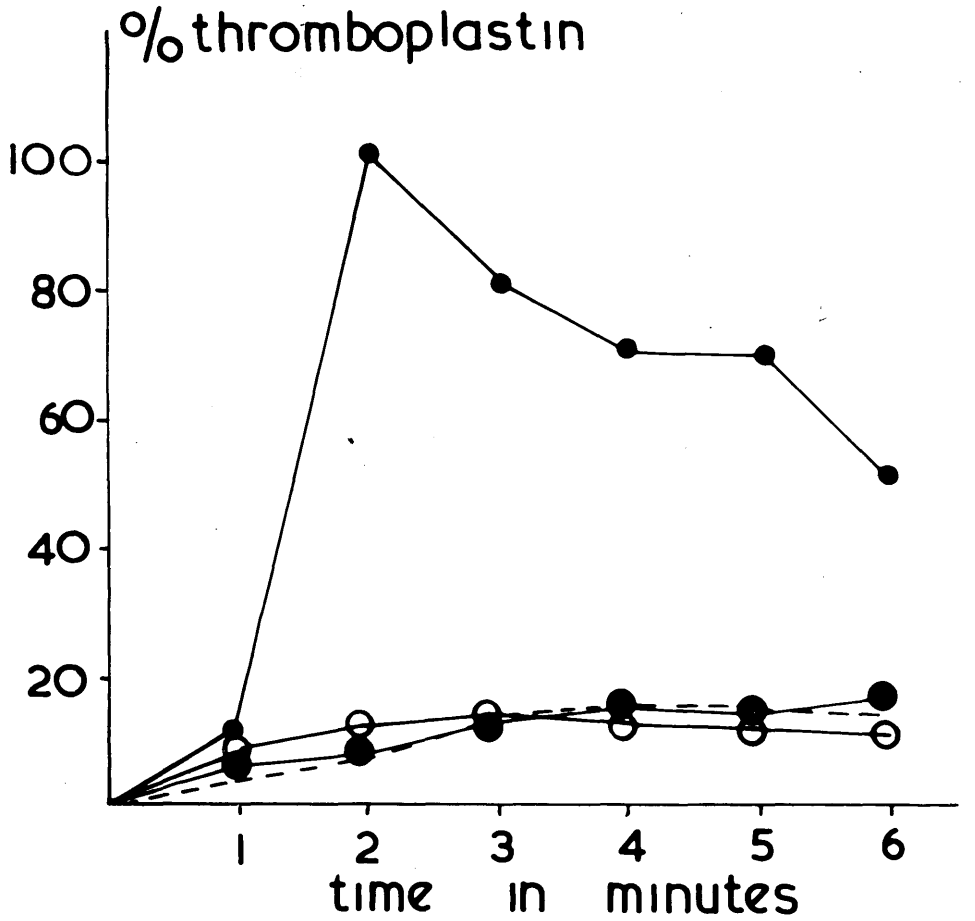

% thromboplastin
100
80
60
40
20
1
2
3
4
5
6
time in minutes

Figure (111)

Failure of serum in vitamin K deficiency to correct the thromboplastin defect in Christmas disease serum.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation technique with normal adsorbed plasma and platelets constant - serum variable.

●——● normal.

O——O vitamin K deficient.

●——● Christmas.

---- 50% mixture of Christmas and vitamin K deficient.

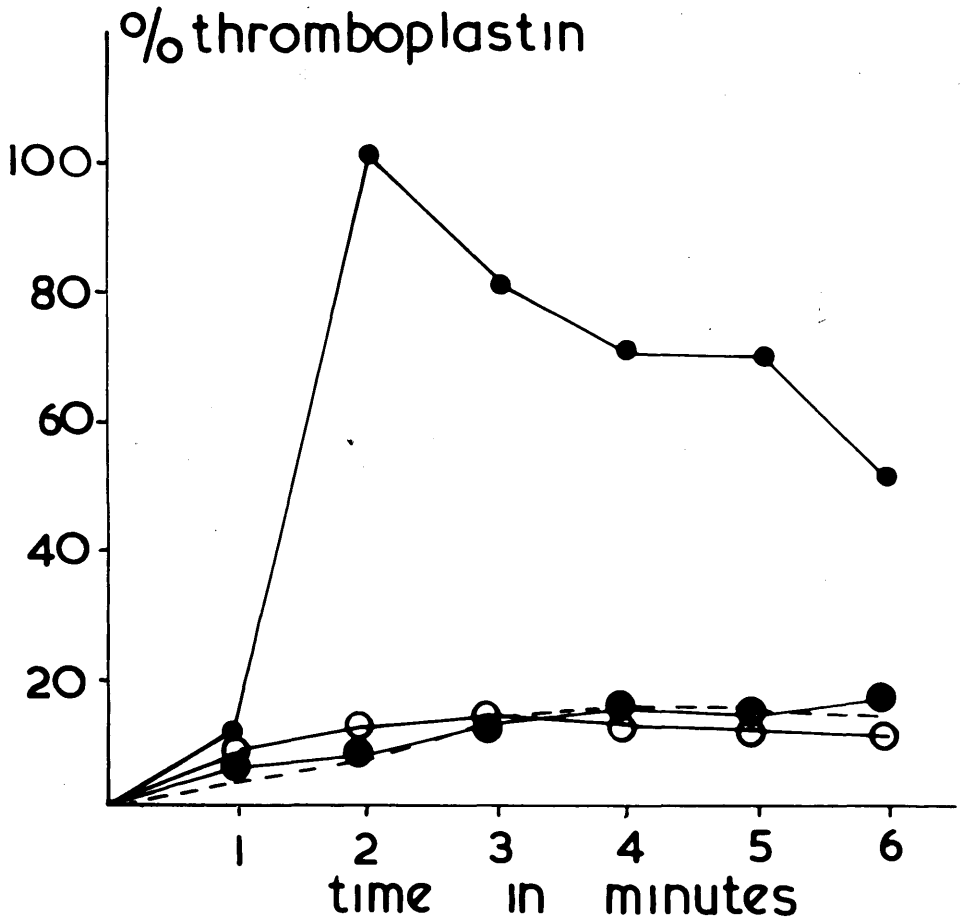

% thromboplastin
100
80
60
40
20
1
2
3
4
5
6
time in minutes

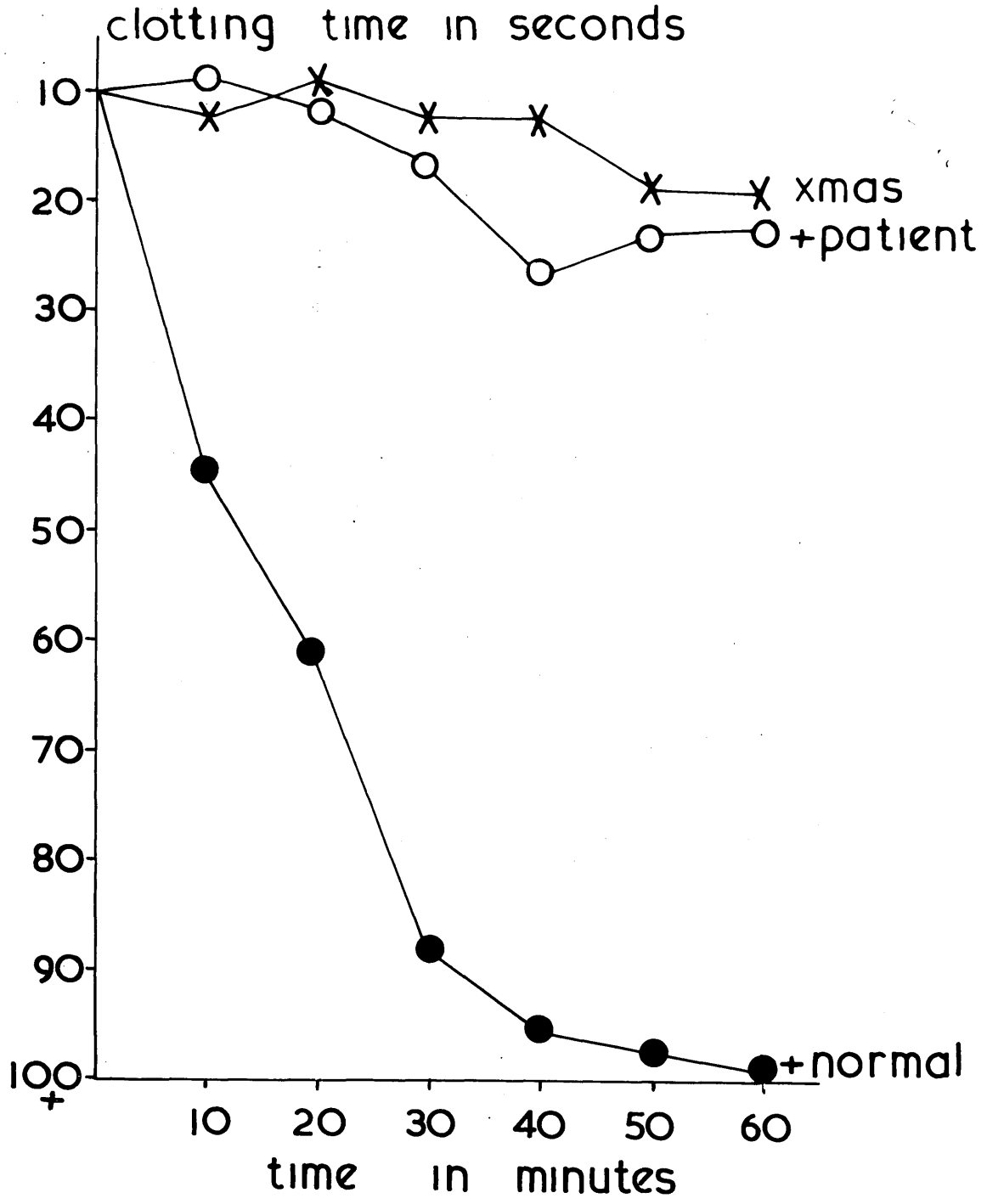
Figure (112)

Failure of vitamin K deficient plasma to correct the defective prothrombin consumption of Christmas disease plasma.

Ordinate - clotting time of fibrinogen.

Abscissa - time in minutes after addition of calcium.

This figure shows the prothrombin consumption over one hour of recalcified Christmas disease plasma, alone and with additions of normal plasma and of vitamin K deficient plasma.


clotting time in seconds
10
20
30
40
50
60
70
80
90
100
xmas
+patient
+normal
10
20
30
40
50
60
time in minutes

Kark et al (1940) concluded from their own case and from those in the literature that the haemorrhagic hypoprothrombinaemia appears in steatorrhoea only after prolonged treatment with almost complete restriction of fatty foods. Such low fat diets may be relatively deficient in the intake of vitamin K and this long continued restriction is considered by Kark et al (1940) to be an important aetiological factor in the production of "hypoprothrombinaemia" in these cases. Such patients have not only difficulty in bacterial synthesis but their intake of the vitamin may be relatively restricted and this may sometimes be the precipitating factor resulting in haemorrhage. This is supported by the evidence of others that the oral administration of vitamin K has been efficacious in controlling the haemorrhagic symptoms and producing an increase in prothrombin concentration (Kark et al 1940, Albright and Stewart 1947, Allen 1941).

Comment: The coagulation defect in steatorrhoea is of deficiencies of factor VII and prothrombin. The serum is defective in its ability to form thromboplastin and fails to correct the abnormality in Christmas disease serum. The plasma is unable to rectify the defective prothrombin consumption of Christmas disease plasma. This represents evidence of Christmas factor deficiency in addition to the lowered concentration of factor VII and prothrombin.

Nutritional vitamin K deficiency

A patient with intestinal obstruction was operated upon for small bowel obstruction and was thereafter on continuous gastric and intestinal drainage with administration of intravenous fluids for several days before the specimens were collected for assessment of the coagulation mechanism. There was no evidence of haemorrhagic phenomena.

Investigation of coagulation abnormality.

Quick's test. Control 16" Patient 23"

plus 1/10 normal serum 17"

plus 1/10 adsorbed plasma 23"

Prothrombin content by globulin fraction technique = 68%

Thromboplastin generation test.

Normal adsorbed plasma Platelets	(1)	(2)	(3)	(4)	(5)	(6)
Normal serum	17	10	10	10	9	10
Patient serum	25	15	14	15	15	16

Restoration of these abnormalities was found 24 hours after the intravenous administration of 50 mg. vitamin K_1.

SUMMARY

Newborn:

The "hypoprothrombinaemia" in the newborn is due to a deficiency both of factor VII and prothrombin; water soluble vitamin K given to the infant, though not producing normal levels of these coagulation factors prevents the deficiency which is normally present on the third day.

In one case of haemorrhagic disease of the newborn it has been shown that there was a marked deficiency of factor VII and prothrombin and an inability of the serum to form blood thromboplastin normally. The serum was unable to correct the serum thromboplastin defect in Christmas disease.

Salicylate Therapy.

There is a deficiency of factor VII and prothrombin and the serum does not form thromboplastin normally.

Steatorrhoea.

The "hypoprothrombinaemia" is a deficiency of factor VII and prothrombin. The serum thromboplastin activity is also abnormal and evidence is presented that this is due to deficiency of Christmas factor.

Nutritional vitamin K deficiency.

The defect here also is a deficiency of factor VII and prothrombin and a failure of the serum to form blood thromboplastin normally.

REFERENCES

Albright, F., and Stewart, J.D. (1940). Hypovitaminosis of all fat-soluble vitamins due to steatorrhoea. New Engl. J. Med., 223, 239.

Aldersberg, D., and Schein, J. (1947). Clinical and pathologic studies in sprue. J. Amer. Med. Ass., 134, 1459.

Allen, J.G. (1941). The comparative prothrombin responses to vitamin K and several of its substitutes in a case of nontropical sprue. New Engl. J. Med., 224, 195.

Biggs, R. and Douglas, A.S. (1953). The measurement of prothrombin in plasma. J. clin. Path., 6, 15.

Biggs, R., and Macfarlane, R.G. (1953). Human blood coagulation and its disorders. Blackwell Scientific Publications, Oxford.

Hay, J.D., Hudson, F.P. and Rodgers, T.S. (1951). Vitamin K in the prevention of haemorrhagic disease of the newborn. Lancet, 1, 423.

Kark, R., Souter, A.W. and Hayward, J.C. (1940). A haemorrhagic diathesis in idiopathic steatorrhoea: observations on its association with vitamin K deficiency. Quart. J. Med., 9, 247.

Jaques, L.B. and Lepp, E. (1947). Action of sodium salicylate on prothrombin time in rabbits. Proc. Soc. exp. Biol. Med., 66, 178.

Link, K.P. (1943). The anticoagulant from spoiled sweet clover hay. Harvey Lect., 39, 162.

Link, K.P., Overman, R.S., Sullevan, W.R., Heubner, C.F. and Scheel, L.D. (1943). Studies on the haemorrhagic sweet clover disease. Hypoprothrombinaemia in the rat induced by salicylic acid. J. biol. Chem., 147, 463.

Loeliger, A., and Koller, F. (1952). Behaviour of factor VII and prothrombin in late pregnancy and in the newborn. Acta Haemat., 7, 157.

Sanford, H.N., Shinigelsky, I. and Chapin, J.M. (1942).
Is administration of vitamin K to the newborn of clinical value?
J. Amer. med. Ass. 118, 697.

Seegers, W.H. (1951).
The influence of certain drugs on blood coagulation and related phenomena.
Pharm. Reviews 3, 278.

Stefanini, M. (1951).
Activity of plasma labile factor in disease.
Lancet, 1, 606.

Tarnoky, A.L. and Steingold, L. (1951).
The action of p-aminosalicylic acid on prothrombin time in man.
J. clin. Path. 4, 478.

CHAPTER 14

COAGULATION DISTURBANCE IN LIVER DISEASE

(obstructive jaundice and parenchymatous hepatic disorders).

CONTENTS.

CHAPTER 14

COAGULATION DISTURBANCE IN LIVER DISEASE

In this chapter an account is given of the disturbances of blood coagulation arising in obstructive jaundice and in chronic parenchymatous disease of the liver (hepatic cirrhosis).

OBSTRUCTIVE JAUNDICE.

Five patients were studied; the vitamin K deficiency was proven by the subsequent response to the intravenous administration of 50 mg. of vitamin K_1. The details of this response to vitamin K_1 therapy are given in Chapter 24 .

A.L. This patient had her common bile duct accidentally ligated during a cholecystectomy. In addition to the other effects of an obstructive jaundice she had steatorrhoea. There were no haemorrhagic phenomena and the prolonged one-stage clotting time was observed during the routine assessment of the patient. The response to vitamin K_1 in this patient is described in Chapter 24 . For the present a description of the coagulation defect is given:-

Quick's one-stage test. Normal 16" patient 25".

Patient's plasma + 1/10 part adsorbed normal plasma = 25"

Patient's plasma + 1/10 part normal serum = 21"

Platelet count = 250,000.

Recalcification time normal $2\frac{1}{2}$' ; 3'

patient $2\frac{1}{2}$' ; $2\frac{3}{4}$'

Prothrombin content by globulin fraction technique = 64%.

Thromboplastin generation test.

	(1)	(2)	(3)	(4)	(5)	(6)
Normal adsorbed plasma Normal Platelets Normal serum	17	9	10	9	8	9
Normal adsorbed plasma Normal Platelets Patient serum	55	35	35	32	32	32
Patient adsorbed plasma Normal Platelets Normal serum	40	9	8	9	9	9

To test ability to correct Christmas disease serum on thromboplastin generation.

Normal adsorbed plasma
Platelets

Serum 0.2 Christmas serum

with 0.1 of other sera

	(1)	(2)	(3)	(4)	(5)	(6)
Normal	10	9	8	9	9	9
Patient	57	40	28	30	25	22
Dindevan	60	32	28	29	30	30

(from patient who had been several weeks on treatment

with dindevan and at a time when the one stage test was 40" against a control of 15".

Conclusion: Inability to correct Christmas disease serum.

Comment:

This patient's coagulation defect had the following features:

(1) Deficiency of prothrombin.

(2) " " factor VII.

(3) Inability of the serum to form thromboplastin and to correct the defect of Christmas disease serum in this respect.

This is an identical defect to that in dindevan therapy. The immediate response of these abnormalities to the administration of vitamin K_1 is described in Chapter 24 .

Patient with obstructive jaundice.

Female patient aged 61 years with obstructive jaundice of 2 weeks' duration. No haemorrhagic phenomena. Response to vitamin K_1 is described in the appendix.

Investigation of coagulation defect.

Quick's one-stage test.

Patient 33" Control 15"

Prothrombin assay by globulin fraction technique.

Patient 22" Control 10"

Prothrombin = 45%.

Thromboplastin generation technique.

	(1)	(2)	(3)	(4)	(5)	(6)
Normal adsorbed plasma Platelets constant Normal serum	11	8	8	9	9	9
Patient serum	65	33	18	13	13	12
Christmas serum	93	41	38	31	25	24
12.5% Normal serum in Christmas serum	12	9	9	10	10	10
12.5% Patient serum in Christmas serum	72	20	14	16	16	16

Patient with obstructive jaundice due to carcinoma of the head of the pancreas. Vitamin K_1 studies on this patient are described later. Evidence of obstruction had only been present for 10 days prior to this investigation. There was no evidence of any haemorrhagic phenomena.

Investigation of coagulation defect.

Quick's test. Patient 35". Control 15"

Prothrombin assay by globulin fraction technique.

Patient 20". Control 10"

= 50% prothrombin.

Thromboplastin generation technique.

	(1)	(2)	(3)	(4)	(5)	(6)
Normal adsorbed plasma Platelets Normal serum	8	10	9	9	9½	9½
Normal adsorbed plasma Platelets Patient's serum	44	20	15	15	16	16
Normal adsorbed plasma Platelets with						
Christmas serum	40	40	30	34	28	30
75% Christmas serum) 25% Normal serum)	14	11	11	10	11	11
75% Christmas serum) 25% Patient serum)	48	31	27	26	26	28
Dindevan serum	40	42	40	42	39	40
50% Christmas serum) 50% Dindevan serum)	36	30	26	28	30	28
50% Christmas serum) 50% Patient serum)	70	38	26	22	20	20

Factor VII.

One-stage technique.

Dindevan plasma	30"
Patient plasma	35"
50% mixture	36"

Patient with obstructive jaundice as a consequence of Hodgkin's disease. No haemorrhagic phenomena.

Investigation of coagulation defect.

Quick's test. Patient 31". Control 15".

Prothrombin assay by globulin fraction technique

= 45% prothrombin.

Thromboplastin generation technique.

	(1)	(2)	(3)	(4)	(5)	(6)
Normal adsorbed plasma Platelets Normal serum	80	33	10	10	10	11
Varied as follows						
Patient serum	90	80	65	40	22	15
Christmas serum	66	46	60	45	32	32
50% Christmas serum) 50% Normal serum)	18	10	10	11	10	11
50% Christmas serum) 50% Patient serum)	65	16	14	16	15	18

PARENCHYMATOUS LIVER DISEASE.

Results.

In a series of 16 consecutive cases of liver disease admitted to Professor Davis' wards in the Royal Infirmary, Glasgow, a detailed assessment of the coagulation mechanism was made. The results are shown in Table 22 .

In a further 8 patients seen in the Radcliffe Infirmary, Oxford, during 1951-52 and suffering from hepatic cirrhosis the factor VII and prothrombin only were estimated. The results are shown in Table 23 .

Comments.

Factor VII and prothrombin: Both these factors were usually deficient, the factor VII in general to a greater extent than the prothrombin. Occasionally the deficiency of prothrombin was greater than the reduction in the concentration of factor VII. Parenchymatous liver disease was the only example of "hypoprothrombinaemia" where this reversal of the order of greater deficiency was found. In all other conditions the deficiency of factor VII was greater than the reduction of prothrombin. This observation has also been made by Donald et al (1954) and Hunter and Walker (1955).

Diagnosis	Lag Time	Lag Time	Horse test	Count (thousands cu.mm.)	A.M.S. Titre agglutination %
Typhosis	3	8	positive	97	100
Typhosis	5	8	negative	88	100
Abortive Undiagnosable Biliary Typhosis	3'	10	positive	46	100
Typhosis	4	14	negative	148	100
Typhosis	2	10	negative	224	75
Typhosis (Biliary)	3	8	negative	440	100
Typhosis	3	9	negative	136	100
Infective Paratyphosis	3	8	negative	225	100
Typhosis	2	10	negative	275	100
Typhosis	3	12	negative	346	100
Abortive Undiagnosable Diplegam	2	3'	negative	164	100
T.Hepatitis	3	3	negative	204	100

TABLE 22

Case	Diagnosis	Bleeding Time	Clotting Time	Hess test	Platelet count (thousands cu.mm.)	A.H.G. assay %	Christ. Fa assay %
(1)	Cirrhosis	3	8	positive	97	100	100
(2)	Cirrhosis	3	8	negative	88	100	50
(3)	Obstructive Jaundice Biliary Cirrhosis	2'	10	positive	46	100	2
(4)	Cirrhosis	4	14	negative	149	100	100
(5)	Cirrhosis	2	10	negative	224	75	100
(6)	Cirrhosis (Biliary)	3	5	negative	440	100	100
(7)	Cirrhosis	3	9	negative	156	100	50
(8)	Infective hepatitis	3	8	negative	225	100	20
(9)	Cirrhosis	2	10	negative	275	100	100
(10)	Cirrhosis	3	12	negative	346	100	100
(11)	Obstructive jaundice neoplasm	2	5'	negative	164	100	15
(12)	Inf.Hepatitis	3	5	negative	204	100	25
(13)	Carcinoma of stomach with hepatic metastases	2	7	negative	201	100	75
(14)	Cirrhosis	4	6	negative	120	100	100
(15)	Cirrhosis	17	11	negative	243	100	100
(16)	Unexplained hepatomegaly ?myelofibrosis	3'	8	negative	237	100	100

- = no lysis
+ = lysis present.

	Factor VII assay %	Factor V assay %	Prothrombin assay %	Fibrinogen assay	Fibrinolytic activity 6 hrs.	Catubes Thrombin Tubes 24 hrs.
)0	10	5	60		---+	++++
					----	++++
					++++	++++
50	6	50	44		++++	++++
					----	----
2	0	12	13		----	----
					---+	++++
)0	25	6	80		----	-+++
					----	++++
)0	12	35	69		----	--++
					----	---+
)0	50	100	95		----	----
					++++	++++
50	25	35	42	189 mg.	++++	++++
					----	----
20	12	30	65		----	----
					++++	++++
)0	12	50	50		--++	--++
					---+	++++
)0	40	100	80		----	++++
					----	----
.5	12	100	100		----	----
					----	--++
25	25	25	48	206 mg.	----	---+
75	20	35	95			
					-+++	++++
)0	25	10	95		----	++++
)0	6	10	64	206 mg.	----	----
					----	--++
)0	40	45	95		----	---+

TABLE 23

Observation	Quick %	VII %	Prothrombin %
1	within normal limits	24	45
2	"	44	69
3	"	65	48
4	38	55	20
5	within normal limits	100	32
6	30	64	33
7	within normal limits	100	60
8	25	62	52
Mean	45	64	45

Factor V - This was frequently deficient but not so constantly as was the factor VII. Deficiency of factor V has been observed previously by Owren (1949) and Stefanini (1950) in parenchymatous liver disease.

Christmas factor: There was often a deficiency of the Christmas factor but this was not so constant nor of the extent of the deficiency of factor VII.

Antihaemophilic globulin: It is of interest that the

concentration of this factor was normal throughout the series of patients.

Platelets: In three of the patients there was appreciable thrombocytopenia.

Fibrinolysis. There was considerable activation of the fibrinolytic mechanism in chronic hepatic cirrhosis, less so in infective hepatitis and almost absence of activity in obstructive jaundice (see figure 113). The numbers of cases are too small to permit of any final conclusions. This activation of fibrinolysis in chronic hepatic disease was first reported by Goodpasture in 1914.

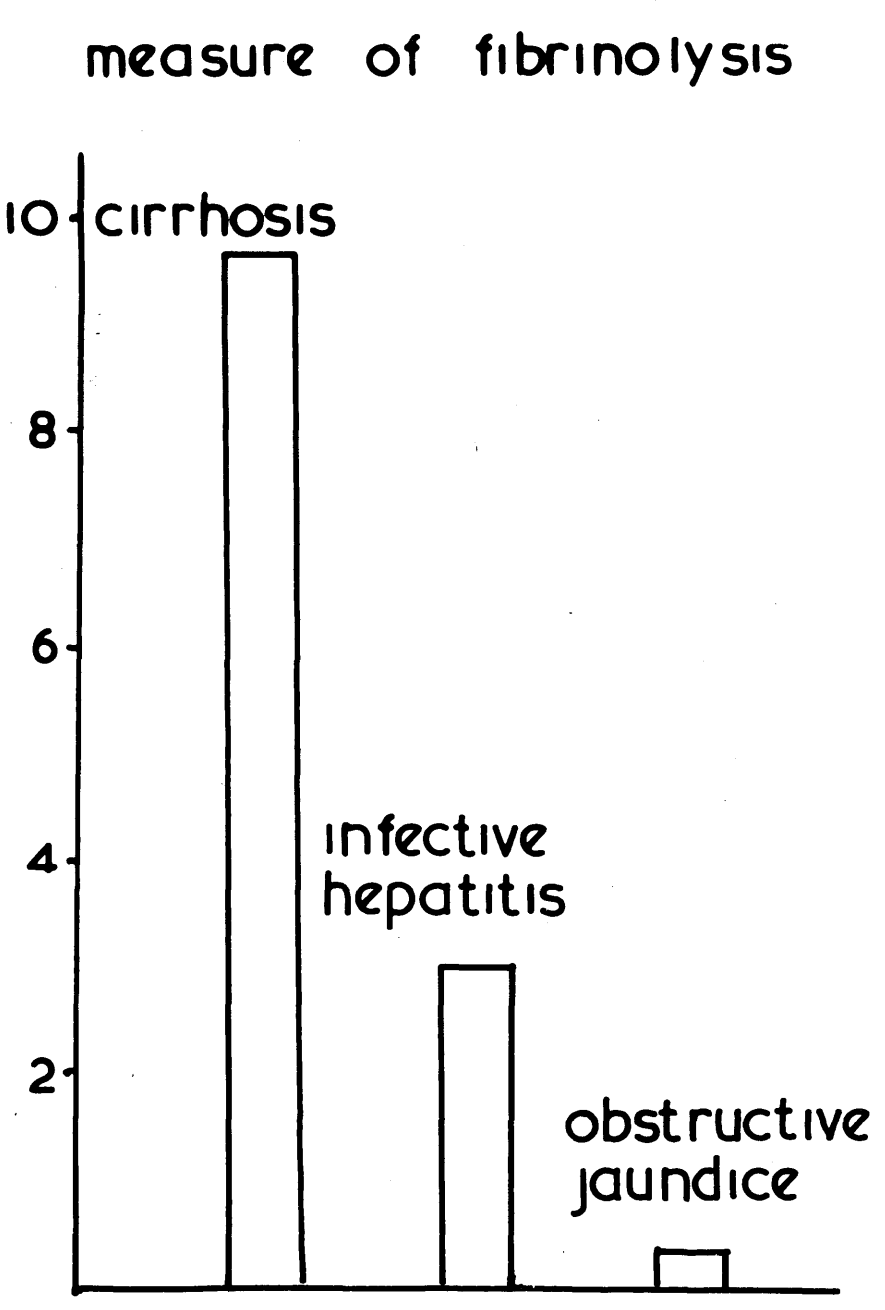
CASE REPORT.

This male patient aged 70 was admitted to hospital unconscious. On examination there was evidence of meningeal irritation and lumbar puncture revealed subarachroid haemorrhage. On other parts of the body there were fairly large and numerous haematomata. He recovered consciousness without evidence of residual neurological damage. During the next few weeks he had numerous spontaneous bruises and ecchymosis. Venepuncture always resulted in a large haematoma at the site. He became oedematous and slightly icteric and some weeks after admission slowly deteriorated and died. Autopsy examination revealed advanced hepatic cirrhosis.

Figure (113)

Fibrinolysis in Liver Disease.

Ordinate - measure of fibrinolysis; this measure is obtained as the number of thrombin and calcium tubes shows lysis, at the specified dilutions, at 6 hours and 24 hours.


measure of fibrinolysis
10
8
6
4
2
cirrhosis
infective hepatitis
obstructive jaundice

The results on the investigation of this patient were:

Whole blood coagulation time - 15' (very poor clot formation)
(Technique (2) - normal range 10'-25')

Hess test - negative.

Bleeding Time (Ivy) - 5'.

Platelet count - mean of two readings 63,000.

Recalcification Time:

			Later observation	
Patient	4'	4½'	10'	11'
Control	2½'	2¾'	1½'	1½'

The fibrin formed was very poor in the patient's plasma. It collapsed to a tiny thready affair when the tube was agitated and very quickly lysed on incubation at 37° C.

Quick's one-stage test.

Patient	29"	30"
Control	14"	15"

- again the fibrin formation was very poor.

Factor V assay - deficiency established-15% of normal.

	(1)	(2)	(3)	(4)	(5)	(6)
0.3 Pro 1/5 0.3 Saline 0.3 Brain 0.3 $CaCl_2$	3'+	3'+	3'+	3'+	3'+	3'+
As above except Factor V in place of saline.						
Normal	30"	14"	11"	11"	12"	11"
Patient	3'+	3'+	90"	55"	40"	27"
½ Normal	3'+	44	17	14	12	11
¼ Normal	3'+	90	50	22	14	13
⅛ Normal	3'+	3'+	95	72	35	20
1/16 Normal	3'+	3'+	3'+	3'	72	60

Factor VII assay - deficiency revealed.

Dindevan plasma - one-stage test 49".

Plus 1/10 Patient's plasma = 41
" " " serum = 35"

Plus 1/10 Normal plasma	= 30"	= 100%
	= 33"	= 50%
(Doubling	= 37"	= 25%
dilutions in	= 42"	= 12%
dindevan plasma)	= 44"	= 6%
	= 45"	= 3%
	= 49"	= 0%
Plus 1/10 Normal serum	= 22"	= 100%
(Doubling dilutions	= 29"	= 50%
in dindevan plasma)	= 31"	= 25%
	= 33"	= 12%
	= 42"	= 6%
	= 43"	= 3%
	= 49"	= 0%

Reading the patient's factor VII content on this curve

- from plasma curve 15%
" serum curve 10%

Mean 12.5%

- approximately $\frac{1}{8}$ of normal concentration.

Prothrombin assay -

by globulin fraction method = 24%

Thromboplastin Generation Technique.

	(1)	(2)	(3)	(4)	(5)	(6)
Normal ad. plasma Normal serum Platelets	45	10	10	11	10	11
Patient ad. plasma Normal serum Platelets	10	10	11	11	8	8
Normal ad. plasma Patient serum Platelets	45	35	27	27	28	26
Normal ad. plasma Platelets 50% normal serum in Christmas serum	11	11	11	11	12	11
33% "	12	12	11	11	13	12
25% "	12	12	13	11	11	12
10% "	53	23	17	15	17	17
50% patient serum in Christmas serum	24	14	14	15	16	15

Reveals - no deficiency of antihaemophilic globulin

deficiency of Christmas factor - approximately 10% of normal.

Fibrinogen assay - 48 mg. per cent.

Fibrinolytic activity.

(done a few days before death - at a later date than the other observations)

	Calcium Tubes			Thrombin Tubes		
	1/16	1/32	1/64	1/16	1/32	1/64
Patient	No fibrin formation.					
5 hrs.	"	"	"	completely lysed		
15 hrs.	"	"	"	"		"
Normal						
5 hrs.	-	-	-	-	-	-
15 hrs.	-	-	-	-	-	+

As above except that 0.1 of a solution of fibrinogen added to test systems and serum used in place of plasma.

	Calcium Tubes			Thrombin Tubes		
	1/16	1/32	1/64	1/16	1/32	1/64
Patient	Complete lysis at 5 hours			Complete lysis at 5 hours		
Normal	No lysis.			No lysis.		

Thrombin - fibrinogen Reaction.

Standard thrombin.

Thrombin	1/1	1/3	1/1	1/3
Normal	12"	30"	5"	6"
Patient	3'+	2¼'	1½'	3'+

This abnormality of the thrombin-fibrinogen reaction is

probably a manifestation of fibrinogen deficiency.

Antithrombin assay (Douglas and Biggs 1953).

	½'	1	2	3	4	5
Patient	10"	15"	17"	19"	25"	35"
Normal	25"	45"	95"	150"	3'+	3'+

Apparently from this the patient has much less antithrombin than the control. This again is probably a manifestation of fibrinogen deficiency, fibrin being a powerful adsorbent of thrombin.

Comments: Investigation of this patient's haemostatic defect revealed that this had many facets. There was deficiency of prothrombin, factor VII, Christmas factor, factor V, fibrinogen and platelets. There was also an abnormally active fibrinolytic mechanism. The only coagulation component present in normal concentration was antihaemophilic globulin.

The nature of the disorder resulting from vitamin K deficiency in obstructive jaundice has essentially the same features as the conditions discussed in Chapter /3 - deficiencies of factor VII and prothrombin and impaired serum thromboplastic activity with the inability of this serum to correct the defect in Christmas disease serum. The disturbance which arises in parenchymatous liver disease is often

much more complex. There may be deficiencies of any of the coagulation components with the possible exception of anti-haemophilic globulin. To what extent the increased fibrinolytic activity contributes to abnormal haemorrhage is problematical.

SUMMARY

(1) The "hypoprothrombinaemia" of obstructive jaundice is caused by deficiencies of prothrombin and factor VII. The ability of the serum to form blood thromboplastin is reduced and it is unable to correct the thromboplastin defect in Christmas disease.

(2) Parenchymatous liver disease can produce deficiencies of prothrombin, factor VII, Christmas factor, factor V, fibrinogen and platelets. There may also be an abnormally active fibrinolytic mechanism.

(3) A.H.G. concentration remained normal even in patients dying of liver failure in chronic hepatic cirrhosis.

(4) Fibrinolysis was found to be very active in parenchymatous liver disease but not in obstructive jaundice.

REFERENCES

Donald, A.C., Hunter, R.B., Tudhope, G.R., Walker, W. and Whitton, I. (1954)
Prothrombin and haemorrhage.
Brit. med. J. 2, 961.

Hunter, R.B. and Walker, W. (1954)
The coagulation defect in liver disease thrombose and embolic. International tagung Basel 1954, page 116.

Goodpasture, E.W. (1914).
Fibrinolysis in chronic hepatic insufficiency.
John Hopkins Hosp. Bull. 25, 330.

Owren, P.A. (1949).
The diagnostic and prognostic significance of plasma prothrombin and factor V levels in parenchymatous hepatitis.
Scand. J. clin. lab. Invest. 1; 131.

Stefanini, M. (1950).
Haemorrhagic diathesis of liver dysfunction and obstructive jaundice. Proc. IIIrd Congress, Intern. Soc. Haematology. New York, Grune & Stratton, 1951.

Figure (114)

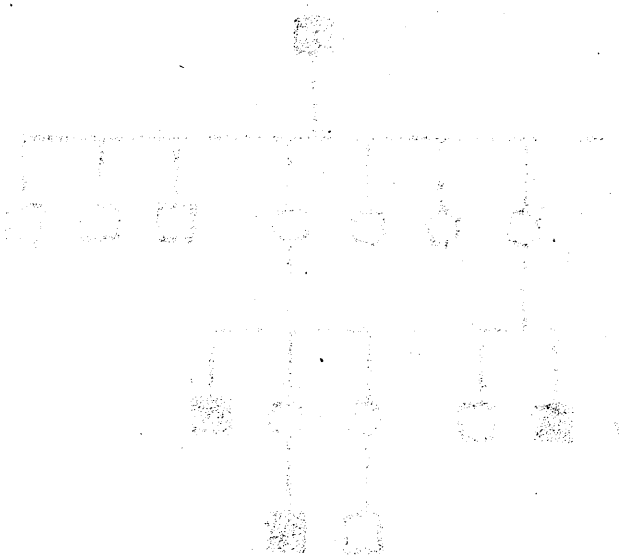


Figure (114)

Illustrative family tree in haemophilia.

□ unaffected male.

■ affected male.

O female.

III 1 is Case (2) in the series.

IV 1 " Case (9) " " "

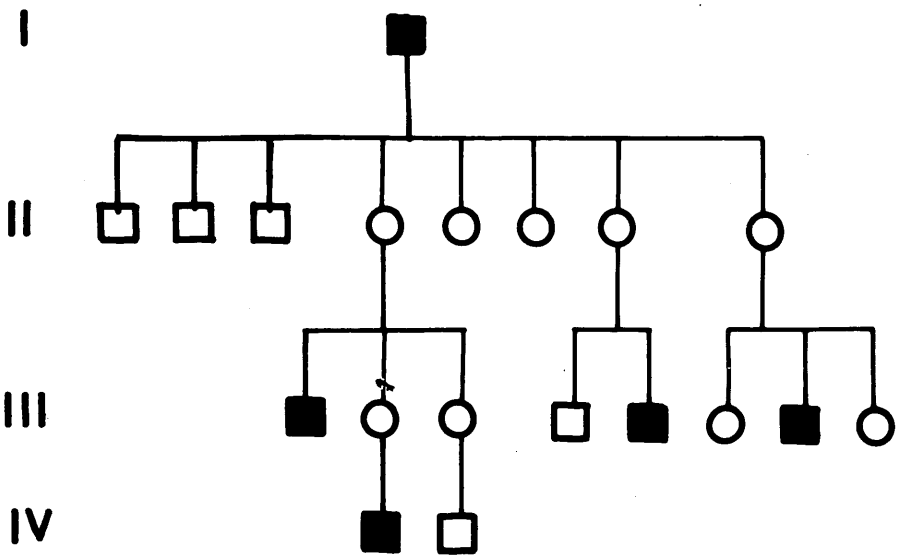

I
II
III
IV

Figure (115)

Figure (115)

Illustrative family tree in haemophilia.

□ unaffected male.

■ affected male.

O female.

III 5 is Case (16) in the series.

IV 2 is Case (51) in the series.

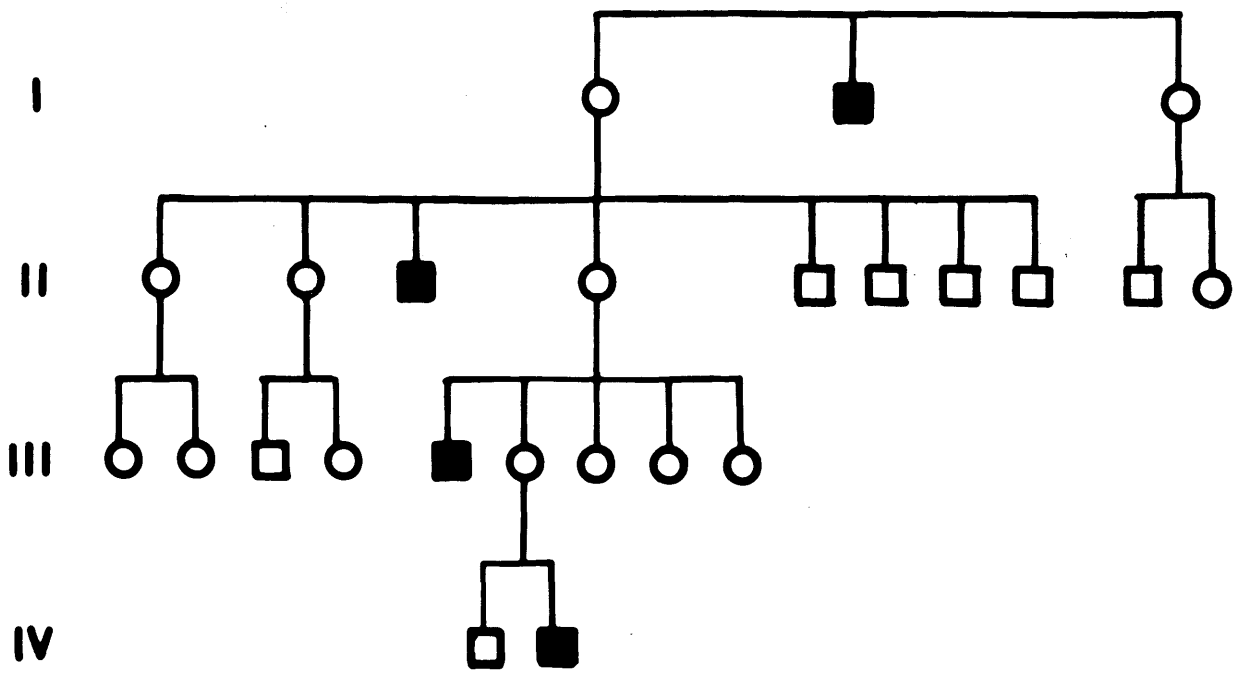

I
II
III
IV

Figure (116)

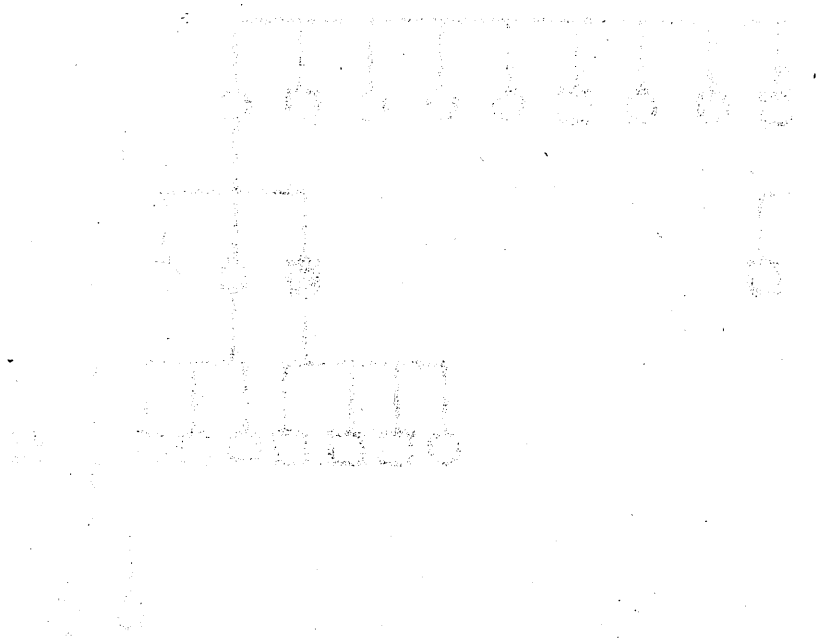


Figure (110)

Illustrative family tree in haemophilia.

□ unaffected male.

■ affected male.

O female

II 5 is Case (68) in the series.

II 8 is Case (85) in the series.

III 2 is Case (19) in the series.

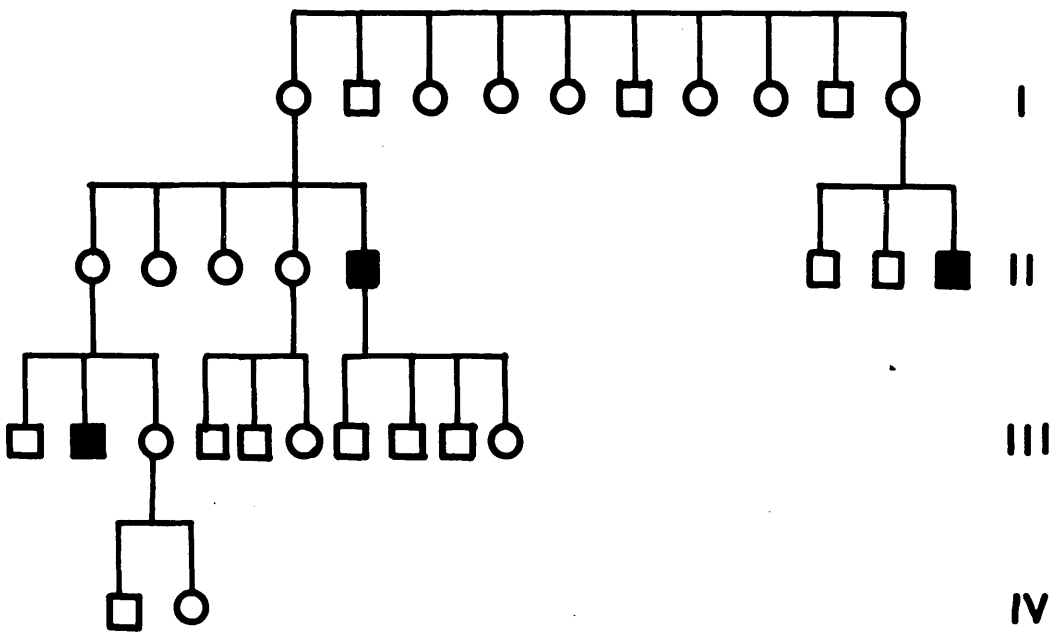

I
II
III
IV

Figure (117)

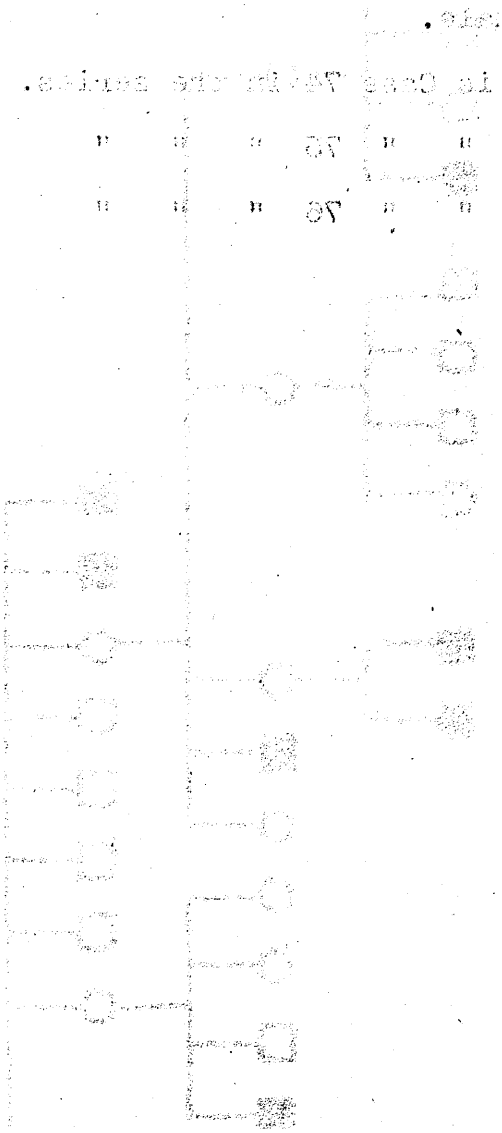


Figure (117)

Illustrative family tree in Christmas Disease.

□ unaffected male.

■ affected male.

O female.

IV 1 is Case 74 in the series.

IV 2 " " 75 " " "

IV 7 " " 76 " " "

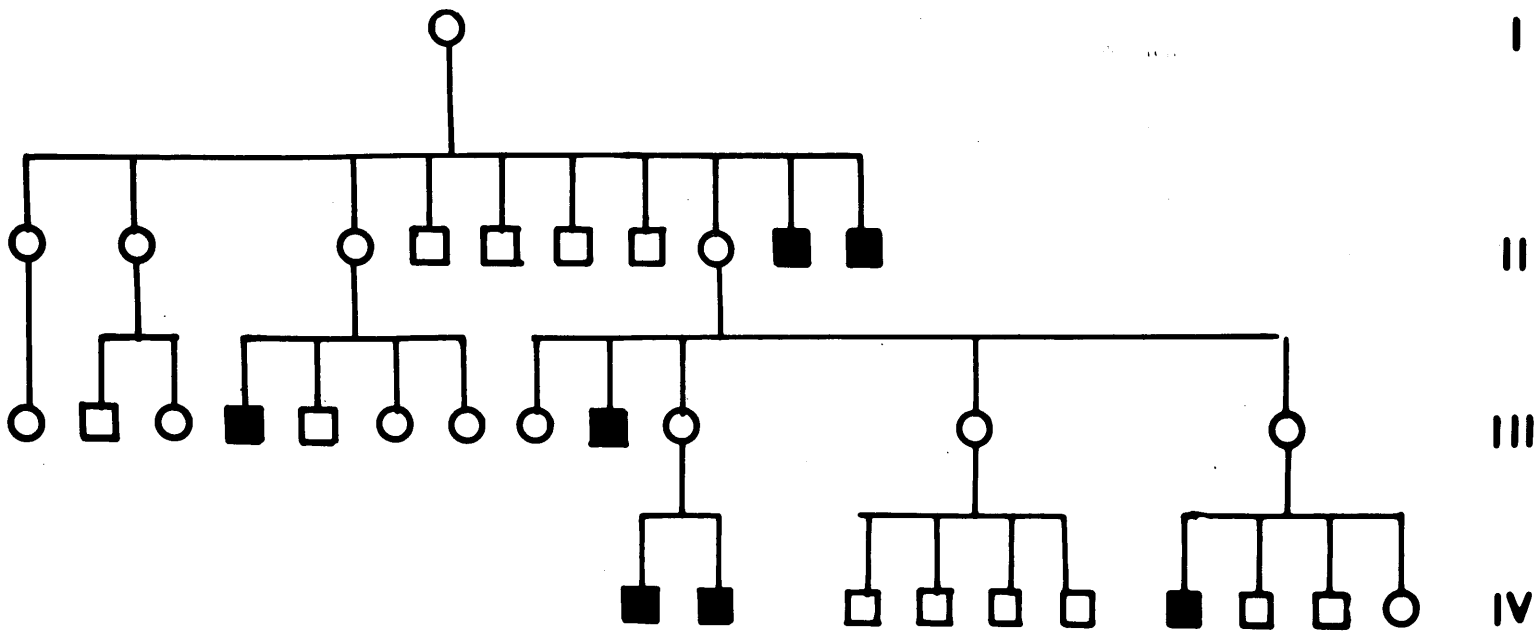

I
II
III
IV

Figure (118)

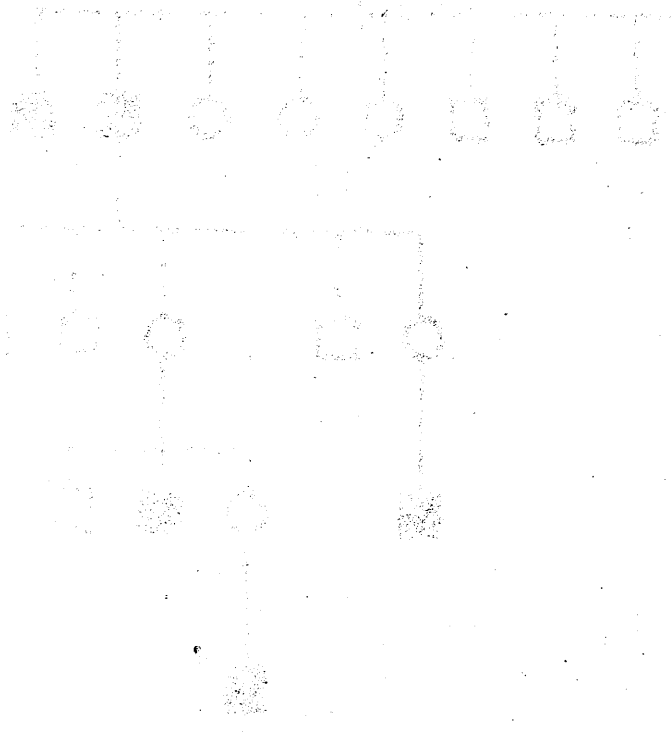


Figure (111)

Illustrative family tree in Christmas Disease.

□ unaffected male.

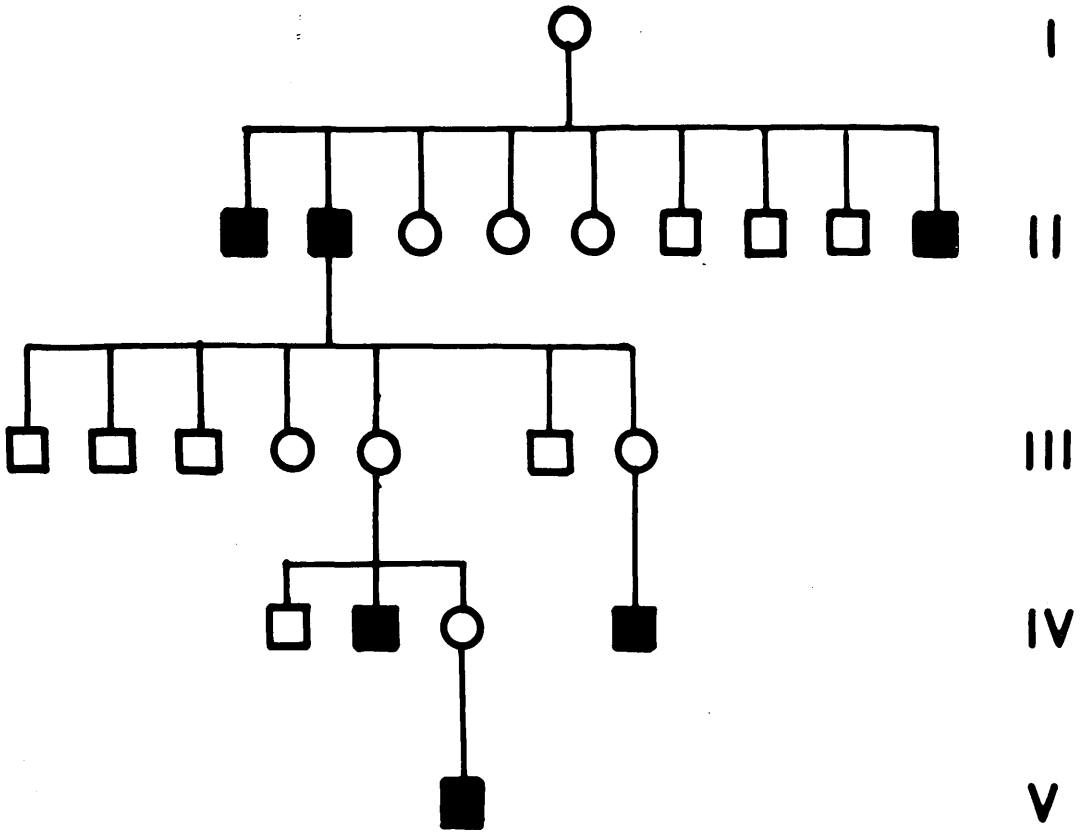
■ affected male.

O female.

IV 2 is Case 5 in the series.

IV 4 " " 50 " " "

V 1 " " 78 " " "


I
II
III
IV
V

(8) Neurological manifestations.
(9) Operations.
(10) Fractures.
(11) Miscellaneous.

CHAPTER 15

HAEMOPHILIA AND CHRISTMAS DISEASE

A clinical study of eighty-seven patients.

The object of this chapter is to record the findings obtained in 87 cases of haemophilia or Christmas disease during a survey of the condition in the West of Scotland. A few aspects of the laboratory investigations will be described but it was the main objective of this present chapter to record some of the details of the clinical features of these cases.

Until recent years haemophilia has been defined as a constitutional anomaly of blood coagulation depending on the hereditary transmission of a sex-linked recessive trait and having as its feature a lifelong liability to haemorrhage in the affected males. Many of the genetic and clinical features of the disease have long been recognised. Thus it has been accepted that females are almost exclusively immune from the disease but capable of transmitting it to their male offspring and that the manifest disease is characterised by the occurrence of bleeding in joints and deep tissues either spontaneously or following minor trauma. The time-honoured laboratory criterion for the diagnosis of haemophilia is a prolongation of the whole blood clotting time.

The investigations described in Chapter 5 have thrown fresh light on our knowledge of this disease and it is now generally accepted that the condition originally thought to be one abnormality may be deficiencies either of anti-haemophilic globulin (A.H.G.) or of the Christmas factor (C.F.)

The objects of this present survey were to study the aspects enumerated in the list of contents at the start of this Chapter.

The incidence of haemophilia and Christmas disease in the population.

The population studied was in the West of Scotland, the hospitals referring the cases being under the Western Regional Hospital Board and the population served by these hospitals being almost three million. 87 patients were seen and there was reason to believe that there were another 9 in the region. Some of these were relatives of patients examined. This gives an incidence of one per 30,000-35,000 of the population. Most of the patients were of Scottish extraction, the family extractions being Scottish in 76, English in 8 and Irish in 3. This is probably not of any particular significance as the great majority of the population in the region are of Scottish extraction.

The incidence in the population as a whole is probably representative of this country and of nationalities of

European extraction. The occurrence in negro and oriental races is probably less. In negro races Christmas disease has been identified amongst the haemophilic population by Epstein et al (1954).

The incidence of Christmas disease in the haemophilic population.

Of the 87 patients examined 74 lacked antihaemophilic globulin and 13 were deficient in the Christmas factor. There was one case of Christmas disease to every 5-6 with haemophilia. Of the total haemophilic population, 15 per cent were suffering from Christmas disease. In this investigation the nature of the defect remained constant throughout the affected families. When one member of a family was found to lack A.H.G. other affected members also had this deficiency. The same is true of Christmas disease. Families have recently been reported where affected relatives have suffered either from A.H.G. or C.F. deficiency (Verstraete 1955, Fantl and Sawers 1956).

When the incidence of the two conditions was considered in families 56 were found to lack A.H.G. and 7 the C.F.; this was an incidence of one Christmas disease family to 8 haemophilic families; of the total number of affected families approximately one in eight were suffering from the Christmas defect.

Pitney and Dacie (1955) from cases reported in the literature concluded that there were 51 patients with Christmas disease amongst 330 "haemophiliacs". From this they concluded that the incidence of haemophilia compared with that of Christmas disease was about 5.5:1. The incidence in the present series is identical with this.

The incidence in small series naturally varies from centre to centre. Koller (1954) for example in Switzerland recorded nine cases of Christmas disease to ten of haemophilia. Pitney and Dacie (1955) in London found 4:1 (haemophilia : Christmas disease) and Biggs and Macfarlane in Oxford 12:1. Fantl and Sawers (1954) in Australia found 5:1.

Family History.

Of the series of patients 36 out of the total of 87 had no family history; in one out of every two or three patients seen there was no other known affected relative. An analysis was made of the incidence of a positive family history in respect to the number of generations affected. Sometimes the history was positive in only one generation the patient having an affected brother. The results are shown in Table 24 .

TABLE 24

	Negative	1 generation	2 generations	3 generations	4 generations
Haemophilia (74)	33	13	11	15	2
Christmas (13)	3	2		8	

Often only one member of a family was seen but sometimes the opportunity arose to see two or three affected members of a family. On one occasion the number seen was nine. These figures are shown in Table 25 .

TABLE 25

Number of members seen per family	1	2	3	9
Haemophilia	47	6	2	1
Christmas Disease	3	2	2	-

According to the accepted mechanism of inheritance of these diseases there should be an equal number of affected and unaffected males amongst the sons of a transmitter. When the patients' generation was considered it was found that in the case of haemophilia there were 82 affected as compared with 45 unaffected and in Christmas disease 15 were

affected and 9 unaffected. This discrepancy remains unexplained.

Social and personal aspects.

Occupation:

50 of the patients were over the age of 16 and the problem of employment was discussed with each of them. 9 were permanently unemployed on account of their condition: 12 were employed but were finding considerable difficulty in retaining their job while only 29 held their job without much inconvenience from their disease. In the latter group were many of the milder cases. Of those employed a few of the milder cases were doing heavy labouring jobs, but the majority had studied a trade and were engaged in this. A few were motor drivers, a few were salesmen, while a small number were in the professions or training for them. Many had found by experience of loss of employment that it was frequently better on application for a job to conceal that they were haemophiliacs. Only one third of those of employable age were on the Disabled Persons Register while two-thirds were not. Three of the patients had served in the forces - one because the diagnosis was not made until some years subsequent to service, one because he had purposely concealed the condition at the time of enlistment and one because the examining doctor dismissed the statement that he was a

haemophiliac.

Marriage:

This is a difficult problem on which to advise the individual haemophiliac. Since all his daughters are transmitters the disease is liable to appear in his grandchildren. Among the patients there were 36 over the age of 20 years. Seventeen were married and nineteen were unmarried. Of those who were married only 6 had appreciated at the time of marriage, that transmission would result and nine had not appreciated this possibility. Two had not been diagnosed as haemophiliacs at the time of marriage. Of the nineteen unmarried patients eleven did not realise at the time of interview that they could transmit the condition. The interesting feature is the high incidence of those patients who did not appreciate that they could transmit the disease. This is related to the dictum, that the female transmits the condition. It is difficult to know what advice to give the haemophiliac about marriage. If a family is wanted there is a case to be made for the adoption of children. The wife should have a training for some gainful occupation as she may often require to go out to work in support of the home at times when the husband is unable to work.

Age Distribution of the patients.

The age distribution of the patients in the series is shown in Table 26, and for comparison figures representing a part of the population in the West of Scotland.

TABLE 26

0-5	5-10	10-15	15-20	20-25	25-30
12	15	10	9	11	7
137,838	118,086	113,965	97,673	98,806	107,332

30-35	35-40	40-45	45-50	50-55	55-60
7	3	6	1	4	2
95,857	101,744	101,573	95,213	79,846	64,900

As would be expected the figures suggest that the expectation of life is considerably diminished for the sufferers from these disorders. The figures show that there are nearly twice as many patients in the group below 25 years of age as compared with the numbers over 25 years of age. It is tempting to assume that the development of the transfusion services may in part be responsible for this.

The gradation of severity of the disease-Haemophilia.

One aspect of haemophilia which is of interest is the variation between cases in the degree of severity of the

condition. It is indeed strange that this genetically determined condition should be so variable in its severity.

From the patients in this series it would appear that there are three grades of the condition. Two of these are quite clearly defined. The mild case has very little trouble with his condition apart from the haemorrhage at tooth extraction; his whole blood clotting time is normal and the abnormality is only clearly demonstrable on the thromboplastin generation test. The patients have no joint disability and lead a normal life. The severe case leads a life of chronic invalidism severely disabled by his condition. The whole blood clotting time is markedly prolonged. The patients in this grade of severity have considerable trouble with their joints being crippled by the effects of recurrent haemarthroses. In between these mild and severe cases are a group of less well defined patients who clinically have a degree of severity intermediate between the mild and the severe. These patients have some joint disability but not nearly so much as the severely affected haemophiliac. They can retain employment without much difficulty. The whole blood clotting time of this group of patients was also short, the mean value being the same as that for the mild group.

When plasmas from the mild and the severe case are compared on thromboplastin generation there is a demonstrable difference

in the degree of the defect (see page 1067 of the appendix). Figure 119 illustrates the differences in the plasma of three haemophiliacs of varying severity examined in the one day by the thromboplastin generation test. Plasma from the severe case forms even less thromboplastin than from the mild one.

In figure 120 is shown by histogram the results of the whole blood clotting time on the series of patients. It will be seen that there is a gap in the numbers between the group with normal or slightly prolonged clotting times and those with markedly prolonged whole blood coagulation times. Approximately one half of the patients have clotting times within or just beyond the normal range and the other half have markedly prolonged whole blood clotting times. A similar phenomenon has been reported by Merskey (1950) and Fantl and Sawers (1954).

In addition to these three groups of patients - the severe, the mild, and the moderate - there were three patients who presented rather distinctive features. They had recurrent and very troublesome bleeding from mucous membranes at particular sites - recurrent epistaxis and haematemesis in one and haematemesis and melaena in the other two. Two of these three patients in addition to having deficiency of antihaemophilic globulin on thromboplastin generation also had prolongation of the bleeding time.

Figure (119)

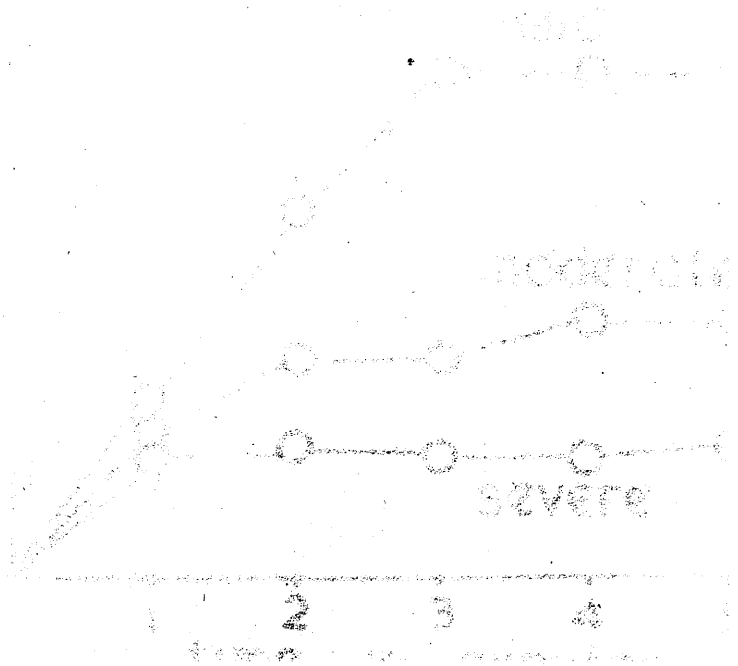


Figure (119)

Comparison of the thromboplastin generation test on haemophiliacs of varying clinical severity.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation technique with normal serum and platelets constant.

●——● normal adsorbed plasma.

O——O haemophilic adsorbed plasma from patients of clinical severity as indicated.

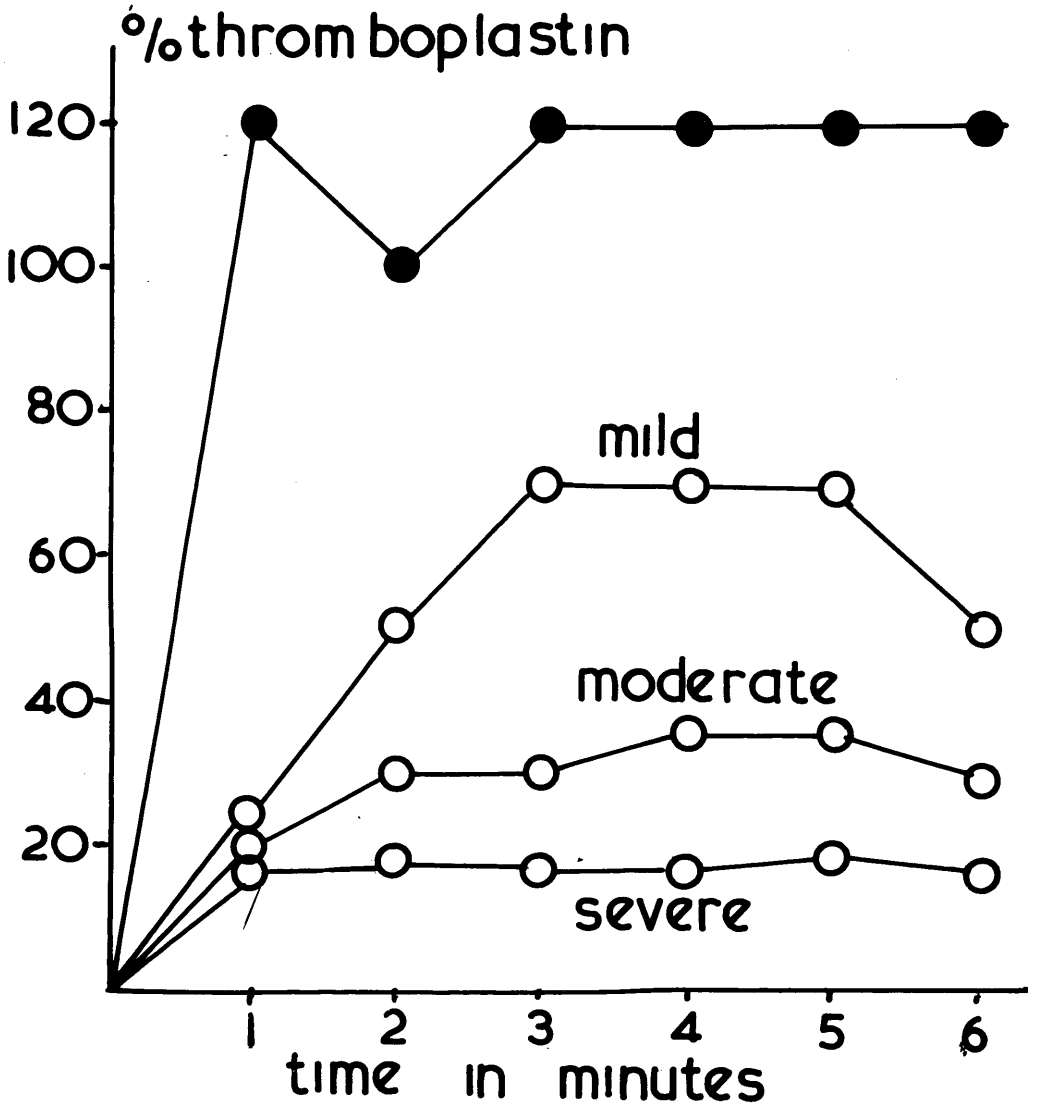

% thromboplastin
120
100
80
60
40
20
mild
moderate
severe
1
2
3
4
5
6
time in minutes

Figure (120)

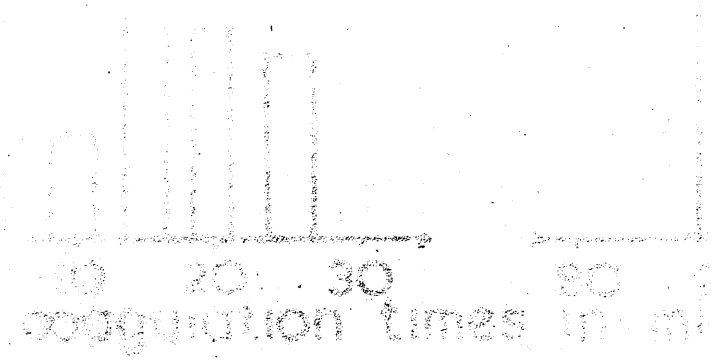


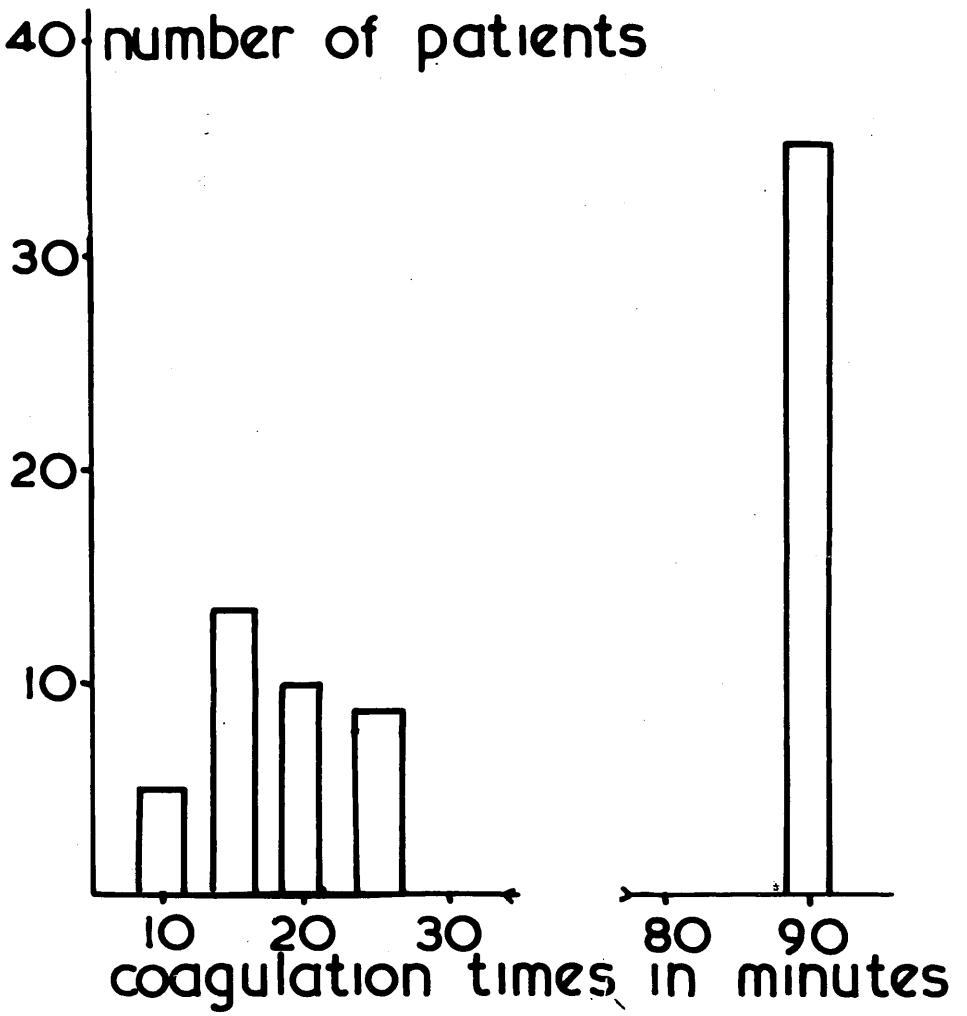
Figure (100)

Whole blood clotting times in haemophilia.

Ordinate - number of patients.

Abscissa - whole blood clotting times in minutes.

The whole blood coagulation times were estimated by method (2).


40
number of patients
30
20
10
10
20
30
80
90
coagulation times in minutes

Christmas Disease:

Taken as a group these patients were somewhat less severely affected than the haemophiliacs. Of the 13 patients only two were of comparable severity with the group of severe haemophiliacs. These two had prolonged whole blood clotting times and considerable joint disability. The remainder were comparable in severity with the moderate haemophiliac having a similar degree of joint disability. The whole blood clotting time in this group was generally short. None of the patients with Christmas disease were as mild as the mildest of the haemophiliacs who have no trouble with their condition apart from tooth extraction.

The grade of the disease was in general the same throughout the one pedigree but there were some exceptions to this, one brother having considerably more trouble than the other.

The nature of the first haemorrhagic incident and the age at which the diagnosis was established:

The next feature in this survey was to assess the nature of the first haemorrhagic incident leading to the establishment of the diagnosis and the age at which this was made. It was possible in retrospect after the establishing of the diagnosis to obtain a history of incidents which were certainly attributable to the condition. There was a tendency for the diagnosis to be established earlier in those children where there was already a family history of the condition and

Figure (121)

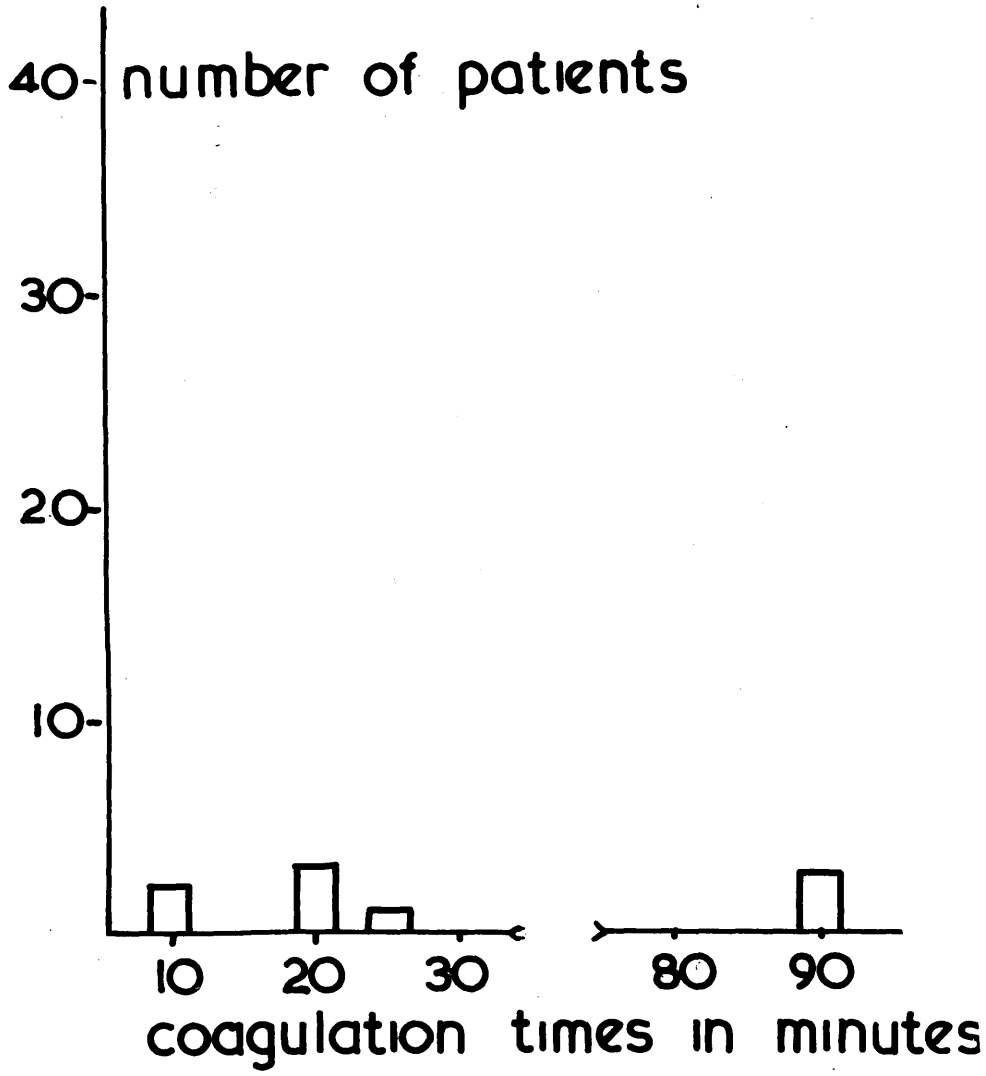
Figure (121)

Whole blood clotting times in Christmas disease.

Ordinate - number of patients.

Abscissa - whole blood clotting times in minutes.

The whole blood coagulation times were estimated by method (2).


40-
number of patients
30-
20-
10-
10
20
30
80
90
coagulation times in minutes

the parents were on the outlook for the disease. The presenting incidents are shown in order of frequency in table 27.

TABLE 27

87 Patients

External bleeding from small cuts - scalp, face, mouth, lips, tongue, hands and feet	40
Haematomata - especially scalp and throat	18
Circumcision	7
Tooth extraction	6
Haemarthrosis	5
Epistaxis	4
Tonsilliectomy	1
Operation - glands of neck	1
Injection for diphtheria-whooping cough immunisation	1
Gastro-intestinal bleeding	1
Bleeding from chickenpox vesicle	1
On assessment by laboratory examination being the member of an affected family	1
Uncertain	1

The most common presenting symptom was of bleeding from cuts particularly of the scalp, face and lips when the child starts to crawl and walk. The slow healing of these is often demonstrated by the subsequent scarring. The next most common presenting feature was of haemotomata affecting particularly

the scalp.

The ages at which the diagnosis was made are summarised in Table 28 .

TABLE 28

Ages at which diagnosis made

0-1	-2	-3	-4	-5	-6	-7	-8	10-11	12-13	18	20	25	34
27	15	8	6	6	11	3	2	2	1	2	1	1	1

The diagnosis refers to the labelling as a haemophiliac - the differentiation into lack of antihaemophilic globulin or of the Christmas factor having been made only during the last three years.

The causes of admission to hospital.

It is often difficult for the older haemophiliac to remember all the incidents which had caused his admission to hospital but an analysis of the reasons for hospitalisation is represented in Table 29 . There had been almost 500 admissions in the series of 87 patients and the reasons for these are shown in Table 29 .

TABLE 29

489 admissions to hospitals

Haemarthrosis	129
Teeth extraction	115
Haematomata	84
Cuts	55
Gastro-intestinal bleeding	41
Haematuria	26
Epistaxis	24
Operations (Tonsillectomy, appendicectomy, glands of neck, enucleation of eye, drainage of abscess, cyst of eyelid).	6
Fractured femur	3
Tonsillar bleeding	2
Haemorrhoids	1

The number of admissions to hospital of a haemophiliac is not necessarily an indication of the severity of the condition. Particularly where a mother for example has gained some experience in the management of the condition either from the care of the individual patient or of other affected members of the family she often elects to manage the great majority of the incidents at home. One severely affected patient had his first admission to hospital at the age of 48 years.

Not infrequently similar haemorrhagic incidents were the cause of admission in the individual case - for example repeated epistaxis in one haemophilic child or recurrent haematemesis and melaena in another.

The transfusion history with reference to the group, genotype and development of immune antibodies.

The group and genotype of each of the patients was determined and tests made for the presence of immune antibodies. The details of these are given in the appendix pages 1062 - 1066. The group and genotype do not show any particular features the distribution being similar to that of the remainder of the population in the West of Scotland.

12 out of 87 patients had never been transfused. The incidence of transfusion is not necessarily an indication of the severity of the condition; one severely affected patient aged 57 had never been transfused. In general however the greater the number of transfusions the more severe the degree of the condition.

TABLE 30

Number of transfusions	None	1	2	3	4	5	6	10+
Number of patients	12	17	9	9	3	6	6	25

Six of the patients had rhesus antibodies as a consequence of previous transfusions - four of these were anti-D alone, one

was anti-C+ anti-D and one was anti-E. One patient had an anti-Kell antibody.

Some of the manifestations of the disorder.

Haemarthrosis:

Of the 74 patients with haemophilia, 42 had severe joint involvement, 14 had minimal joint symptoms and in 18 there was no history of any haemarthrosis. In those 13 patients with Christmas disease 3 had severe joint disability, 3 had minimal joint symptoms and in 7 there was no history of joint lesions.

X-Rays were taken routinely of the knee joints but there was nothing new to add to the already established knowledge of the radiological appearances in these conditions. The features variably seen in relation to the knees were osteoporosis, coarse trabeculation, scalloping of intercondylar area, widening of intercondylar notch, degeneration of articular cartilage, degenerative cyst formation and irregularity of articular surface. There was occasionally thickening of the joint capsule with calcification of soft tissues around the joint, calcification of ligamentosis capsular attachment and of the suprapatellar pouch. Secondary osteoarthritis was common in the older patients and enlargement of the epiphysis was sometimes seen in the younger patients.

The description of both acute and chronic haemophilic

joint disease by "Konig" is the classical one, but there have been numerous other accounts. Caffey and Schlesinger (1940) report on epiphyseal overgrowth and precocious ossification. Other valuable accounts of the condition include Fonio (1938), Newcomer (1939), Lamy (1942), Keefer and Myers (1933) and MacDonald and Lozner (1943).

In Table 31 is set down an analysis of the incidence of involvement in particular joints.

TABLE 31

62 patients with joint involvement

Knees	55
Elbows	36
Ankles	36
Shoulders	15
Wrists	12
Hips	7

The knees are the joints most commonly afflicted and after them the elbows and the ankles with equal frequency.

Enquiry was made as to the time taken for each joint incident to settle down again. Each fresh incident of joint bleeding disabled the patient generally for one to two weeks.

Haemorrhage at tooth extraction.

Primary dentition:

The milk teeth in general cause much less difficulty than the permanent teeth. 38 out of the 87 patients had some significant haemorrhage at the time of the loss of the milk teeth. In general this was much greater if the teeth were extracted than when they were allowed to shed by themselves. Sometimes there was some bleeding when the tooth was shed but considerably less than that following extraction of the teeth. Occasionally when the teeth were "cutting" there was minor haemorrhage. It is probably advisable to allow the primary teeth to shed by themselves rather than to have them extracted.

Secondary dentition:

Certain aspects of this are described in greater detail in Chapter 20 . It should be impressed on all parents of haemophilic children that regular dental treatment is essential. Careful conservative dentistry will often prevent the necessity for extractions at a later date and should be carried out in every case. There is no danger of haemorrhage from the dental procedure of filling of teeth provided care is taken not to damage the gum.

Tooth extraction forms a severe test of haemostasis in these patients and haemorrhage may persist for 2-3 weeks

following these extractions. The patients should certainly be hospitalized and certain measures described in detail in Chapter 20 adopted for the control of bleeding.

Haematoma:

In the severe patient these occurred either spontaneously or following minor trauma whereas in the mild case there was usually a history obtained of some injury. There was no particular site, bruising occurring at any area. The thighs, buttocks and shins were amongst the commonest sites. More dangerous was haematoma formation in the floor of the mouth or in the neck. Iliopsoas haematoma with femoral nerve damage is another, not uncommon site. Cases of this are mentioned below in the section on the neurological complications. When this occurs on the right side it presents diagnostic difficulty on account of its similarity to an appendix abscess.

Another surprising feature was the very marked loss of blood which can occur into tissue. Not uncommonly patients have been seen who have bleeding into tissues where the haemoglobin has fallen quickly to 30-40% without evidence of any external blood loss.

Massive scalp haematomata are characteristic of the condition in childhood presumably on account of the greater risk of sufficient injury to that region to produce such a lesion. These can become large and the risk of skin

ulceration is then present.

Haemorrhage from wounds:

The prolonged haemorrhage from small cuts of the scalp, face, lips or tongue in early childhood often constitutes the presenting feature of the condition. The haemorrhage from the tongue may be the consequence of accidentally biting the tongue. The wounds are slow to heal and permanent scarring common. Any wound will cause this prolonged bleeding but those most commonly seen are following tooth extraction, circumcision or other minor operations.

Gastro-intestinal bleeding:

30 of the patients (i.e. about one third) had suffered from haematemesis or melaena. Generally there was no known cause for this but it was thought to be due on one occasion to the ingestion of A.P.C. tablets for toothache and on another to alcoholic beverage. It occurred in childhood in association with the vomiting of whooping cough and with gastro-enteritis. In 11 of the series there had been repeated incidents of gastro-intestinal bleeding and on occasion this constituted the main disability of the condition. In the other patients who had suffered from this type of bleeding it had only occurred once. Not infrequently haematemesis had occurred subsequent to the swallowing of blood from the bleeding of tooth extraction or of epistaxis.

Renal disease including haematuria:

About one third (31) of the patients had suffered at least one incident of haematuria. In seven of the patients repeated incidents of haematuria occurred. In three of these patients renal calculi were present. This is a relatively high incidence of calculi. It is possible that the bleeding results in sufficient fibrin deposit to act as a nidus for stone formation.

Epistaxis:

The nose was a very common site for haemorrhage. 51 of the series of patients had suffered from troublesome epistaxis. 26 of these had frequent and recurrent epistaxis and on occasion this constituted the main disability of their condition.

Neurological complications:

A clinical study has been made of the neurological lesions resulting from the pressure of haematomata on nerve tissue. 8 patients in this series developed this type of complication and these are described in Chapter 16 .

Operations:

It is well known that surgical procedures in haemophiliacs carry a high mortality rate and should be avoided at all costs. One mild haemophiliac survived tonsillectomy carried out before

it was appreciated that he was affected with the condition. Another mildly affected patient had an enucleation of an eye subsequent to an injury to the eye. In severe haemophiliacs there had been on one occasion drainage of glands of neck and on another of an abscess of leg. An extensive laminectomy was performed under the haemostatic cover of bovine and pig antihaemophilic globulin in the Radcliffe Infirmary, Oxford. This was a severe haemophiliac sent to Oxford to be under the care of Dr. R. G. Macfarlane and Dr. J. B. Pennybacker subsequent to the development of a transverse lesion of the cord by extrathecal bleeding. This patient is described in the section dealing with the neurological complications of these conditions.

Fractures:

There were four fractures of femur in the series, three of these having developed as a consequence of trivial injury and could be classified as pathological fractures. They occurred as a result of stumbling and affected the shaft of the femur. Two of these pathological fractures were in patients with haemophilia and the other was in a patient with Christmas disease. One haemophiliac fractured both wrists. The healing of these fractures did not present serious difficulty there being abundant callus formation.

MISCELLANEOUS.

Haemophilic pseudotumour:

One patient had a very large swelling the size of a football in the left thigh and this had been present for some years. This is a recognized complication of the condition referred to as a haemophilic pseudotumour. It is believed to arise as a consequence of recurrent bleeding around bone. It is converted over the course of months or years into a swollen tense sac of old blood and destroyed tissue. The X-Ray appearance is readily mistaken for a sarcoma on account of the soft tissue, swelling and spicules of calcification arising from the shaft of the bone. The condition probably starts as a subperiosteal haematoma.

Firor and Woodhall (1936) have reviewed the literature of this condition and reported a case of their own. This was a 15-year old boy who developed a gradually progressive swelling of the right thumb over 18 months following injury. X-ray revealed absorption of bone and a diagnosis of bone sarcoma was made. Successful amputation was done with the aid of electric coutery. Other cases are reported by Starker (1918), Echternacht (1943) and Davidson et al (1949).

Tonsillar bleeding:

Quite apart from bleeding following tonsillectomy bleeding sometimes occurs from the tonsils - this was troublesome in

two of the patients.

Haemoptysis:

This was relatively rare occurring in only seven patients of the series. In two this haemoptysis was a complication of an acute respiratory infection - whooping cough in one and an acute bronchitis in the other in association with a head cold.

SUMMARY

The patients with haemophilia or Christmas disease have been surveyed in the West of Scotland.

(1) The incidence in the population is one per 30,000-35,000.

(2) There was one case of Christmas disease to every 5-6 with haemophilia.

(3) In two-fifths of the patients no family history of the condition was found.

(4) Problems of occupation and marriage were investigated.

(5) Laboratory differentiation of grades of severity by thromboplastin generation and whole blood clotting time are described.

(6) The nature of the first haemorrhagic incident and the causes of admission to hospital are enumerated.

(7) The transfusion history of the patients with reference to group, genotype and development of immune antibodies is discussed.

(8) The clinical manifestations of the condition are described under these headings:
- (a) haemarthrosis
- (b) tooth extraction
- (c) tissue haematoma
- (d) wound haemorrhage
- (e) gastro-intestinal bleeding
- (f) haematuria
- (g) epistaxis
- (h) neurological complications
- (i) operations
- (j) fractures
- (k) miscellaneous
  - haemophilic pseudo tumour
  - tonsillar bleeding
  - haemoptysis.

REFERENCES

Aggeler, P.M., White, S.G., Glendining, M.B., Page, E.W., Leake, T.B. and Bates, G. (1952).
Plasma thromboplastin component (P.T.C.) deficiency. A new disease resembling haemophilia.
Proc. Soc. exp. Biol. & Med. 79, 692.

Biggs, R., Douglas, A.S., Macfarlane, R.G., Dacie J.V., Pitney, W.R., Merskey, C. and O'Brien, J.R. (1952).
Christmas disease. A condition previously mistaken for haemophilia.
Brit. med. J. 2, 1378.

Biggs, R. and Macfarlane, R.G. (1954).
Personal communication.

Caffey, J. and Schlesinger, E.R. (1940).
Certain effects of haemophilia on the growing skeleton. Some roentgenographic observations on overgrowth and dysgenesis of the epiphyses associated with chronic haemarthrosis.
J. Pediat. 16, 549.

Davidson, C.S., Epstein, R.D., Miller, G.F. and Taylor, F.H.L. (1949). Haemophilia. A clinical study of forty patients.
Blood, 4, 489.

Echternacht, A.P. (1943).
Pseudotumor of bone in haemophilia.
Radiology, 41, 4651.

Epstein, B., Bersohn, I., Ibler, I. and Bloch, H. (1954)
Christmas Disease. Report on a case.
South African med. J., 28, 95.

Fantl, P. and Sawers, R.J. (1954).
"Haemophilia": a syndrome under review.
Aust. Ann. Med. 3, 245.

Fantl, P. and Sawers, R.J. (1956)
Occurrence of different prothromboplastin deficiencies in related male bleeders.
Brit. J. Haemat. 2, 102.

Firor, W.M. and Woodhall, B. (1936).
Haemophilic pseudotumor: diagnosis, pathology and surgical treatment of haemophilic lesions in the smaller bones and joints.
Bull. John Hopkins Hosp., 59, 237.

Fonio, A. (1938)
Das Blutergelink mit besonderer Birucksichtigung der Berner Bluter.
Arch. f. Klin. Chir. 191, 171.

Keefer, C.S. and Myers, W.K. (1933).
Haemophilic arthritis.
New Eng. J. med. 208, 1183.

Koller, F. (1954).
Is haemophilia a nasologic entity?
Blood 9, 286.

Koller, F., Krüsi, G. and Luchsinger, G. (1950)
Ueber eine besondere Form hamorrhagischer Diathese.
Schwuz. med. Wchnschr., 80, 1101.

König, F. (1892)
Die Gelenkirkrankungun bei Blutern mit besonderer Berücksichtigung der Diagnose.
Klinische Vortrage 36, 233.

Lamy, M. (1942).
L'Aspet. radiologique des osteo-arthropathus hemophiliques.
Bull et. mim. Soc. Med. de Hop. de Paris, 58, 45.

McDonald, E.J. and Lozner, E.L. (1943)
Haemophilic arthritis; roentgenographic studies in fifteen adult patients with haemophilia.
Amer. J. Roent. & Rad. Ther. 49, 405.

Merskey, C. (1950)
The laboratory diagnosis of haemophilia.
J. clin. Path. 3, 301.

Newcomer, N.B. (1939).
The joint changes in haemophilia.
Radiology, 32, 573.

Pavlovsky, A. (1947)
Contribution to the pathogenesis of haemophilia.
Blood, 2, 185.

Pitney, W.R. and Dacie, J.V. (1955)
Haemophilia and allied disorders of blood coagulation.
Brit. med. Bull. 11, 11.

Schulman, I. and Smith, C.H. (1952)
Haemorrhagic disease in an infant due to deficiency of a previously undescribed clotting factor.
Blood, 7, 794.

Starker, L. (1918), Knochenusur durch ein hemophilis subperiostalis Hematom. Mitt. a.d. Grenzgeb. d. Med. v. Chir. 31, 381.

Verstraete, M. (1955).
De Vorming van Plasma-thromboplastine. Studie over het. haemophilic-syndroom.
Arscia Edit. Stoofstraat, 60, Brussels (Belgium)

CHAPTER 16

NEUROLOGICAL COMPLICATIONS OF HAEMOPHILIA AND CHRISTMAS DISEASE

CONTENTS

A clinical account of 10 patients with haemophilia or Christmas disease who have developed neurological damage as a consequence of their haemostatic defect.

Case 1 - Right hemiplegia.

2 - Spinal cord compression.

3 - Median and ulnar nerves.

4 - Femoral nerve.

5 - Anterior tibial nerve.

6 - Femoral, posterior cutaneous of thigh and sciatic nerves.

7 - Sciatic nerve.

8 - Left cerebral haemorrhage.

9 - Femoral nerve

10 - Subarachnoid haemorrhage.

CHAPTER 16

NEUROLOGICAL COMPLICATIONS OF HAEMOPHILIA AND CHRISTMAS DISEASE

During the survey of haemophilia and Christmas disease described in the previous chapter, a detailed clinical study was made of the neurological complications which had developed amongst these patients as a consequence of their disorder.

Eight patients with neurological lesions were observed among the eighty-seven patients (74 with haemophilia and 13 with Christmas disease). Of these eight patients 6 had haemophilia and 2 had Christmas disease. A further two patients are reported who died as a consequence of the development of fatal neurological complications of the disease; one of these belonged to a Christmas disease family but the exact nature of the defect in the other fatal case was not established. The whole blood clotting time, together with the results of the thromboplastin generation test and the degree of severity of the cases are noted in Table 32 .

Case 1. Male aged 51 years. Diagnosis: Haemophilia. At the age of 10 years this patient developed a right hemiplegia. His recollection of events at that time are vague,

but he remembers that he lost the power of speech and that his face and leg were affected, but his arm less so. The power of speech had returned to normal by the age of 14 years when he took his first job.

On examination in 1955 he was found to have an upper motor neurone lesion of the right arm and leg. A flexion deformity of the right wrist made him unable to use the right hand. Muscle power at the elbow and shoulder was reduced, but power in the right leg was extremely good. Muscle tone was increased in the affected limbs, and the tendon reflexes on the right side were very brisk. The right plantar reflex was extensor. Abdominal reflexes were present. There was no sensory loss and cranial nerves were normal. No abnormality of speech was detected.

Comment: Intracranial haemorrhage in the left cerebral hemisphere resulting in a right hemiplegia.

Case 2. Male aged 16 years. Diagnosis: Haemophilia. On March 10th, 1955, this patient fell a distance of about twenty feet on to sand. On rising he had slight back pain but was able to walk. For the succeeding twenty-four hours he had retention of urine which required catheterisation on March 11th. At this time he was observed to move both legs. By March 12th he had lost the power of both legs and examination revealed a sensory level at the ninth thoracic root with

absence of sacral spacing.

A diagnosis of cord compression by a haematoma was made and he was transferred to the Radcliffe Infirmary, Oxford, under the care of Mr. J. Pennybacker and Dr. R. G. Macfarlane. An extensive throaco-lumbar laminectomy was performed with removal of extrathecal clot under the haemostatic cover of bovine antihaemophilic globulin.

Six months later there was little appreciable change in the sensory level and the paralysis was remarkably spastic the extremities being held tightly in the scissor position.

Comment: Intrathecal haemorrhage over the course of forty-eight hours, following trauma to the back resulted in spinal cord compression with signs of transection at the level of the ninth thoracic root.

Case 3. Male aged 26 years. Diagnosis: Haemophilia. In March, 1951, the patient fell, bruising the right forearm which rapidly became swollen and very painful. The following day he noticed that the tips of the fingers were numb, and that when he touched them 'pins and needles' were felt. The numb sensation rapidly spread over the hand to the level of the wrist. Initially hand movements were limited by the pain in the forearm, but as this became less severe he found that he could use neither hand nor wrist and that he had developed a claw hand.

Examination revealed loss of sensation over the right hand in both ulnar and median nerve distribution to the level of the wrist. He was treated initially by light massage and passive movements, progressing to active exercise and occupational therapy. Complete recovery took place in the course of a year.

Comment: Median and ulnar nerve palsies were produced by the pressure of a haematoma in the right forearm.

Case 4. Male aged 44 years. Diagnosis: Haemophilia. Two episodes of bleeding in the muscles of the right groin, the first 20 years ago and the second 12 years ago, were followed by loss of sensation over the front of the thigh and leg.

On examination in 1955 there was obvious wasting of the right quadriceps muscle. Haemarthrosis affecting both knee joints made interpretation of the reflexes impossible. Sensation to pin prick and touch was lost throughout the distribution of the right femoral nerve. The central nervous system was otherwise normal.

Comment: The right femoral nerve was involved on two occasions by haemorrhage in the right groin, resulting in extensive sensory loss and wasting of the right quadriceps.

Case 5. Male aged 4 years. Diagnosis: Haemophilia. This small boy had been subject to haemorrhagic episodes since infancy. In 1954, at the age of three years, he was admitted to hospital with bruising of the dorsum of the right foot, the right forearm and of the abdomen.

On examination he was found to have paralysis of the dorsiflexors of the right foot. He was treated by physiotherapy and an appliance to prevent stretching of the anterior muscles. Complete recovery took place within one year.

Comment: A right drop foot was an incidental finding in a child admitted with extensive bruising. The lesion was presumed to be due to haemorrhagic involvement of the anterior tibial nerve.

Case 6. Male aged 20 years. Diagnosis: Christmas disease. In July, 1952, after some unusually strenuous activity, he was aware of stiffness in the muscles of the left upper thigh, although this did not preclude him from walking. Two weeks later, on going upstairs, he felt a wrench in his left groin, which rapidly became very painful. He was transferred to hospital, where the left hip was immobilised for three weeks in a plaster spica. When the plaster was removed the patient noted loss of sensation over the front of the left thigh and medial surface of the left leg.

In January, 1955, without apparent cause, the patient

developed a swelling in the left buttock. Initially the affected side was swollen, painful and tense, but later bruising became obvious. Since this episode the patient has been aware of loss of sensation over the back of the left thigh.

On examination in the summer of 1955, there was partial ankylosis of the left knee from an old haemarthrosis, with some diminution of power in the left quadriceps muscle, which showed one and three quarter inches of wasting. Both the left knee and ankle jerk were absent, while other tendon reflexes were normal.

Sensation to pinprick was lost over the anterior surface of the lower third of thigh, and impaired over the same aspect posteriorly. Medially, sensation to pinprick, touch and temperature was lost from the level of the knee joint to the medial malleolus of the left ankle.

No other abnormality was detected in the nervous system. The diagnosis of a haemorrhagic diathesis was not established in this patient until 1955.

Comment: In 1952, haemorrhage in the left femoral triangle caused extensive damage to the femoral nerve with resulting sensory loss over the skin distribution of its three main divisions.

In 1955, haemorrhage in the left buttock impaired the

function of the posterior cutaneous nerve of the thigh in its position deep to the gluteus maximus. As this nerve lies in close proximity to the sciatic nerve, damage to motor fibres in the latter would account for absence of the left ankle jerk.

Case 7. Male aged 26 years. Diagnosis: Christmas disease (by inference from investigation of the coagulation defect in affected relations). This patient was admitted to hospital in coma and died. He was known to suffer from a haemorrhagic disthesis and had been labelled as a "haemophiliac", the family history being positive. At the time it was not appreciated that there were two types of haemophilia and the patient was not classified by thromboplastin generation into antihaemophilic globulin or Christmas factor deficiency. Subsequent assessment of affected relatives established Christmas factor deficiency and the available evidence indicates that the defect is generally constant in affected families.

The history was obtained from the father who stated that his son had complained of frontal headache and retro-orbital discomfort for three days, but had remained at work until lunch time on the day of admission to hospital. At this time he had returned home and gone to bed. Two hours later he was found unconscious.

On examination he was deeply comatose with pallor, cyanosis and stertorous breathing. The pupils were unequal the left larger than the right, and unreactive to light. A divergent squint was present. All four limbs were spastic, the tendon reflexes exaggerated, and plantar reflexes extensor. Clonus was elicited at both ankles. Optic fundi were normal. The blood pressure was raised to 204/100 mm.Hg, but the heart was not enlarged clinically.

The patient remained in coma until his death two hours later.

Autopsy: A large haemorrhage was present occupying the anterior two thirds of the left cerebral hemisphere, with several punctate haemorrhages within the substance of the pons.

Macroscopically, the kidneys appeared normal, but microscopic examination revealed thickening and hyaline change of many afferent arterioles and glomerular capillaries, indicative of arterial hypertension.

Comment: Fatal intracerebral haemorrhage of the left hemisphere.

Case 8. Male aged 27 years. Diagnosis: Christmas disease. This patient has had three separate episodes of haemorrhage in the left buttock. The first, in 1942, resulted in weakness of his left foot. The second, in 1944, was followed by impaired sensation in the lateral aspect of

the left leg and over the dorsum of the foot. The third and most severe episode was in 1946, since which the patient has dragged the left foot on walking.

Examination in 1955 revealed involvement of the left knee and ankle joint by intra-articular haemorrhage. Compared with the right leg, the left thigh showed two and a half inches, and the left calf three and a quarter inches, of wasting. Power to dorsiflex the left ankle was lost, while plantar flexion was very weak. The left ankle jerk was absent, but the left knee jerk was normally brisk.

Sensation to pinprick, touch, and temperature was lost over the dorsum of the foot, except for a strip along the medial border. Sensation to pain and temperature was impaired over the lateral aspect of the leg.

Comment: Damage to the sciatic nerve in the left buttock occurred on each of three occasions, resulting in motor weakness and sensory loss below the knee.

Case 9. Male aged 9 years. Diagnosis: Christmas disease.

On the morning of the day before admission to hospital, the patient felt pain in the left knee. A few hours later he became aware of discomfort in the left groin and observed that the front of the left thigh had become numb. A few days before the onset of these symptoms he had received a very

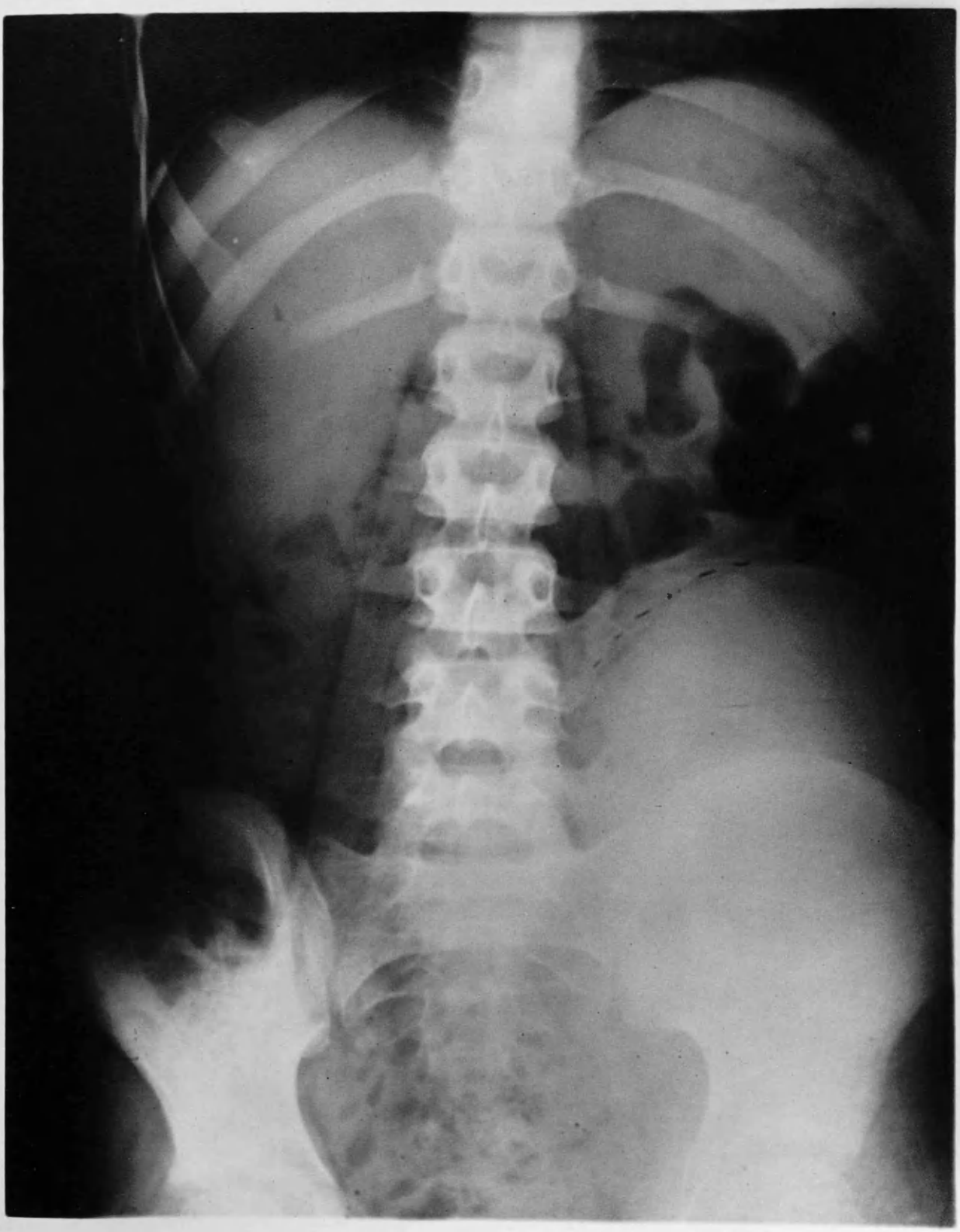
trivial injury to the left groin. When admitted to hospital there was evidence of severe haemorrhage, the patient being pale and restless. The pulse was 160/minute, blood pressure being systolic 95 and diastolic 45 mms. Hg. and haemoglobin 40%. A large swelling both visible and palpable was present in the left iliac forsa. This was firm and tender to the touch. Examination of the nervous system revealed sensory loss throughout the distribution of the left femoral nerve with weakness of the left quadriceps muscle and loss of the left knee jerk. The shock responded to the administration of two pints of blood on two separate occasions but the femoral nerve lesion has persisted. An X-Ray of the abdomen is shown in figure 122.

Comment: Femoral nerve involvement by a haematoma of left ilio-psoas muscle.

Case 10. Male aged 48 years. Diagnosis: undetermined. This patient had been a known "haemophiliac". Since infancy he had been subject to numerous episodes of bleeding from the gums, nose, ears, renal and gastro-intestinal tracts and into the joints. He had never been able to work. A maternal uncle had been similarly affected and had died following appendicectomy. This patient was seen in 1951 when differentiation into haemophilia or Christmas disease was not yet recognised. No living affected relatives were available for

Figure (122)

X-Ray of abdomen in a boy with Christmas disease and left femoral nerve paralysis. The massive iliopsoas haematoma has been demarcated in pencil on the film.



autopsy being refused.

Comment: Although this patient was afflicted by numerous haemorrhagic episodes in the past, subarachnoid haemorrhage at the age of 48 years was the first episode affecting the nervous system and unfortunately, proved fatal.

TABLE 32

Case	Whole Blood Clotting Time (Range 10-20')	Thromboplastin Generation Test (deficiency of antihaemophilic globulin or Christmas factor)	Severity of Disease	Neurological Lesion
Case (1)	90 mins. +	Antihaemophilic globulin deficiency	Severe	Right hemiplegia
Case (2)	90 mins. +	Antihaemophilic globulin deficiency	Severe	Spinal cord compression
Case (3)	15 mins.	Antihaemophilic globulin deficiency	Moderate	Median and ulnar nerves
Case (4)	50 mins.	Antihaemophilic globulin deficiency	Severe	Femoral nerves
Case (5)	90 mins. +	Antihaemophilic globulin deficiency	Severe	Anterior tibial nerve
Case (6)	20 mins.	Christmas factor deficiency	Moderate	Femoral, posterior cutaneous of thigh and sciatic nerves
Case (7)	15 mins.	Christmas factor deficiency	Moderate	Sciatic nerves
Case (8)	?	Christmas factor deficiency on examination of other members of the family	Moderate	Left cerebral haemorrhage
Case (9)	90 mins. +	Christmas factor deficiency	Moderate	Femoral nerves
Case (10)	90 mins. +	-	Severe	Subarachnoid haemorrhage

DISCUSSION

In 1944 Aggeler and Lucia have published a detailed review of the literature up to that time, on the neurological complications of haemophilia. These complications are not uncommon occurring in ten per cent of the patients seen in the West of Scotland. The serious consequences of such internal haemorrhage can be readily appreciated from the case reports. An analysis was made of the time interval between the onset of these lesions and the fully established neurological damage. From this it was determined that early correction of the haemostatic defect might prevent the fully established neurological trauma.

These neurological lesions serve also to confirm the great importance of blood thromboplastin in physiological haemostasis. A powerful tissue thromboplastin can be prepared from the brain or other tissues of patients who have died from haemophilia or Christmas disease; despite this the patients described have suffered the consequence of bleeding into tissues.

S U M M A R Y

(1) A clinical study has been made of the neurological lesions which may develop as a consequence of internal haemorrhage into tissues, in haemophilia and Christmas disease.

(2) Ten patients showed evidence of neurological damage. The lesions included a right hemiplegia, transection of the cord from compression by haematoma, left cerebral haemorrhage, subarachnoid haemorrhage and peripheral nerve damage-median, ulnar, femoral, sciatic and posterior cutaneous of thigh.

(3) In the management of these patients the value of early replacement therapy with antihaemophilic globulin or the Christmas factor is discussed.

REFERENCES

Aggeler, P.M. and Lucia, S.P. (1944).
The neurologic complications of haemophilia.
J. Nerve and Ment. Dis. 99, 475.

CHAPTER 17

COAGULATION DISTURBANCE IN THE FEMALE RELATIVES OF HAEMOPHILIACS

CONTENTS.

CHAPTER 17

COAGULATION DISTURBANCE IN THE FEMALE RELATIVES OF HAEMOPHILIACS

Homozygous and heterozygous haemophilic defect in females.

One of the most striking features of haemophilia is its mode of inheritance and this has been described in greater detail in Chapter 15 . One of the accepted features of the condition is its almost exclusive limitation to the male. Since the female transmitters are generally heterozygous for the defect they do not exhibit any abnormality. In the extremely rare event of the marriage of an affected male and a transmitter female, a homozygous female can theoretically occur. The female patients described by Merskey (1951), Israels, Lempert and Gilbertson (1951) and Pinniger and Franks (1951) are almost certainly examples of this type of inheritance. These patients had the clinical features of haemophilia and as assessed by the laboratory methods available at the time had the necessary criteria for making the diagnosis. They were however investigated before the recognition of the Christmas defect - whether they represent deficiencies of antihaemophilic globulin or of the Christmas factor or of both is not known.

Merskey and Macfarlane (1951) studied the prothrombin

consumption in haemophilic transmitters and found that in the series investigated there was an overall deficiency in prothrombin utilisation but that individual differences could not be demonstrated with certainty.

Description of three female members of haemophilic families with haemostatic defects.

Three female patients belonging to haemophilic families, have been investigated by me on account of an abnormal tendency to haemorrhage. The first of these (Case (1)) was seen in 1949 at a time when it was not possible to make the detailed assessment which was made on the other two. Cases (2) and (3) were investigated in greater detail using modern techniques and deficiency of A.H.G. demonstrated. Case (1) was clinically more severely affected than Case (2), who in turn was more so than Case (3). Case (1) had from the age of two years recurrent haemarthroses, bleeding after tooth extraction and from scalp wounds. In the second case the defect was only established in adult life following tonsillectomy and in the third following tooth extraction. The first patient was the daughter of a sister of a haemophiliac and had several affected male cousins (see figure /23). The second was the daughter of a haemophiliac and was therefore potentially a transmitter of the condition; the third was the sister of two mildly affected haemophiliacs (Cases 79 & 80 in the main series).

Figure (123)

Figure (123)

Haemorrhagic state in a female member of a haemophilic family; family tree.

□ = unaffected male.

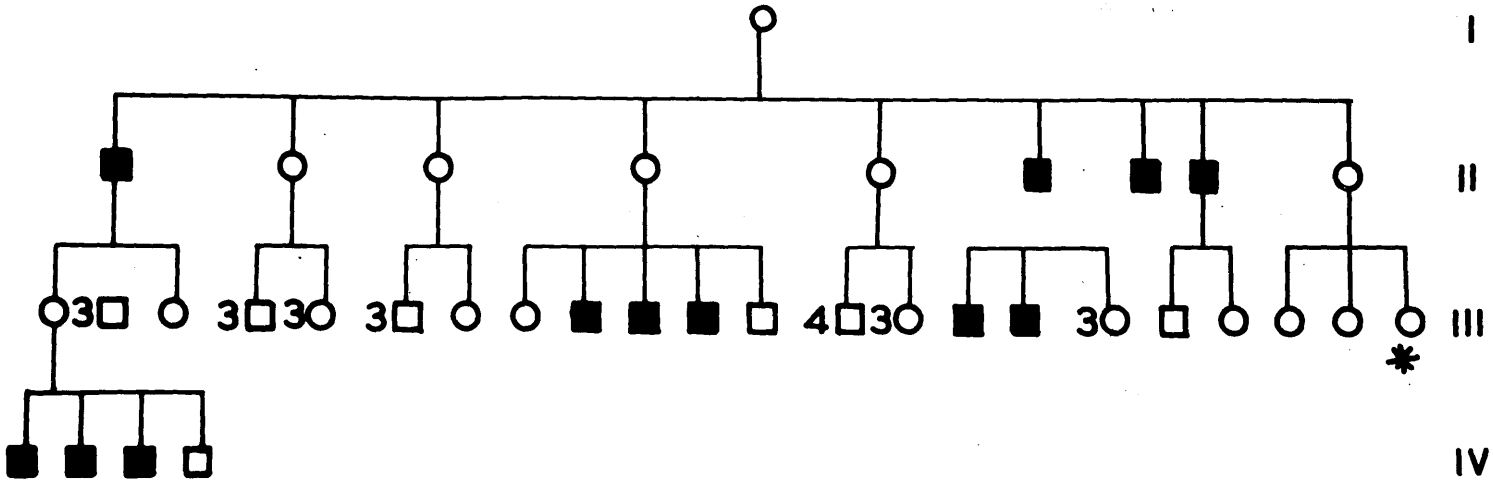
■ = affected male.

O = female.

	Case number in series
II 1	= 72
III 17	= 82
III 18	= 83
III 19	= 53
III 28	= 42
III 29	= 43
IV 1	= 27
IV 2	= 28
IV 3	= 29

The numbers inserted were to reduce the space e.g. 3□ = 3 male unaffected.

III 37 was the female with the significant haemorrhagic tendency - marked O


I
II
III
IV
3
3
3
3
4
3
3

Case (1) Aged 15.

It will be seen from figure 123 that this girl's mother had an affected brother in whom A.H.G. deficiency was established. On the maternal side also she had several affected male cousins. There was a history of stiffness and swelling of the knee joints from the age of 2 years. Between the ages of 5 and 12 years she was in the Royal Hospital for Sick Children, Glasgow, on many occasions - with haemarthrosis, bleeding from tooth sockets and scalp wounds. The right knee was most frequently the site of haemarthrosis and she developed a deformity of this necessitating orthopaedic treatment over a period of two years. Menstruation started at the age of 14 years and the menstrual periods had been regular lasting 3-4 days every month and the loss had never been excessive.

On physical examination the only abnormality was of well marked crepitus on movement of both knees. She was admitted to Professor Davis' wards in the Royal Infirmary when three teeth were extracted. The patient bled for 14 days thereafter, the haemoglobin at one stage being down to 49%. She required 7 pints of blood by transfusion at this time.

Some weeks after her discharge from hospital she was unfortunately injured in a street accident and died as a consequence, preventing her subsequent reassessment by modern

methods. The whole blood clotting time was abnormal and on one occasion was over one hour.

Case (2).

This interesting patient was referred to see me by Dr. Iain Cook of the Southern General Hospital in Glasgow. She was an unmarried woman of 28 years and was seen in October 1955. Her father was said to have died of haemophilia in 1927, a few months before the patient was born. From the patient's mother some account was obtained of her husband's condition. The details provided suggested that he was a haemophiliac of only moderate severity as there was no history of haemarthrosis. He had served for two years in the Cameron Highlanders during the 1914-18 war before being discharged on account of his condition. In 1918 and again in 1924 he had to be admitted to hospital because he bled for two weeks following tooth extractions. An injury to the toe also bled for two weeks. He was admitted to the Royal Infirmary, Glasgow, in 1927 on account of gastro-intestinal bleeding and died three days after admission; there was no autopsy. The family history was in keeping with the inheritance of haemophilia - see figure 124.

The patient herself gave a history of being very readily bruised all her life. At 9 years of age she fell astride a

Figure (124)

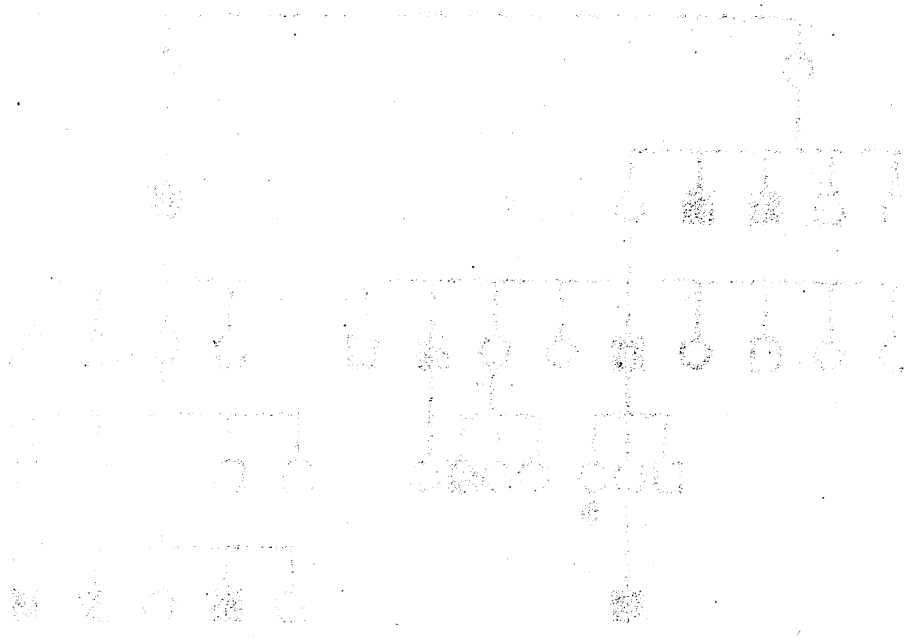


Figure (124)

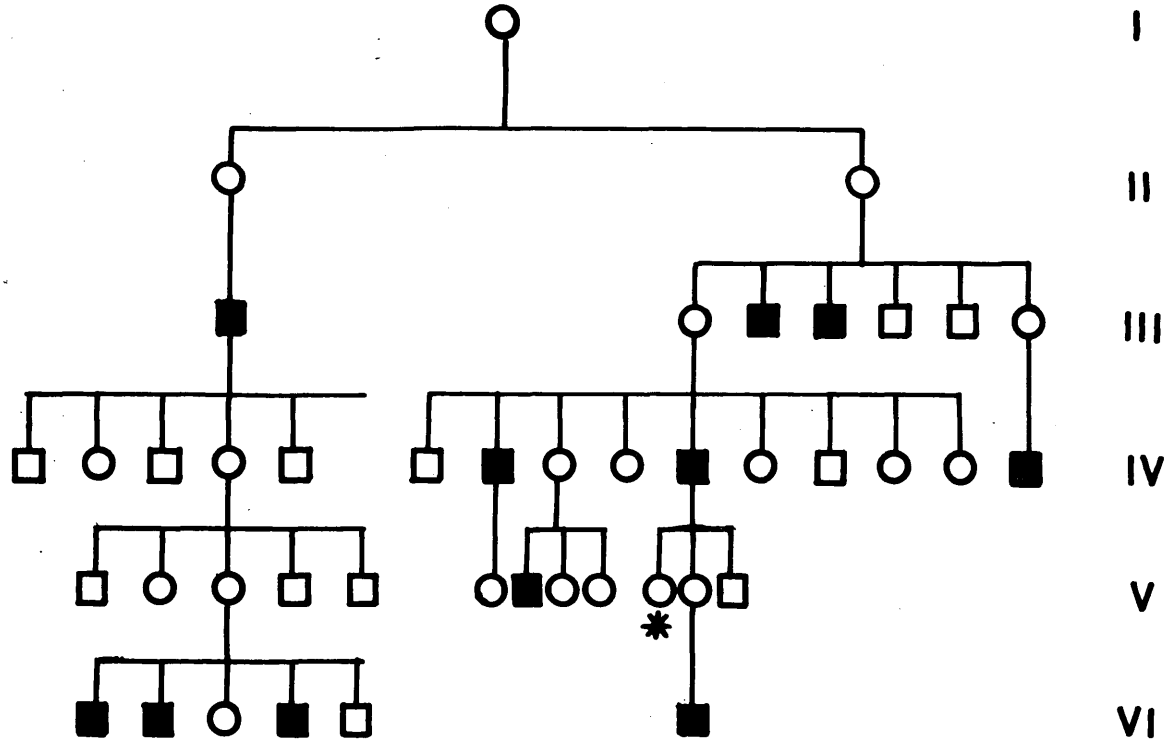
Haemorrhagic state in a female haemophilic transmitter; family tree.

□ unaffected male.

■ affected male.

O female.

V 10 was the female with significant haemorrhagic tendency.


I
II
III
IV
V
VI

desk at school and injured the vagina, bleeding from this for nine days; she was seriously ill at that time. She has had three teeth out on separate occasions and has bled for 5 days from each of these extractions. She had an operation for appendicectomy and at operation it was found that the cause of the pain was haemorrhage from one of the ovaries. The periods formerly lasted 9-10 days but since a "D & C" they have only lasted 4-5 days.

The patient came under review on account of severe haemorrhage following tonsillectomy, this requiring the transfusion of 4 pints of blood.

The patient's sister is said to be similarly affected but unfortunately as she lives in England, has not yet been examined. She also bleeds for five days following tooth extraction and had severe haemorrhage following tonsillectomy. This bleeding continued for at least a week after the removal of the tonsils. There was one brother who showed no abnormality.

The following are the results on the patient:-

Hess test - negative.
Bleeding time - 4 minutes.
Quick's test C = 19 P = 19.
Whole blood clotting time (method (1)) = 20'
Prothrombin consumption index (Merskey)= 50%
Platelet count = 320,000 per cu. mm.

Calcium clotting times:-

0.5 Normal = 3'
0.5 Haem. = 8'
0.5 Xmas = $10\frac{1}{2}$'
0.5 Patient = $4\frac{1}{2}$'

0.475 Haem.
0.025 Nor. = $3\frac{1}{2}$'

0.475 Haem.
0.025 Patient = $6\frac{1}{2}$'

0.475 Xmas
0.025 Nor. = $4\frac{1}{4}$'

0.475 Xmas
0.025 Patient = 4'

Volumes in ml.

Thromboplastin generation test.

Platelets constant.

Nor. al. plas. Nor. serum	42	11	9	10	11	11
Pat. al. plas. Pat. serum	20	20	19	17	17	18
Nor. al. plas. Pat. serum	11	11	10	10	11	10
Pat. al. plas. Nor. serum	76	57	29	15	13	15

Demonstration of A.H.G. deficiency.

A.H.G. assay - Tpl. gen. at 6'.

Nor.	100%	12"
	50%	15
	25%	15
	12%	18
	6%	22
	3%	30
	0	60
Pat.	50%	28
	25%	31

Figure 125 illustrates the inability of this patient's adsorbed plasma to correct the thromboplastin defect in adsorbed haemophilic plasma.

Christmas factor assay:

100%	Normal	12
50%	"	13
25%	"	14
12%	"	20
6%	"	25
3%	"	21
0%	"	43
50%	Patient	13
25%	"	13

Figure 126 shows the results on the thromboplastin generation test when comparing the adsorbed plasma from the patient, the normal and several haemophiliacs. It will be seen that the defect in this patient is comparable with that of a mild haemophiliac.

Figure 127 shows the failure of plasma from this patient to correct the defective prothrombin consumption of recalcified haemophilic plasma.

Figure (125)

Figure (193)

Haemorrhagic state in a female haemophilic transmitter - results of thromboplastin generation test.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation technique with normal serum and platelets constant. Adsorbed plasma variable.

●——● severe haemophilic + normal.

X——X severe haemophilic + patient.

O——O severe haemophilic alone.

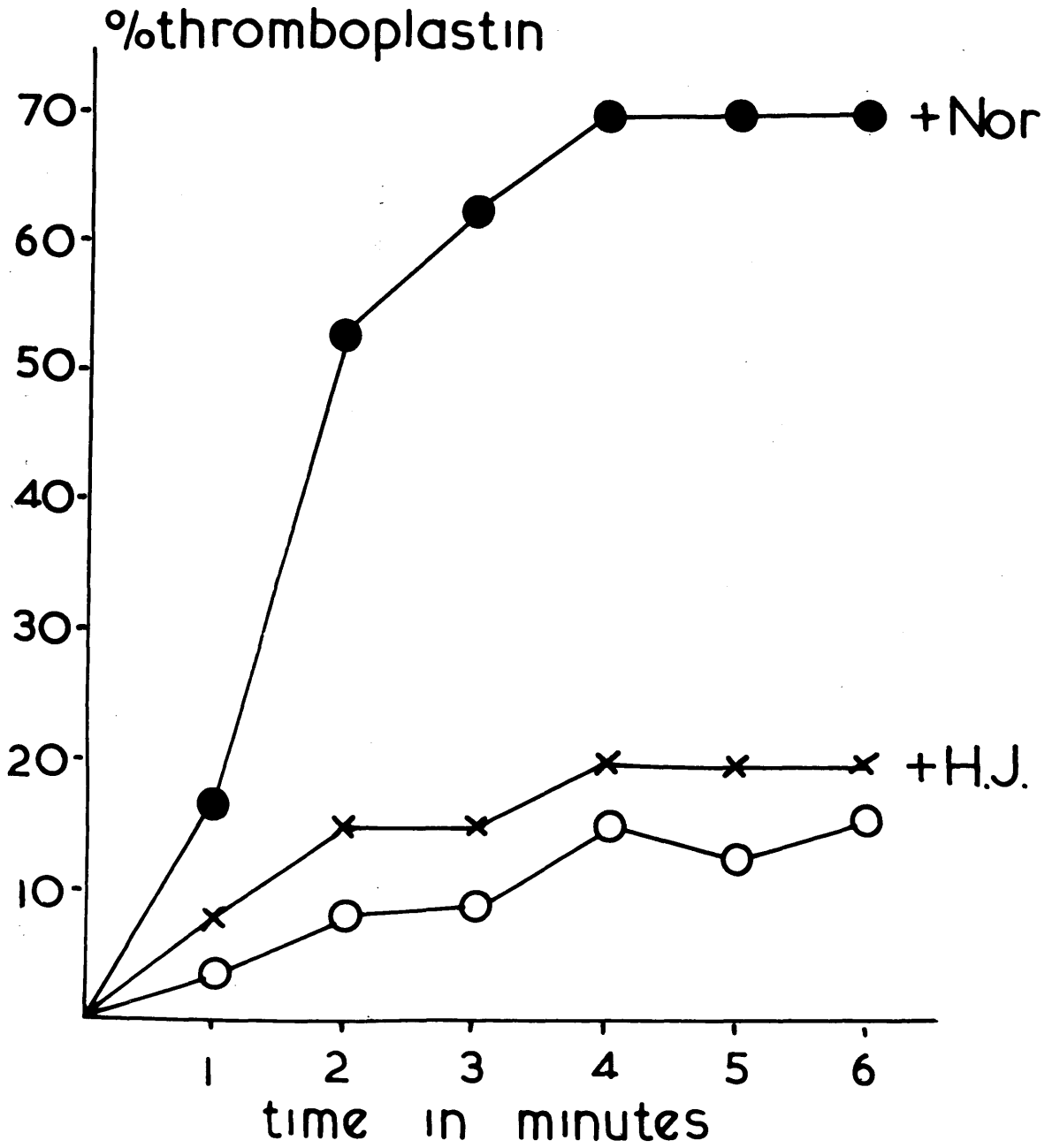

%thromboplastin
70
60
50
40
30
20
10
+Nor
+H.J.
1
2
3
4
5
6
time in minutes

Figure (126)

Figure (125)

Haemorrhagic state in a female haemophilic transmitter - results of thromboplastin generation test in comparison with haemophiliacs of varying severity.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation technique with normal serum and platelets constant. Adsorbed plasma variable.

●——● normal.

X——X female transmitter being studied.

O——O haemophiliacs of varying clinical severity.

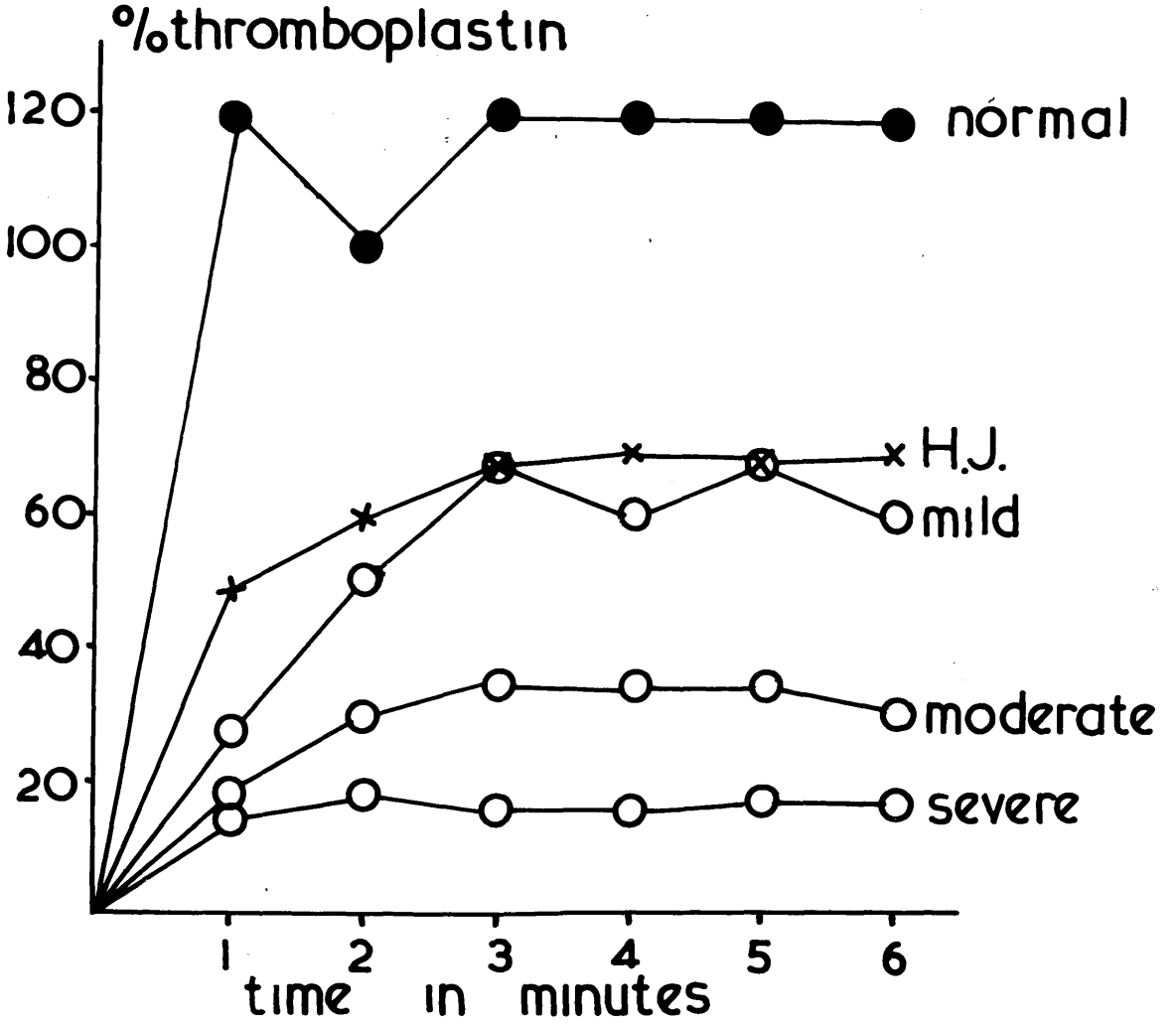

%thromboplastin
120
100
80
60
40
20
normal
H.J.
mild
moderate
severe
1
2
3
4
5
6
time in minutes

Figure (127)

Figure (127)

Haemorrhagic state in a female haemophilic transmitter; failure of this patient's plasma to correct the defective prothrombin consumption of haemophilic plasma.

Ordinate - clotting time of fibrinogen.

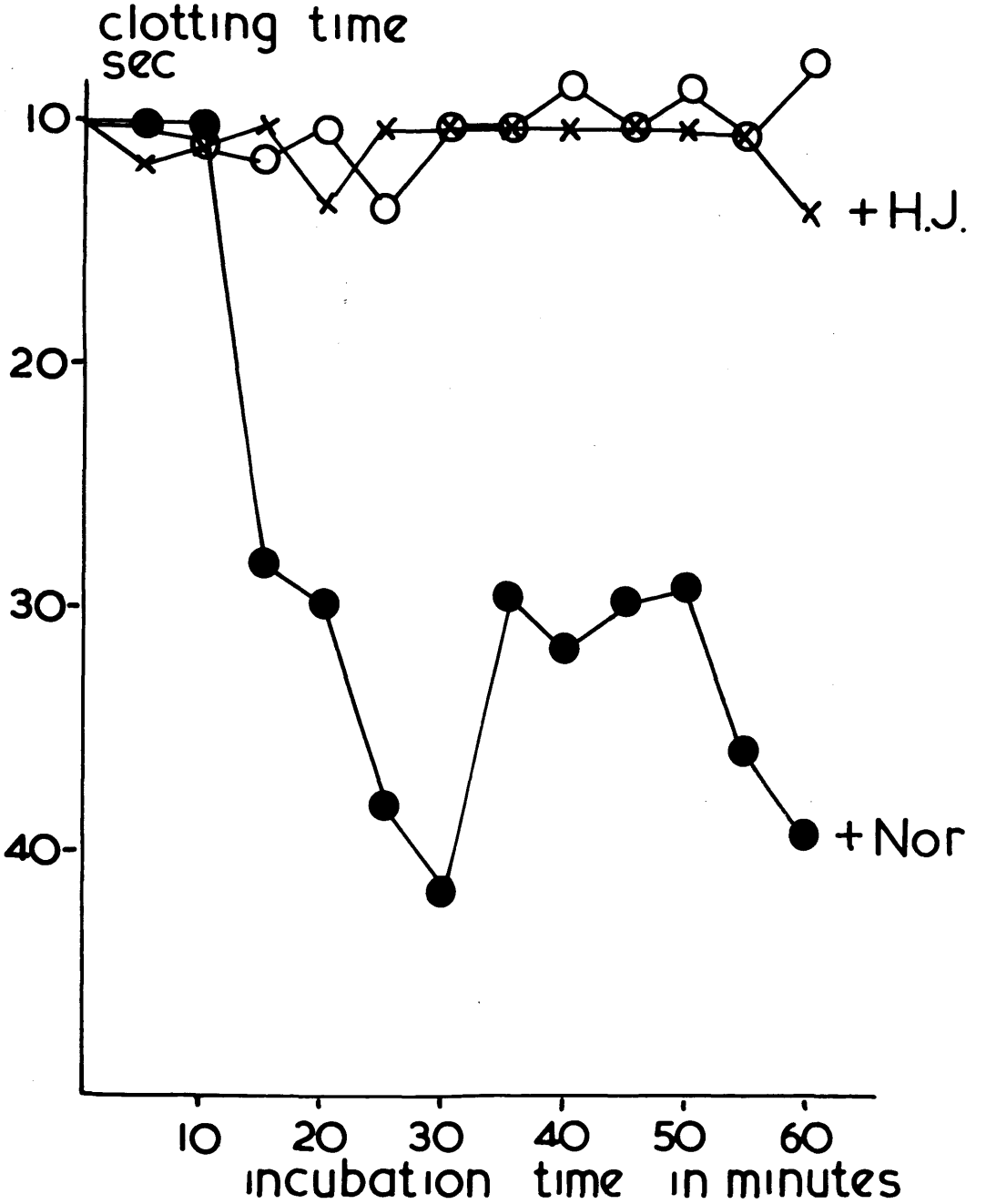
Abscissa - time in minutes after addition of calcium.

O——O haemophilic plasma alone.

X——X haemophilic plasma + plasma from this transmitter.

●——● haemophilic plasma + normal plasma.

This figure shows the prothrombin consumption over one hour.


clotting time
sec
10
20
30
40
+H.J.
+Nor
10
20
30
40
50
60
incubation time in minutes

Comment. These results establish that this patient had a significant deficiency of A.H.G. There was defective prothrombin consumption in the patient's blood and an inability to correct the defective prothrombin consumption of recalcified haemophilic plasma. The adsorbed plasma from the patient fails to form blood thromboplastin normally in the thromboplastin generation test. The A.H.G. assay on this technique demonstrates the inability to correct the haemophilic defect. The assay procedure recorded less than 5% of the normal amount of A.H.G.

Case (3).

This girl was the sister of two mildly affected haemophiliacs (Cases 79 & 80 in my series). There was a history of haemorrhage for three days following tooth extraction, but no other significant history of bleeding. The deficiency in this girl was not so marked as in the second patient. The results are given in the appendix.

DISCUSSION

The second and third patients have a deficiency of antihaemophilic globulin. The first patient had undoubtedly a haemostatic defect, the exact nature of which was not established. There is no evidence from the family histories to suggest that these patients were homozygous for the haemophilic defect. The likely explanation is that they are transmitters of the condition, where for some unknown reason the defect is not so recessive as is usual.

It is of interest that these three female patients belonged to haemophilic families in whom the male affected members were not suffering from the most severe grade of the condition. The problem of A.H.G. deficiency in female transmitters has not yet been completely investigated but a number of transmitters in severely affected families have been investigated with negative results. (see pages 1084-1090 of the appendix).

In a limited study there was no demonstrable A.H.G. deficiency in female transmitters belonging to families where the affected male members were severely afflicted with the disease.

SUMMARY

(1) Three female members of haemophilic families have been studied (Cases (1), (2) and (3)). These three patients each had good clinical evidence of a haemostatic disturbance.

(2) In two of these three patients (Cases (2) and (3)) an A.H.G. deficiency was demonstrated. The other patient (Case (1)) belonged to a family where the affected males were subsequently shown to have an A.H.G. deficiency. The patient herself died some years before the development of modern methods of detecting A.H.G. deficiency.

(3) Case (2) was investigated in considerable detail. She was a 28 year old woman who came under observation as a result of prolonged haemorrhage following tonsillectomy. She was the daughter of a haemophiliac and had an A.H.G. deficiency; this was demonstrated on the thromboplastin generation technique and on the failure to correct the defective prothrombin consumption of recalcified haemophilic plasma.

(4) There was no evidence from the family histories that these patients were homozygous for the haemophilic defect. It is thought that they were transmitters where the defect is not so recessive as is usual.

REFERENCES

Israels, M. C. G., Lempert, H. and Gilbertson, P. (1951).
Haemophilia in the female.
Lancet, 1, 1375.

Merskey, C. (1951).
The occurrence of haemophilia in the human female.
Quart. J. Med. 20, 299.

Merskey, C. and Macfarlane, R. G. (1951).
The female carrier of haemophilia: a clinical and laboratory study.
Lancet, 1, 487.

Pinniger, J. L. and Franks, R. B. (1951).
Haemophilia in the female.
Lancet 2, 82.

CHAPTER 18

HAEMORRHAGIC STATE IN ASSOCIATION WITH DYSPROTEINAEMIA

CONTENTS.

Haemorrhagic states in association with hyperglobulinaemia.

Illustrative case report demonstrating evidence of interference with blood thromboplastin formation.

CHAPTER 18

HAEMORRHAGIC STATE IN ASSOCIATION WITH DYSPROTEINAEMIA

Haemorrhagic states in association with hyperglobulinaemia.

Haemorrhagic states have been reported in association with conditions where the common denominator is an abnormality of the plasma proteins. This abnormality of the plasma proteins is a hyperglobulinaemia. Such a bleeding tendency has been recorded as due to thrombocytopenia, infiltration of the vascular wall by abnormal protein or to disturbance of the coagulation system (Stefanini and Dameshek 1955). The thrombocytopenia may be the consequence of marrow invasion as in myelomatosis. Abnormal globulins or amyloid may infiltrate or weaken the vessel wall. The abnormality in the coagulation system has been ascribed to a variety of causes. It has been reported that the high globulin concentration of the plasma may interfere both with the formation of thromboplastin and of fibrin (Craddock, Adams and Figueroa 1953, Uehlinger 1949). It has also been suggested that the excess globulin in the plasma "binds" calcium and that there is a resultant disturbance in blood clotting.

The patient to be described had a severe haemorrhagic tendency and died from intracranial haemorrhage. She had a

profound disturbance of her plasma proteins with hyperglobulinaemia. There was no histological proof of the diagnosis but it is likely that she was suffering from myelomatosis. The feature of the investigation of her coagulation mechanism was an interference with thromboplastin formation; both the adsorbed plasma and the serum were defective but there was no evidence of a circulating anticoagulant. Assay of the A.H.G. and of the Christmas factor revealed deficiencies of both of these components. Since in these assays the test plasma is diluted with the haemophilic plasma it is unlikely that the result is to be interpreted as due to the presence of an abnormal protein but rather a deficiency of A.H.G. and Christmas factor. It would seem not unreasonable to suggest that the abnormal production of excessive globulin results in a failure of formation of globulins needed for blood thromboplastin formation.

Illustrative case report.

This patient was admitted to the Royal Infirmary, Glasgow, under Dr. David Smith, on 18th August, 1955. She was 60 years of age and was well until January 1955 when she developed a series of sore throats and headaches. In February she began to feel weak and easily tired. She was seen by another physician, found to be anaemic and given iron, vitamin B_{12} and vitamin C. On 2nd August, 1955, she cut her

finger and this bled for 24 hours and the dorsum of the right hand became bruised. During the two weeks before admission she noticed bruising in several areas. A swelling of the right knee developed which was thought possibly to be a haemarthrosis. On examination there was extensive ecchymosis of the right leg and of the right hand. The patient died a few days after admission from cerebral haemorrhage.

Results - Plasma fibrinogen = 160 mg/100 ml.

Plasma proteins A = 1.6 gm. % 1.4 gm. %
G =10.2 gm. % 8.1 gm. %

Colloidal Gold 000000

Thymal Turbidity 5 McL - Units

Electrophoretic separation showed considerable abnormality in the globulin range consistent with multiple myeloma. X-Ray of skull showed areas of the skull suspiciously like multiple myeloma.

$CaCl_2$ time	normal	$1\frac{3}{4}'$; 2'
	patient	$2\frac{3}{4}'$; 3'
3 days later	normal	$1\frac{1}{2}'$
	patient	$5\frac{1}{2}'$
	haemophiliac	$5\frac{1}{2}'$
	normal + 1/10 patient =	$1\frac{3}{4}'$.
Quick's test	normal	13
	patient	15

	1	2	3	4	5	6
Normal ads. plasma normal serum	40	13	15	14	14	15
Patient ads. plasma patient serum	75	70	45	30	25	20
Normal ads. plasma patient serum	95	95	45	23	22	20
Patient ads plasma normal serum	70	13	14	15	16	16

Platelet count - 120,000 per cu. mm.
Whole blood clotting time 15'
Hess test - negative
Clot retraction - normal
Repeated T.G.T. 3 days later.

Normal ads. plasma, nor. serum	53	11	11	10	11	12
Patient ads. plasma, nor. serum	76	65	28	21	21	20
Haem. ads. plasma, nor. serum	126	93	70	20	17	15

A.H.G. Assay.

50% Normal in haem.	14"
25% " " "	15"
12% " " "	17"
6% " " "	19"
3% " " "	20"
50% patient in haem.	18"

Christmas Assay.

50% normal in Christmas	19"
25% " " "	32"
12% " " "	33"
6% " " "	42"
3% " " "	57"
1% " " "	60"
0 " " "	75"
50% Patient in Christmas	60"

To determine whether addition of extra calcium could correct the thromboplastin defect-T.G.T.

	Ca					
Normal ads. plasma, serum platelets	m/40	25	11	10	10	10	11
Patient ads. plas. Pat. serum, platelets	m/40	45	43	25	16	16	18
" " " " " "	m/32	50	52	28	15	14	16
" " " " " "	m/16	85	75	55	30	25	19

Comment:- This patient had an acquired haemostatic defect in association with hyperglobulinaemia. There was marked interference with blood thromboplastin formation and this was due to deficiencies of A.H.G. and Christmas factor.

There was no evidence of interference with the role of calcium in blood thromboplastin formation. It is unlikely that the results given above could be interpreted as due to interference with reactions within the thromboplastin forming system by some non-specific effect of the abnormal proteins. It is more probable that as a consequence of the production of the abnormal proteins there is deficient formation of antihaemophilic globulin and the Christmas factor.

S U M M A R Y

(1) A patient is described who had a profound disturbance of blood coagulation in association with hyperglobulinaemia.

(2) There was demonstrable interference with blood thromboplastin formation using the thromboplastin generation test. The evidence suggested that this was due to deficiencies of antihaemophilic globulin and the Christmas factor.

REFERENCES

Craddock, C.G., Adams, W.S. and Figueroa, W.G. (1953)
Interference with fibrin formation in multiple myeloma by an unusual protein found in blood and urine.

J. lab. clin. Med. 42, 847.

Stefanini, M. and Dameshek, W. (1955)
The Haemorrhagic Diseases.
Grune and Stratton, New York and London.

Uehlinger, E. (1949)
Concerning a disturbance of blood coagulation with dysproteinemia.
Helv. med. Acta. 16, 508.

CHAPTER 19

CIRCULATING THROMBOPLASTIN INHIBITORS

CONTENTS.

CHAPTER 19

CIRCULATING THROMBOPLASTIN INHIBITORS

An acquired haemophilia - like disease has been recognized for many years. Since 1940 it has been appreciated that some of these have been due to the development of a circulating anticoagulant; these anticoagulants were recognized by finding that the addition of blood from such patients in small amounts to normal blood caused a marked prolongation of the clotting time of the normal blood. Before 1951 the other recognized features of these cases were a prolongation of the whole blood clotting time and defective prothrombin consumption. The application of the thromboplastin generation test to blood from such patients has revealed that they prevent blood thromboplastin formation but not its action once formed. (Biggs and Douglas 1953). A similar type of anticoagulant has been found complicating haemophilia. These anticoagulants are not identical with heparin as they do not interfere with the thrombin-fibrinogen reaction and are not neutralized by protamine sulphate; a naturally occurring coagulation abnormality due to heparin must be an excessively rare cause of haemorrhagic disease in man and is certainly less common than the occurrence of circulating anticoagulants.

Incidence and occurrence of circulating anticoagulants.

The occurrence of circulating thromboplastin inhibitors can be considered as follows:-

(a) in patients suffering already from haemophilia or Christmas disease.

(b) in females following pregnancy.

(c) in a miscellaneous group of patients. (Hougie 1955)

(a) in patients suffering already from haemophilia or Christmas disease.

In a male patient with a haemorrhagic tendency dating from early childhood and shown to have a circulating anticoagulant it may be impossible to determine whether the pre-existing haemorrhagic state was haemophilia or Christmas disease. There have been only 18 case reports of the appearance of circulating anticoagulants in patients presumed to have haemophilia, since the first account of such a case by Lawrence and Johnson in 1941. In the 90 patients with haemophilia or Christmas disease seen in the West of Scotland, there was no evidence of a circulating thromboplastin inhibitor in any. In my experience therefore it is a rare phenomenon. Hougie and Fearnley (1954) found only two cases of circulating anticoagulants in a series of 70 patients. In both these series however the criterion for detection of the circulating anticoagulants was the thromboplastin

generation test using diluted reagents as described in the original technique (Biggs and Douglas 1953). As will be seen from one of the cases about to be described, thromboplastin inhibitors may in consequence have been missed. Frommeyer, Epstein and Taylor (1950) recorded that, in a series of 22 patients presumed to have haemophilia, five out of sixteen who had received injections of an A.H.G. preparation (Cohn's factor I) developed circulating anticoagulants, while no anticoagulants were detected in the remaining six patients of the series, who had received only transfusions with whole blood or plasma. The activity of these anticoagulants was much less than that in the first patient described below. Soulier and Larrieu (1953) in a study of 33 patients found circulating anticoagulants in three. In one of these the thromboplastin inhibitor subsequently disappeared and the authors were able to demonstrate that the underlying defect was in the Christmas factor. Lewis and Ferguson (1953) have studied a patient where also on disappearance of the anticoagulant they were able to show that the underlying defect was Christmas disease.

Illustrative case report.

This patient was a nine year old boy who had been labelled as a "haemophiliac" since the age of one year. He had been admitted to another hospital with a massive haematoma of the

abdominal wall and upper aspect of the right thigh. There had been numerous episodes of bleeding following minor trauma throughout his life including haemarthrosis, intramuscular bleeding and excessive bleeding from cuts. He had been transfused on numerous occasions in the past. The features on assessment of the coagulation defect were:-

Thromboplastin generation test - Patient "N".

Platelets constant.

Alumina Plasma	Serum	1	2	3	4	5	6
Normal	Normal	77	60	27	11	9	11
N	Normal	115	105	101	94	81	67
Normal	N	64	41	51	67	57	59
Normal + Saline 0.3	Normal	30	15	15	14	13	14
Normal + 0.3 50% N	"	67	55	63	59	60	62
Normal + 0.3 25% N	"	84	61	51	48	56	60
Normal + 0.3 10% N	"	63	38	37	38	36	32
Normal + 0.3 5% N	"	40	18	17	18	16	18

Calcium clotting times.

Normal	1.0	0.9	0.7	0.5	0.3	0.1	0
N	0	0.1	0.3	0.5	0.7	0.9	1.0
Immediately	1'45"	12'35"	14'	16'50"	19'15"	19'20"	25' +
One hour later	2'5"	24'-	24'-	25'	19'	19'	30' +

The features of this patient's coagulation defect may be summarised as follows:-

(a) Additions of the patient's plasma to normal plasma caused prolongation of the calcium clotting time.

(b) Both the patient's adsorbed plasma and the patient's serum show an inability to generate blood thromboplastin, when incubated with the appropriate normal component in the thromboplastin generation test.

(c) Additions of the patient's adsorbed plasma or patient's serum to a system containing all the requirements for blood thromboplastin, inhibits the formation of this.

(b) Circulating anticoagulants following pregnancy.

I have not seen a case of this type but 11 cases have been reported in the literature (Madison and Quick, 1945, Ghargaff and West 1946, Fantl and Nance 1946, Hewlett and Haden 1949, Heinle, Weisberger, Vignos and Holden 1949, Dreskin and Rosenthal 1950, Biggs and Macfarlane 1953, Frick 1953, Beaumont 1954). In general these have presented within 12 months after parturition with a haemorrhagic diathesis. It is tempting to assume that some foetal maternal auto-

immunization process may be responsible.

(c) Circulating anticoagulants in miscellaneous cases.

Circulating anticoagulants may be found apart from haemophilia and Christmas disease or unrelated to pregnancy. They have been reported to occur unrelated to any other condition or as a complicating feature of recognised disease. Those reported by Joules and Macfarlane 1938, Pons and de Torregrosa 1952 and Hougie, 1953, Evans 1955, developed in patients otherwise healthy. The remaining cases all had some associated illness:

Tuberculous lymphadenopathy.
(Lozner, Joliffe and Taylor 1940).

Chronic nephritis and syphilis
(Conley, Rathbun, Morse Robinson 1948)

Generalised lymphadenopathy of unspecified aetiology.
(Conley et al 1948)

Myocardial infarction.
(Singer, Mond, Hyman, Levy 1950)

Rheumatoid arthritis.
(Collins and Ferriman 1952)

Rheumatic heart disease.
(Hardisty 1954)

Pemphigus treated with arsenic.
(Quick and Stefanini 1948,
Dieter, Spooner and Pohle 1949)

Arthritis
(Evans 1955)

Lupus erythematosus (2 cases) - Frick 1955.

In association with a skin rash after antibiotic therapy - Frick 1955.

The condition is probably more common than indicated by the relatively small number of cases reported in the literature.

Circulating anticoagulants unassociated with other disorders. Illustrative case report.

The patient, a retired clerk, aged 82, was admitted to hospital in April 1952, with massive subcutaneous and intramuscular haemorrhages involving both arms and legs. He had never previously bruised easily and there was no past history of any other haemorrhagic manifestations such as excessive bleeding following dental extractions or cuts. A prostatectomy had been performed five years previously for benign hypertrophy; this operation was uneventful as regards excessive bleeding and duration of convalescence. The patient had never received transfusions or injections of whole blood or blood products. There was no family history of excessive bleeding or bruising. The diet was adequate. Apart from the manifestations of haemorrhage the only physical abnormality on admission was mild osteoarthritis of both knees. Subsequently he developed a haematoma of the whole of the left forearm and a haemarthrosis of the right ankle. Treatment with Cortisone produced no effect. He was admitted to another hospital with acute abdominal pain and operated

Figure (128)

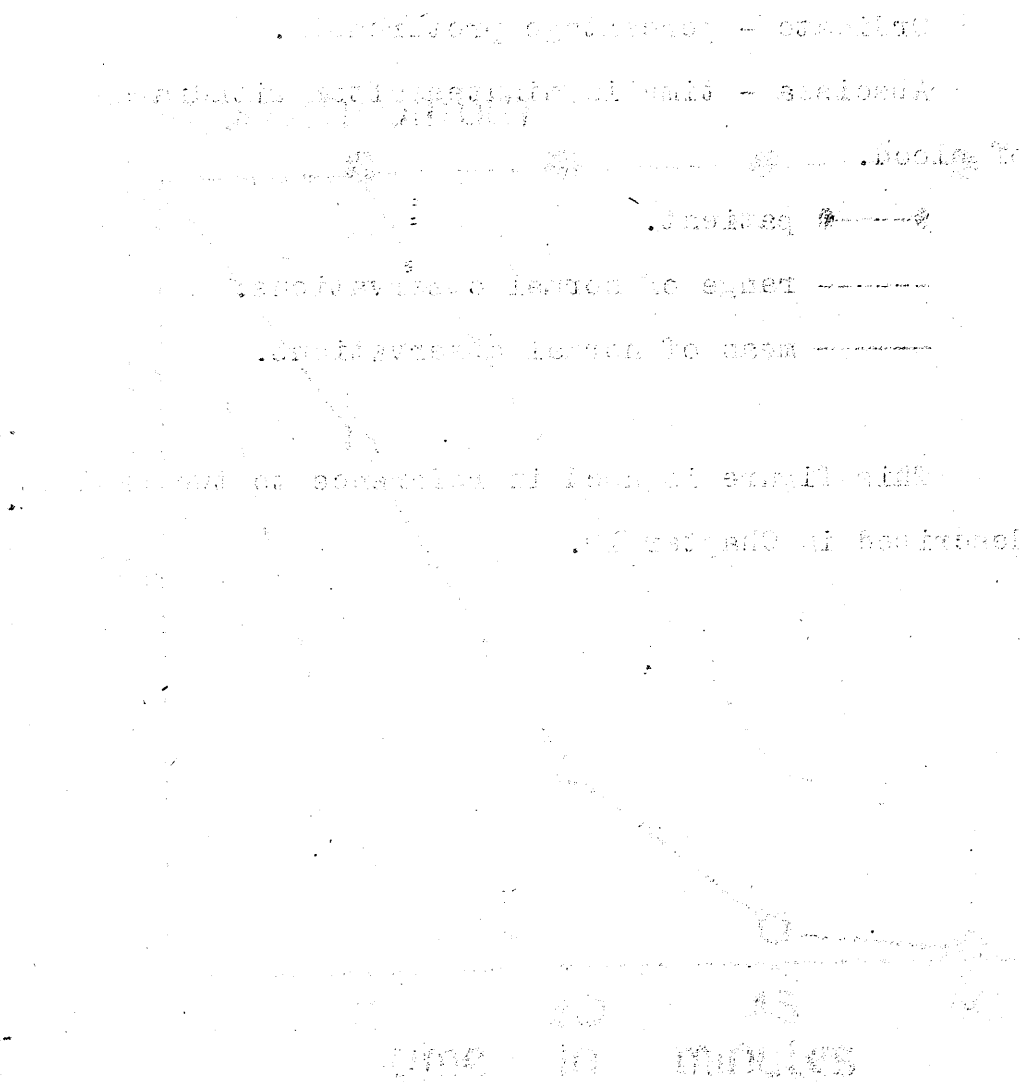


Figure (128)

Defective prothrombin consumption in patients with circulating anticoagulants.

Ordinate - percentage prothrombin.

Abscissa - time in minutes after withdrawal of blood.

●——● patient.

------ range of normal observations.

——— mean of normal observations.

This figure is used in reference to two patients described in Chapter 19.

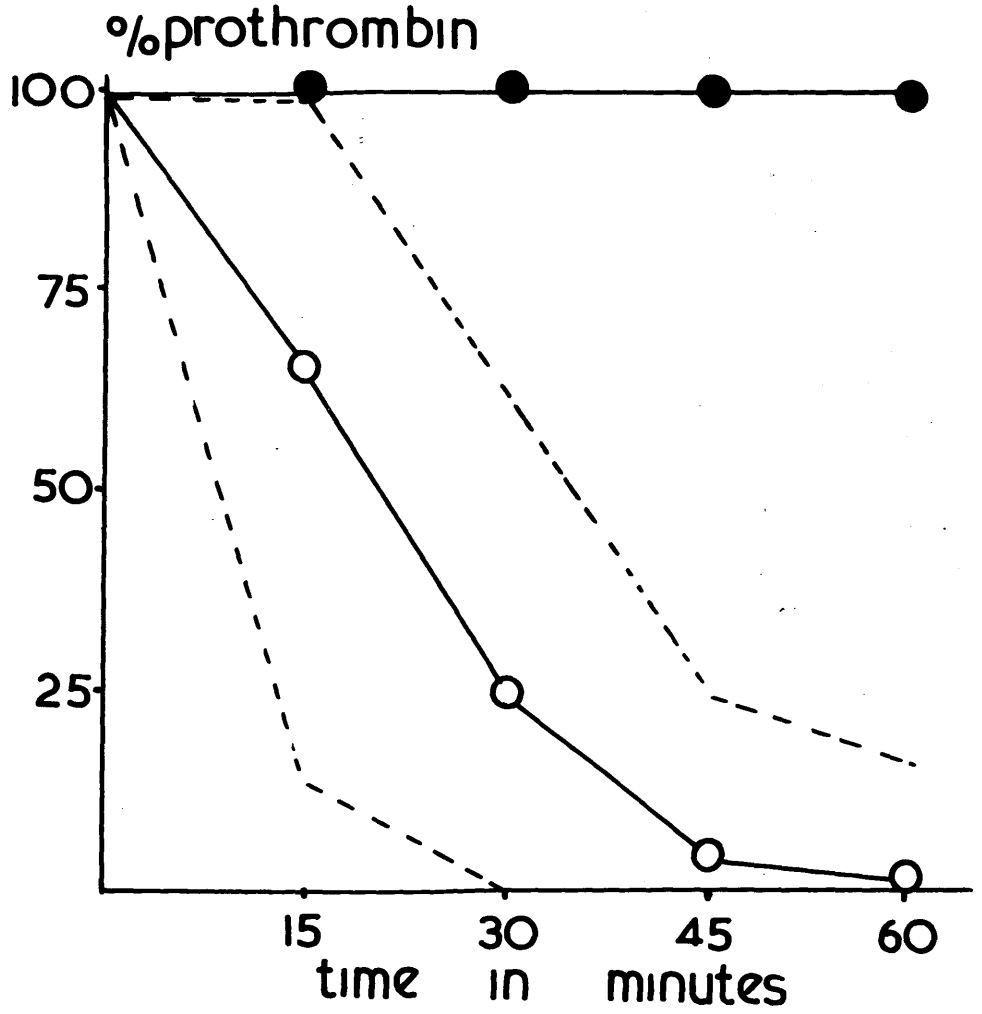

%prothrombin
100
75
50
25
15
30
45
60
time in minutes

Figure (128)

Defective prothrombin consumption in patients with circulating anticoagulants.

Ordinate - percentage prothrombin.

Abscissa - time in minutes after withdrawal of blood.

●——● patient.

------ range of normal observations.

——— mean of normal observations.

This figure is used in reference to two patients described in Chapter 19.

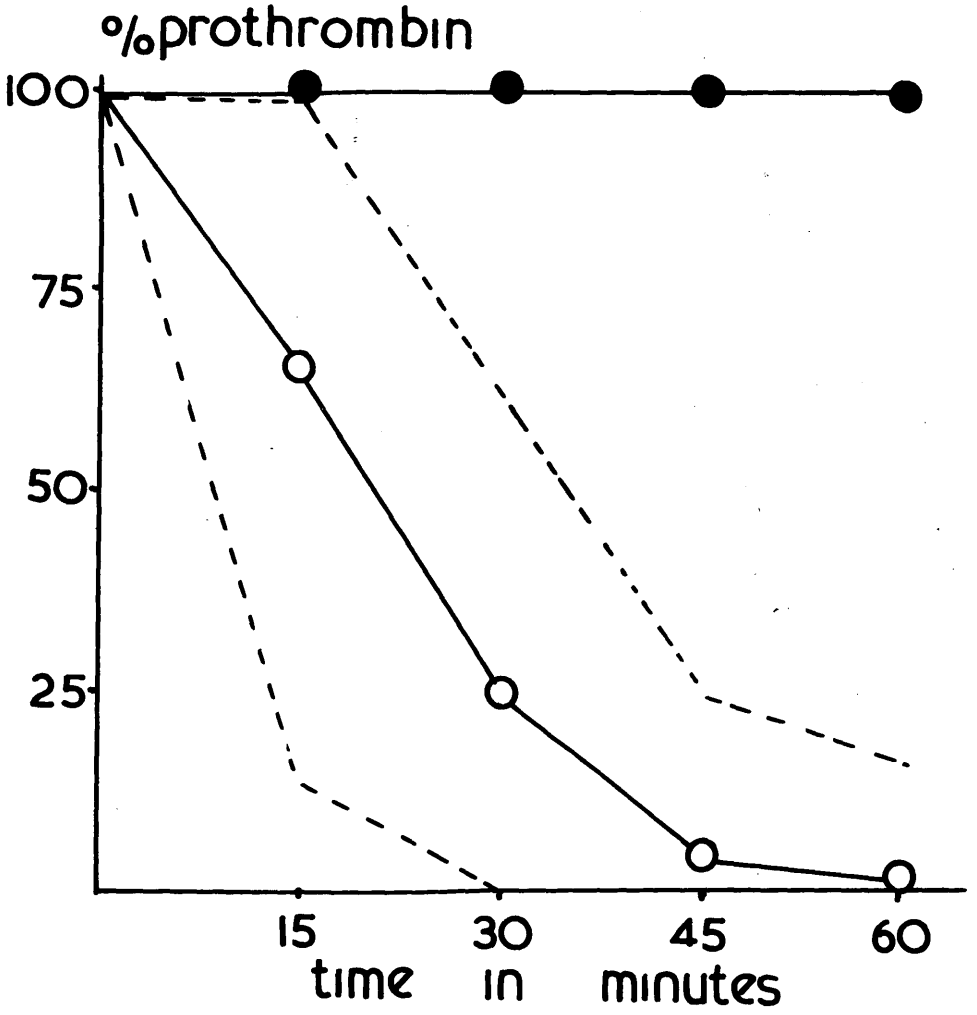

%prothrombin
100
75
50
25
15
30
45
60
time in minutes

Figure (129)

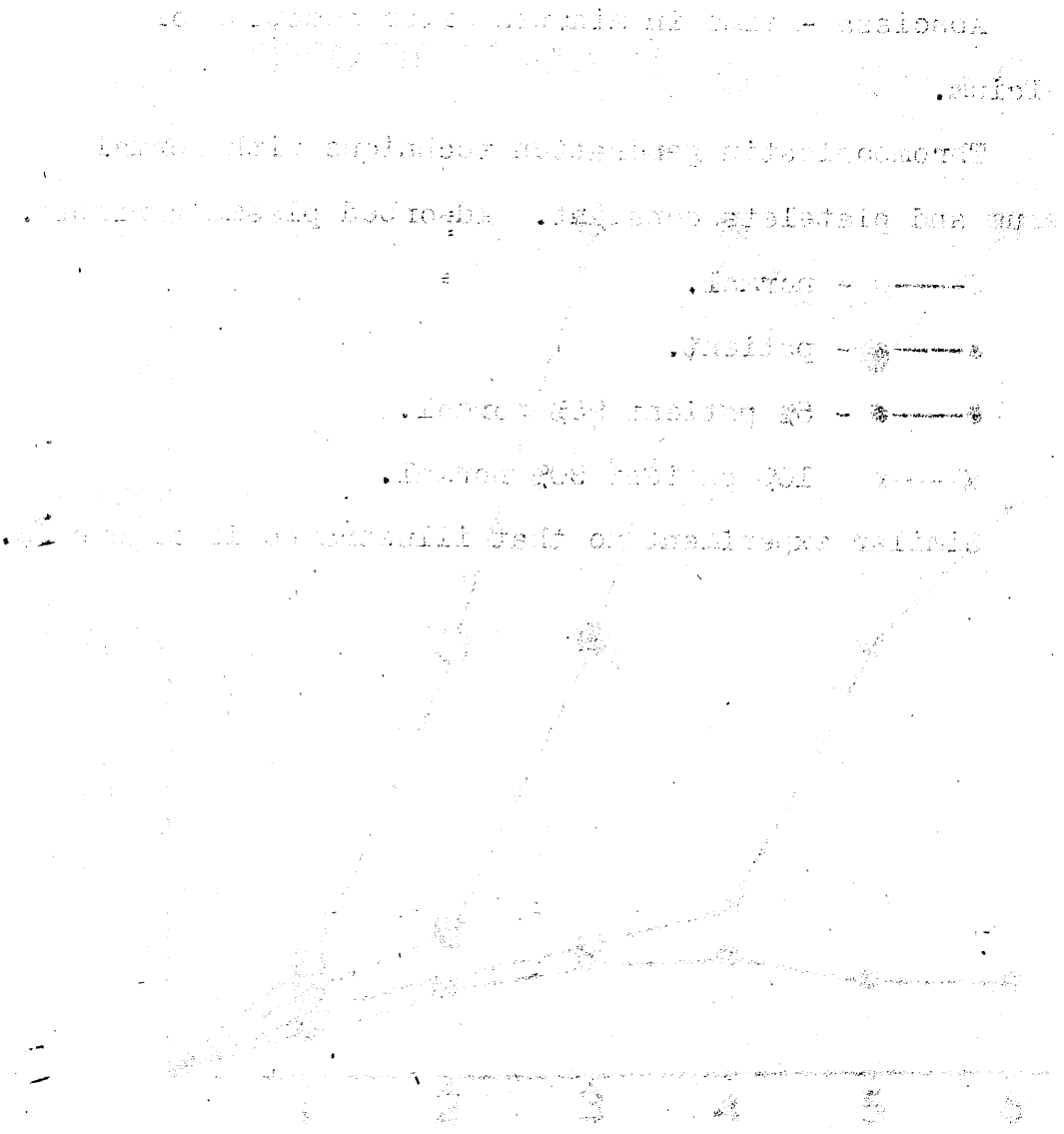


Figure (129)

Circulating thromboplastin inhibitor - thromboplastin generation test.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation technique with normal serum and platelets constant. Adsorbed plasma variable.

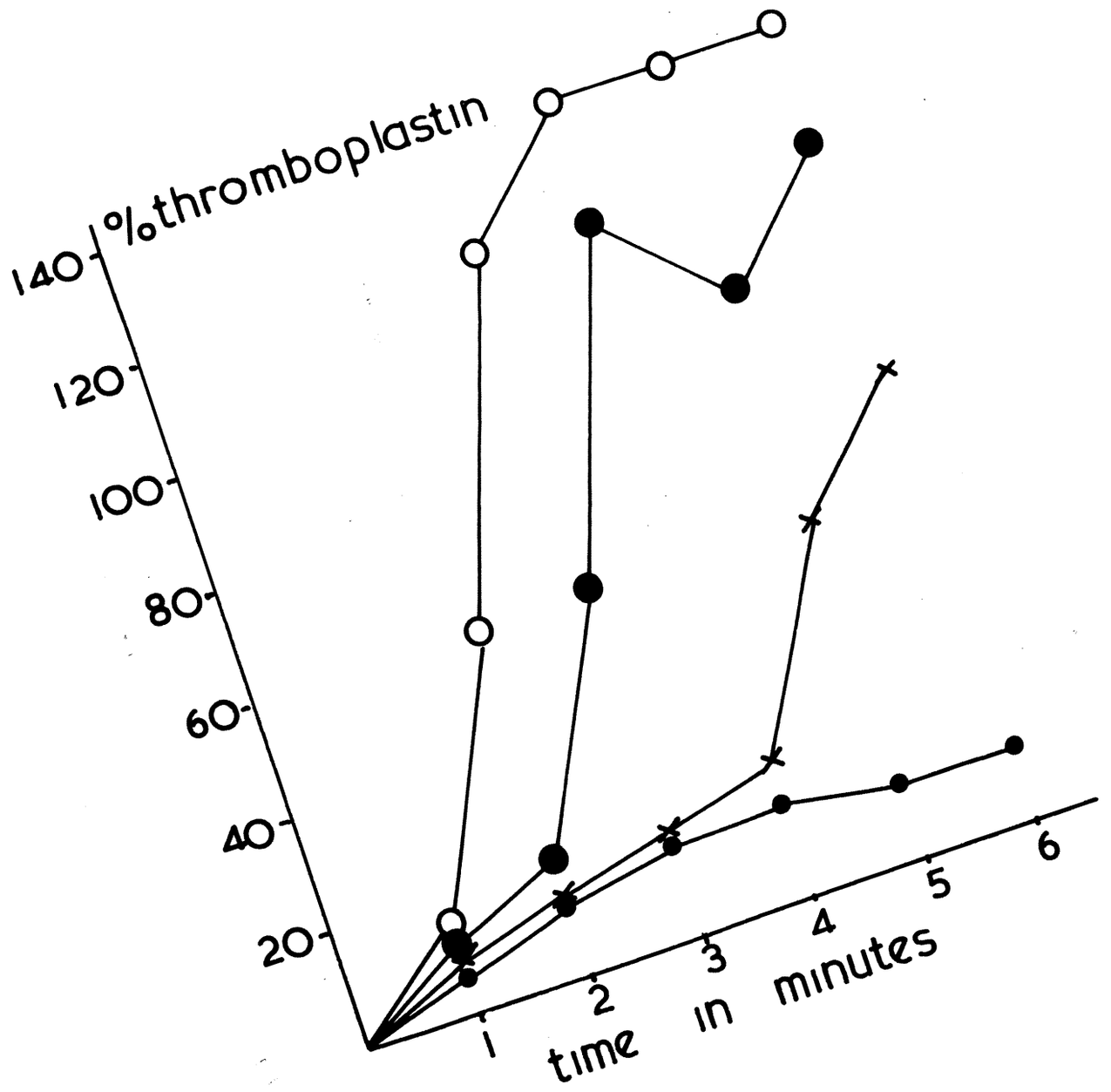
O——O - normal.

●——● - patient.

●——● - 5% patient 95% normal.

×——× 10% patient 90% normal.

Similar experiment to that illustrated in figure 93.


% thromboplastin
140
120
100
80
60
40
20
1
2
3
4
5
6
time in minutes

upon, in ignorance of the coagulation defect and died.

This case is reported by Hougie (J. clin. Path.1953, 6, 30). Specimens were examined in the Department of Clinical Pathology, The Radcliffe Infirmary, Oxford, and investigated by the thromboplastin generation test.

Previous investigation by Dr. Hougie had established a long whole blood clotting time and defective prothrombin consumption. The recalcification time was prolonged and the addition of small proportions of the patient's plasma to normal plasma caused prolongation of the clotting time of the normal plasma. Haemophilic plasma could not be corrected on the recalcification time.

The investigations at Oxford (see appendix page 1097-9) confirmed Dr. Hougie's findings. Figure 128 illustrates the very defective prothrombin consumption using the technique of Douglas and Biggs (1953). Using the thromboplastin generation test it was established that the anticoagulant like the others, inhibited blood thromboplastin formation but not its action once formed. (see figure 129).

Circulating anticoagulants complicating other disorders.
Illustrative case report - in rheumatoid arthritis.

The patient was a man aged 73 with advanced widespread rheumatoid arthritis for ten years, who also had enlarged axillary glands and cutaneous nodules over the right elbow and hip.

His liver and spleen were not enlarged. He had never had any gold therapy. The bleeding disorder which started in January 1951 was manifested by extensive subcutaneous and intramuscular haemorrhages (included two into the root of the tongue, embarrassing respiration), haematemesis, melaena and haematuria. He died of massive haemorrhages in Nov. 1951. Treatment with haematinics, transfusions, antihaemophilic globulin and A.C.T.H. had no effect on the underlying disorder. At necropsy there were large recent ecchymosis of the chest wall and neck, old and recent haemothoraces with adhesions and altered blood in the gut. The liver showed siderosis. The spleen was enlarged to almost twice the normal size, very soft and autolytic and siderosis was increased. Histological examination confirmed these findings and showed amyloid change in the axillary lymph-nodes but none in the liver.

This case is reported by Collins and Ferriman (Lancet 1952, 2, 712). Specimens were sent to the Department of Clinical Pathology, the Radcliffe Infirmary, Oxford, and investigated by the thromboplastin generation system (see page 1095 of the appendix).

Previous investigation of the coagulation abnormality by Dr. Collins had shown prolonged whole blood clotting time with defective prothrombin consumption. The clotting time

of normal whole blood was greatly prolonged by the addition of small proportions of the patient's blood.

The results of the investigations at Oxford on dried fractions from this patient showed that there was inhibition of blood thromboplastin formation but not its action once formed. (see pages 1095 - 1097 of the appendix).

An atypical thromboplastin inhibitor with previously undescribed features.

This patient (D.C.) was a merchant seaman aged 33 years and was first seen by me in December 1955. In 1945 when aged 23 years he had a complaint of having "pains all over". He was diagnosed in 1947 in New Zealand as having ankylosing spondylitis and from that time there had been increasing rigidity of the spine. There was stiffness and pain in the back, stiffness when turning the head and pain in the chest when coughing or sneezing. In 1953 he had X-Ray therapy to the spine for two weeks in Brisbane. In April 1955 he complained of pain in the left shoulder and was given X-Ray therapy to this region. In June-July 1955 he developed pain and swelling of the right knee. This was aspirated and serous fluid obtained - no haemarthrosis was present. In August 1955 he had haematuria for two months, which was painless apart from one occasion when he passed blood clots. This haematuria was investigated in hospital in Australia by

cystoscopy and retrograde pyelogram. These showed no abnormality. A guinea pig innoculation was carried out and this was positive for mycobacterium tuberculosis. He was sent home by sea from Australia, before the result of the guinea pig innoculation had become available. Just before arrival by ship in this country he developed a spontaneous haematoma of the right forearm which lesion was at first thought to be inflammatory and he was treated by intramuscular injections of penicillin. As a consequence of the injection of penicillin he developed a large haematoma of the left thigh. He was admitted to Professor Davis' wards in the Royal Infirmary and developed a further large haematoma of the left forearm which followed the small incision with an automatic pricker for estimation of the bleeding time.

On examination he was of lean build and pale. There was a haematoma of the right forearm and of the left thigh. There was a dorsal kyphosis and the spine was rigid, with very limited movement also of the chest. There was an effusion in the right knee joint.

On admission the haemoglobin was 49%, the red count 3-3 million/cu.mm and the white count 5,200/cu.mm., the peripheral blood film showing little of significance apart from anisocytosis of the red cells. Sternal marrow biopsy revealed a very active marrow with normoblastic erythroporesis.

Leucopoiesis was normal and megakaryocytes present in average numbers. The section was hyperplastic and there was considerable stainable iron present. Blood group O Rhesus + ve.

Radiological assessment showed gross changes due to ankylosing spondylitis in the small joints between the articular facets in the lumbar and lower dorsal spine; in addition to obliteration of the joint space there was extensive ossification. The lower costo-vertebral joints were similarly affected. On the vertebral bodies ligamentous ossification was only to be seen in the region of D.V.10-L.V.2. Both sacro-iliac joints were obliterated.

Biochemical liver function tests were normal. Plasma proteins were normal.

E.S.R. was raised to 87 mms/1 hour.

Urinary examination was negative, both chemically and on microscopic examination and on bacteriological examination. A guinea-pig was again innoculated and on this occasion no evidence of tuberculosis found.

Investigation of haemostatic mechanism.

Platelet count = 290,000 per cu.mm.

Hess test = negative.

Bleeding time (Ivy) - left forearm = 5'
(this procedure caused a haematoma of the left forearm)

Whole blood clotting time was 90'+. (normal range 10'-25')

" " " " (Biggs & Macfarlane 1953)-20'

One-stage "prothrombin time" - Patient 20" Control 17"
" 16" " 16"

Factor VII assay.

Addition of one-tenth part to Dindevan plasma.

Dindevan plasma	=	33"
Plus one-tenth Control	=	22"
Plus one-tenth Patient	=	24"

Factor VII - no significant decrease.

Factor V assay.

See experimental, appendix page 1102

Factor V concentration normal.

Prothrombin consumption.

Merskey technique.
Prothrombin consumption index 108%.

Douglas & Biggs technique
- see figure 128

Prothrombin consumption very defective.

Factor V consumption - as described by Douglas (1956)

Factor V consumption was defective.

Antihaemophilic globulin consumption - as described by Douglas (1956)

A.H.G. consumption was defective.

Thromboplastin Generation Test:

When this was done by the standard technique there was no detectable abnormality. This finding, in association

Figure (130)

Figure (130)

Thromboplastin generation test in a patient with atypical circulating thromboplastin inhibitor.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

●——● patient's adsorbed plasma and patient's serum.

O——O normal adsorbed plasma and normal serum.

Thromboplastin generation test with reagents used at standard dilutions - adsorbed plasma $^{1}/_{5}$ and serum $^{1}/_{10}$

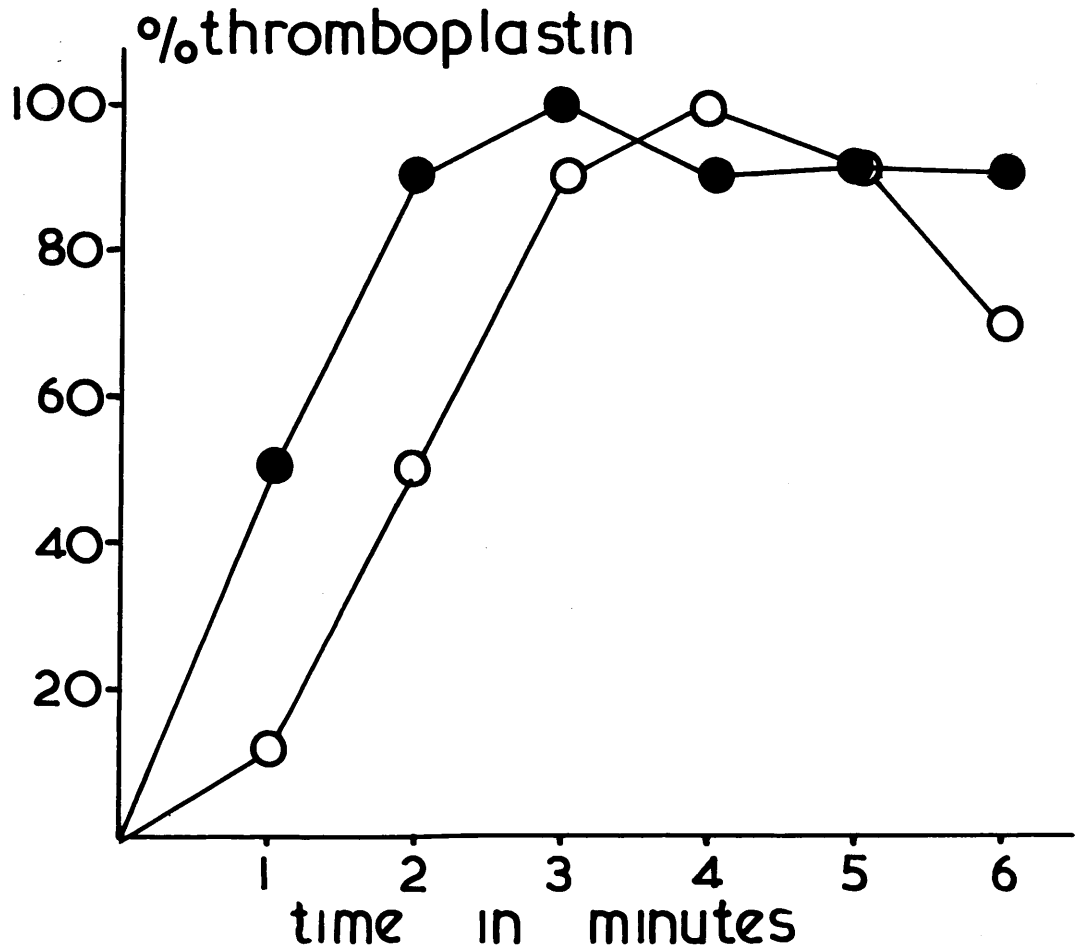

%thromboplastin
100
80
60
40
20
1
2
3
4
5
6
time in minutes

Figure (131)

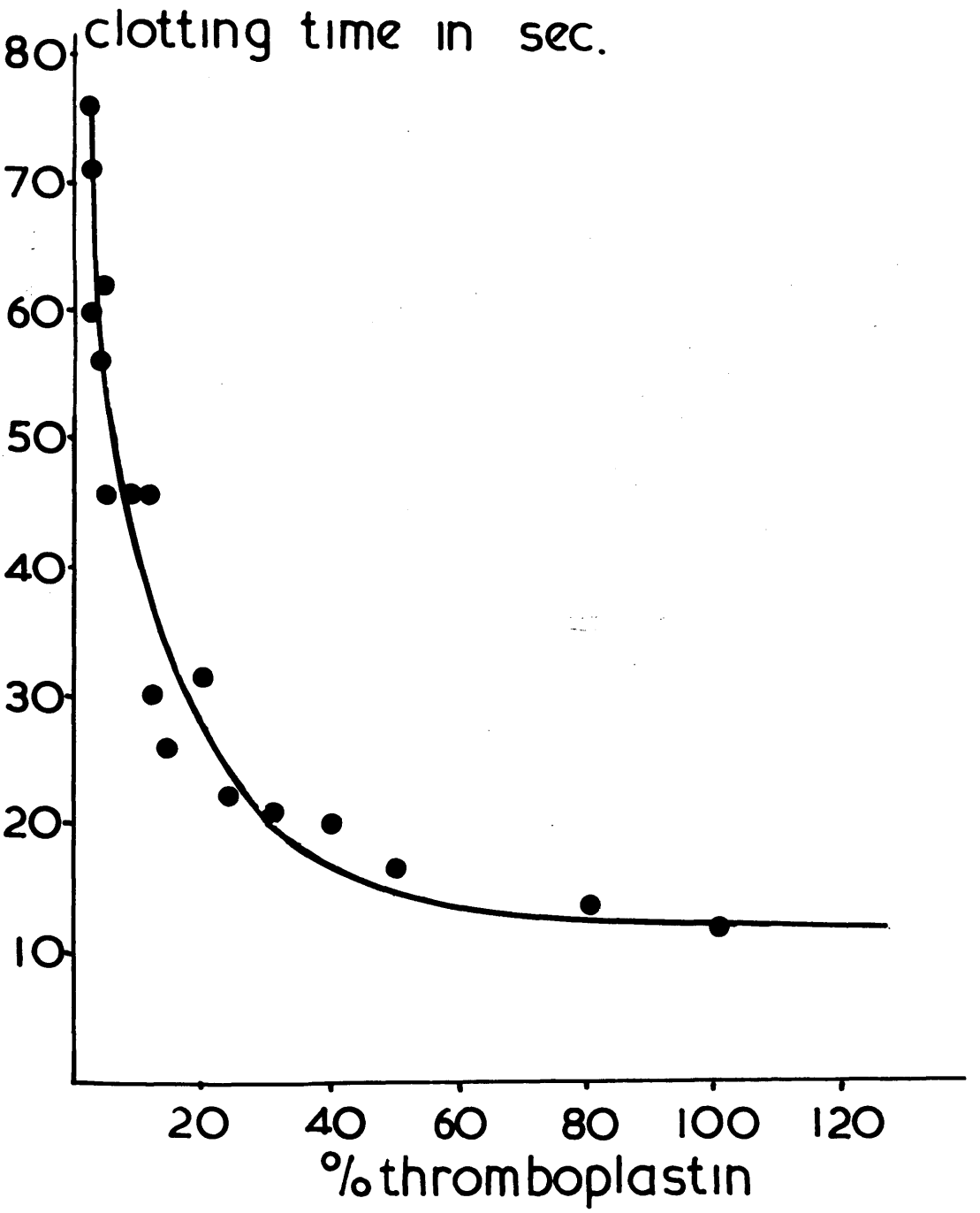
Figure (101)

Thromboplastin dilution curve with substrate 0.1 high spun plasma and 0.3 saline.

Ordinate - clotting time in seconds.

Abscissa - percentage thromboplastin.

This figure shows the thromboplastin dilution curve constructed for the study of the patient with the atypical thromboplastin inhibitor.


clotting time in sec.
80
70
60
50
40
30
20
10
20
40
60
80
100
120
% thromboplastin

with grossly defective prothrombin consumption, represented the interesting feature of this case. The results of this test using the adsorbed plasma (one in five) and the serum (one in ten) are shown in figure 130.

When the adsorbed plasma and the serum were used undiluted and the substrate diluted to give a greater "spread" to the figures it was possible to demonstrate a very marked abnormality. The incubation mixture contained 0.3 ml. of adsorbed plasma (undiluted), 0.3 ml. of normal serum (undiluted), 0.3 ml. of platelets, and 0.3 ml. of m/40 $CaCl_2$. The substrate consisted of 0.1 ml. of high-spun normal plasma and 0.3 ml. of saline. Using this substrate, a dilution curve of thromboplastin was prepared (see figure 131) as described for the thromboplastin generation test. The results of such an experiment are illustrated in figure 133. It will be seen that there is a very marked difference in the ability to form thromboplastin in the normal and the patient. With progressive dilution this difference became less marked so that when each was used in a dilution of one in five, the ability to form blood thromboplastin was the same. The clotting times in this experiment are shown in the experimental appendix.

Using the reagents undiluted and the substrate diluted with saline, it was possible to demonstrate that the inability

Figure (132)

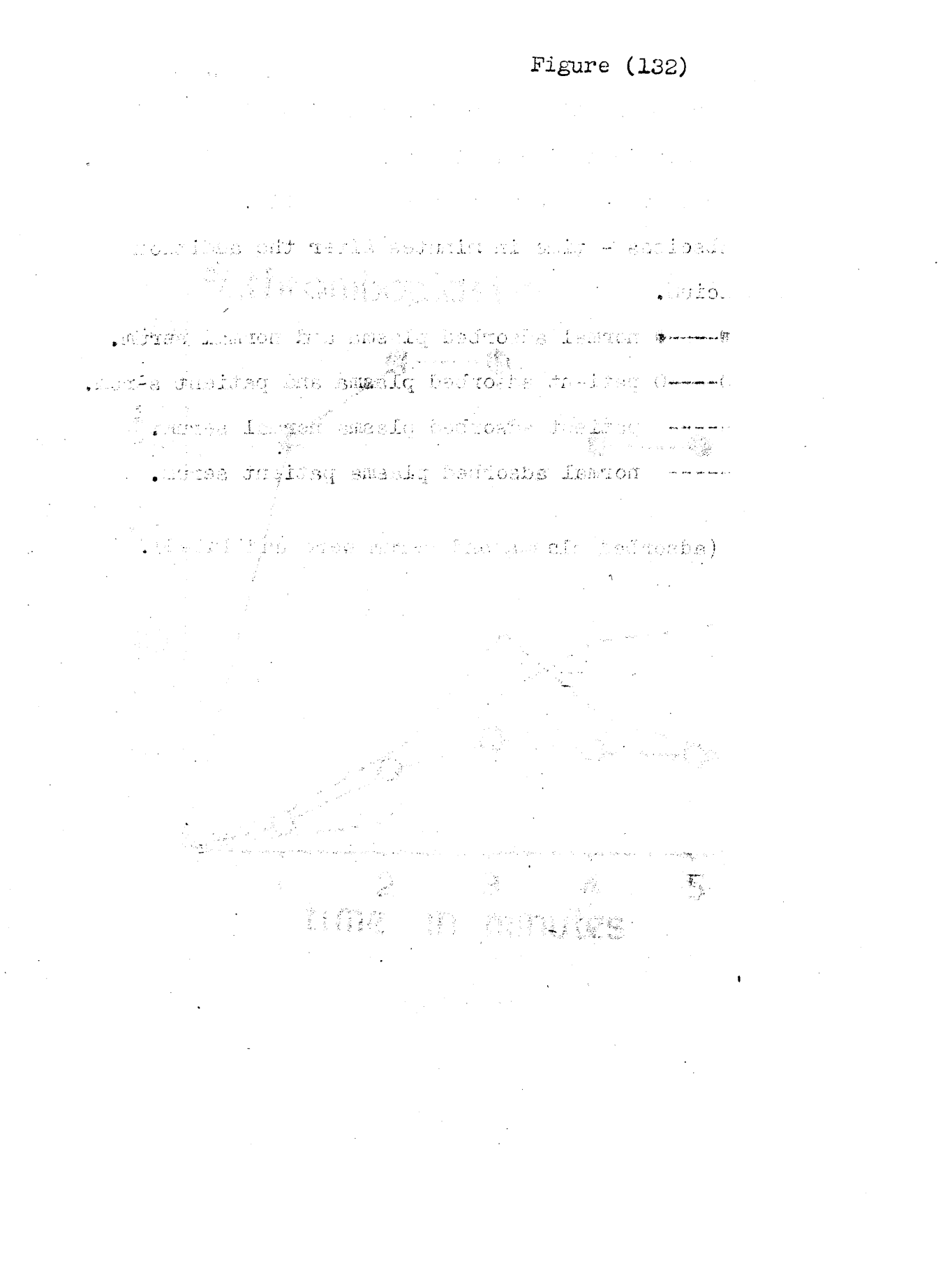


Figure (122)

Thromboplastin generation technique in a patient with an atypical circulating thromboplastin inhibitor.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after the addition of calcium.

●——● normal adsorbed plasma and normal serum.

O——O patient adsorbed plasma and patient serum.

----- patient adsorbed plasma normal serum.

----- normal adsorbed plasma patient serum.

(adsorbed plasma and serum were undiluted).

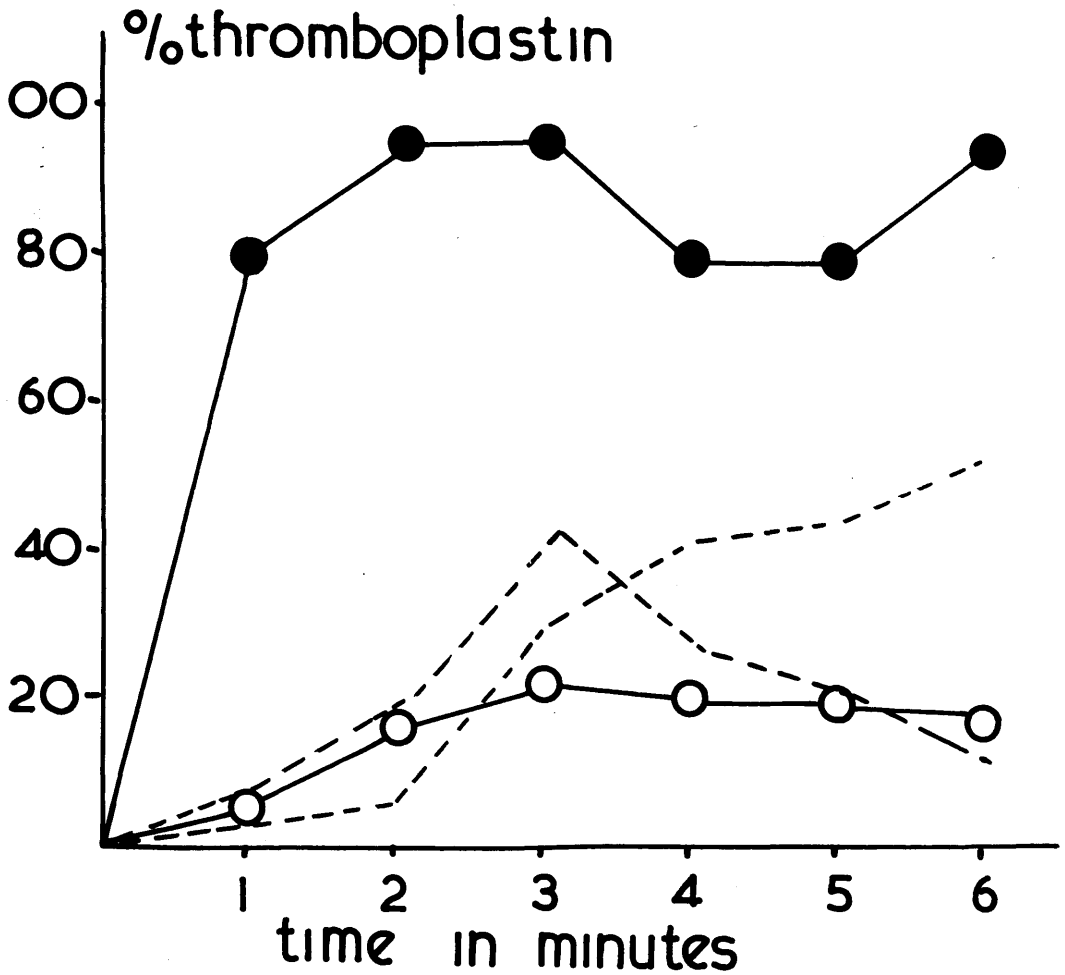
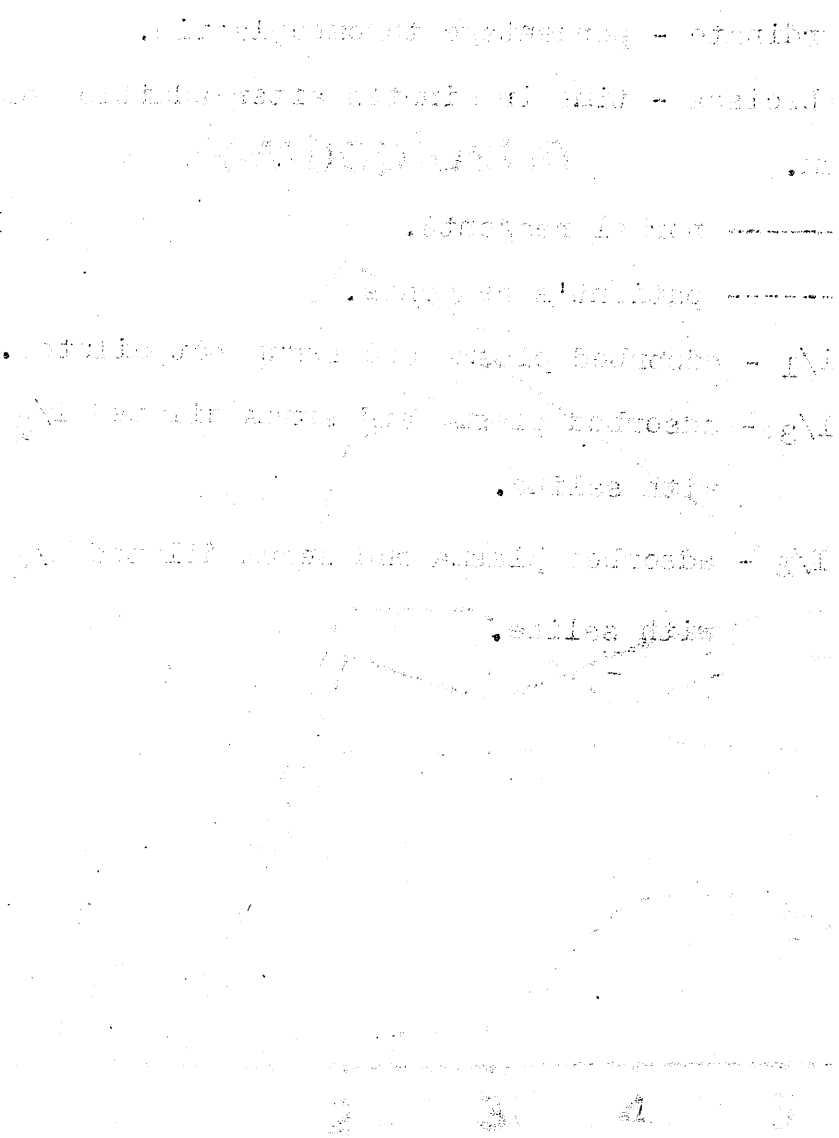

%thromboplastin
00
80
60
40
20
1
2
3
4
5
6
time in minutes

Figure (133)



Thromboplastin generation test in the patient with an atypical circulating thromboplastin inhibitor. Effect of dilution.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

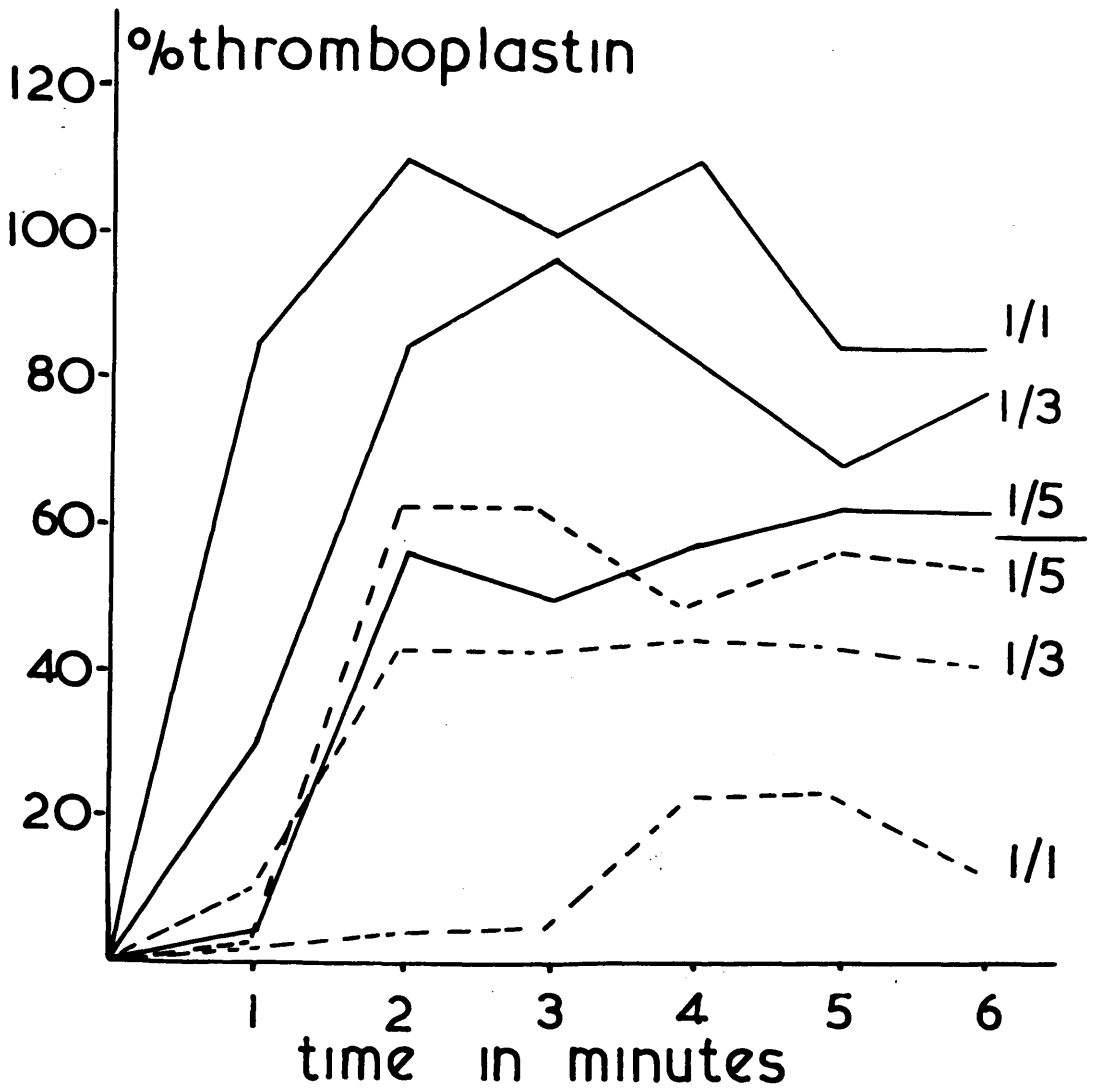
——— normal reagents.

------ patient's reagents.

$1/1$ - adsorbed plasma and serum not diluted.

$1/3$ - adsorbed plasma and serum diluted $1/3$ with saline.

$1/5$ - adsorbed plasma and serum diluted $1/5$ with saline.


%thromboplastin
120
100
80
60
40
20
1/1
1/3
1/5
1/5
1/3
1/1
1
2
3
4
5
6
time in minutes

to form blood thromboplastin was a function of both the adsorbed plasma and the serum - see figure 132 . Results shown in the experimental appendix page 1110 . In such a test system neither the serum nor the adsorbed plasma from the patient will form thromboplastin with its normal counterpart. Figure 134 shows the effect of the undiluted patient's adsorbed plasma on a thromboplastin generating system with diluted reagents.

Antihaemophilic globulin assay.

Using the thromboplastin generation technique the A.H.G. was assayed. The patient's adsorbed plasma used in a dilution of one in five. There was a normal concentration of A.H.G. (see appendix page 1103)

Christmas factor assay.

Using the thromboplastin generation technique the Christmas factor was assayed, the serum being used at a dilution of one in ten. There was a normal concentration of Christmas factor (see appendix page 1105)

Platelet activity.

In generating thromboplastin the activity of the platelets was normal (see appendix page 1115). The platelets were numerically normal.

Figure (134)

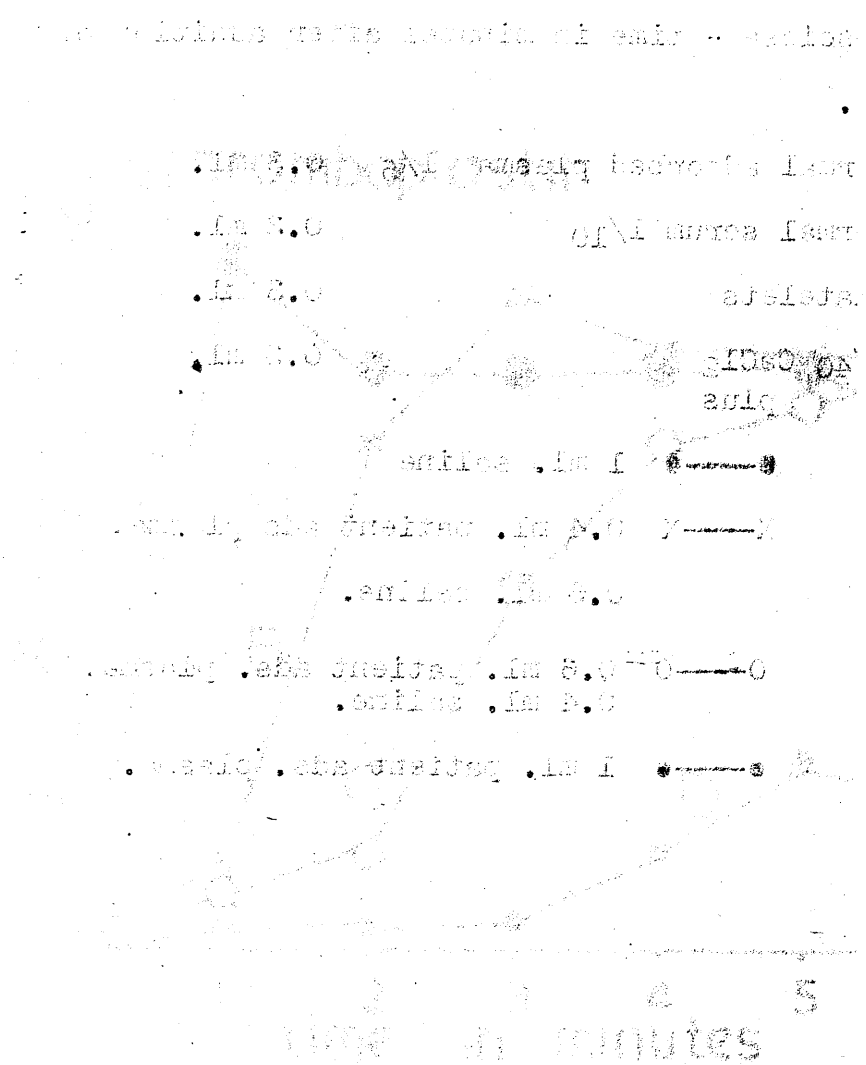


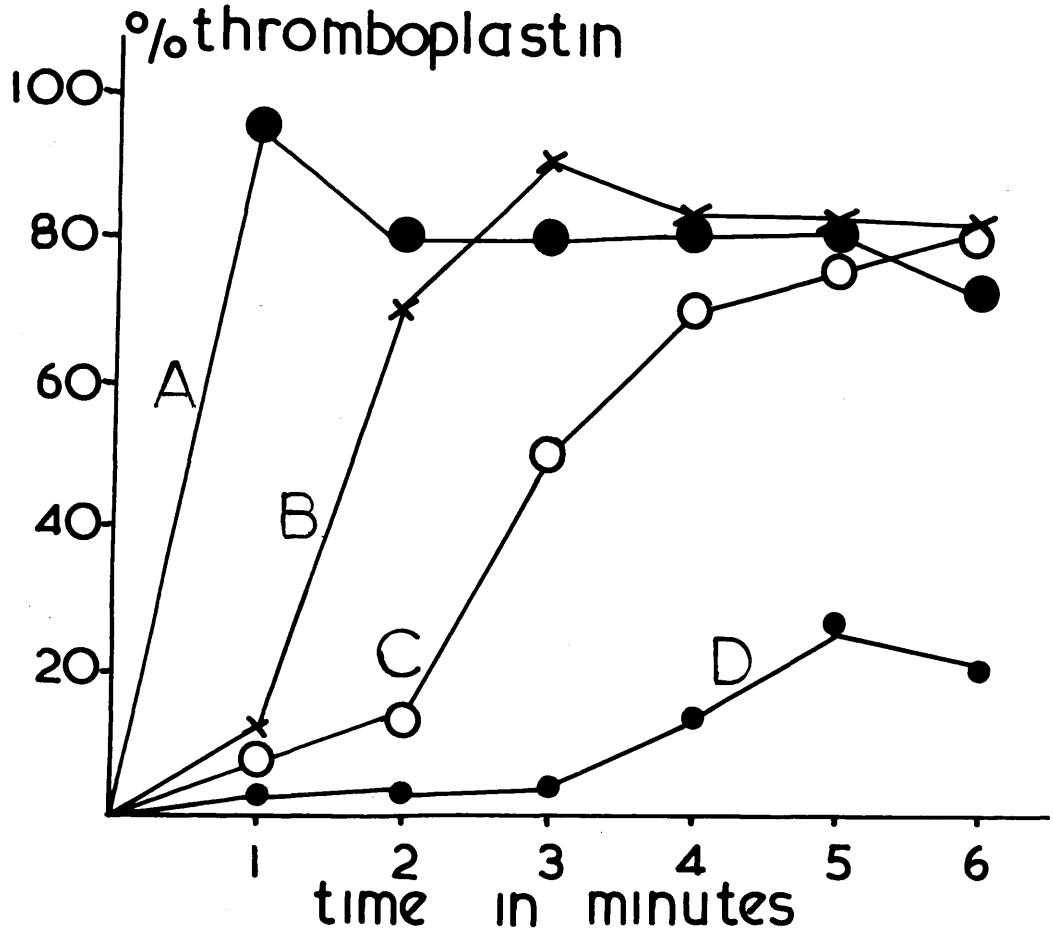
Figure (131)

Thromboplastin generation test in the patient with an atypical circulating thromboplastin inhibitor.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Normal adsorbed plasma	1/5	0.3 ml.
Normal serum	1/10	0.3 ml.
Platelets		0.3 ml.
m/40 $CaCl_2$ plus		0.3 ml.
●——●		1 ml. saline
X——X		0.4 ml. patient ads plasma. 0.6 ml. saline.
O——O		0.6 ml. patient ads. plasma. 0.4 ml. saline.
●——●		1 ml. patient ads. plasma.


% thromboplastin
100
80
60
40
20
A
B
C
D
1
2
3
4
5
6
time in minutes

Prothrombin concentration -

Normal-using the two-stage area method of Biggs and Douglas. Antithrombin concentration in the patient similar to the normal.

Fibrinolysis -

No increase in activity - method described in experimental appendix.

Addition of extra calcium -

This had no effect on the whole blood clotting time - see appendix

Thrombin generation test (whole blood)

(Macfarlane and Biggs 1953)-shows delay in thrombin formation - see figure 135 .

Reaction of patient's plasma to formed blood thromboplastin - normal.

Recalcification times.

Small additions of the patient's plasma are able to correct the calcium clotting time in Christmas disease or in haemophilia. Small additions do not prolong the times of normal plasma. Small additions of normal plasma would not shorten the patient's recalcification time.

Figure (135)

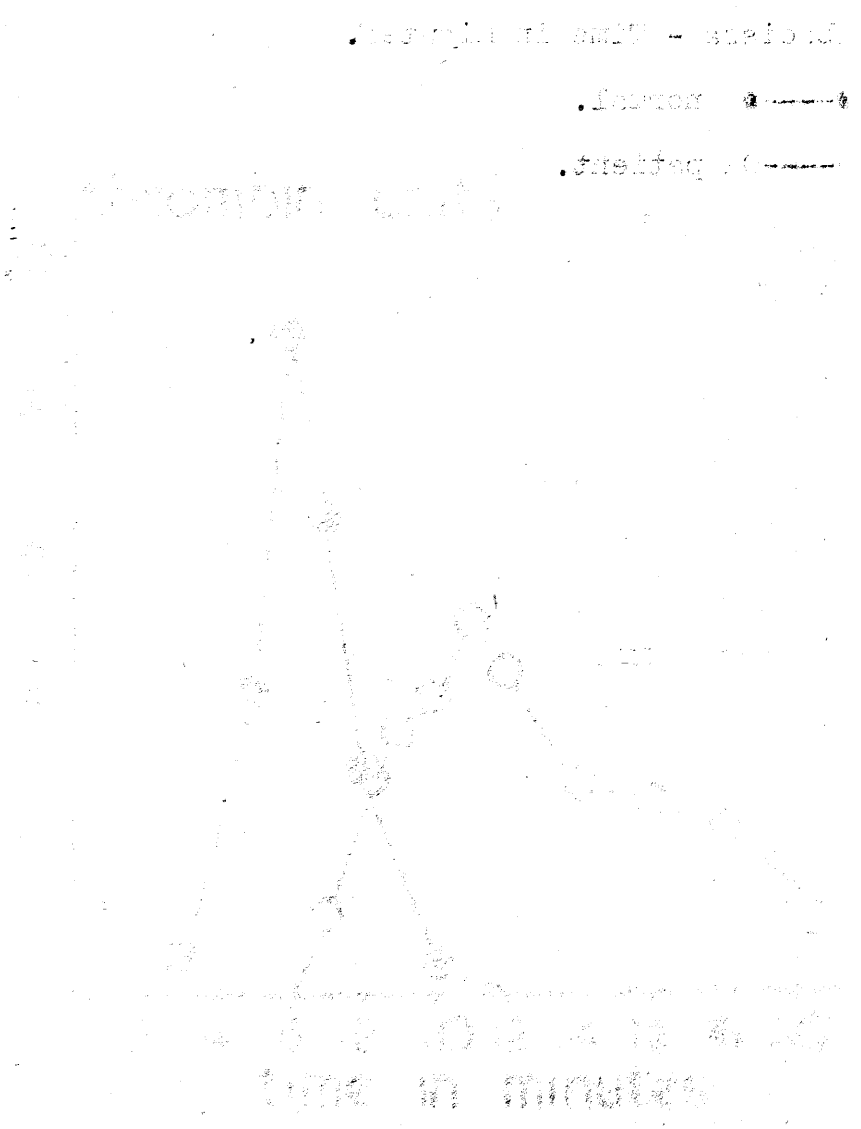


Figure (120)

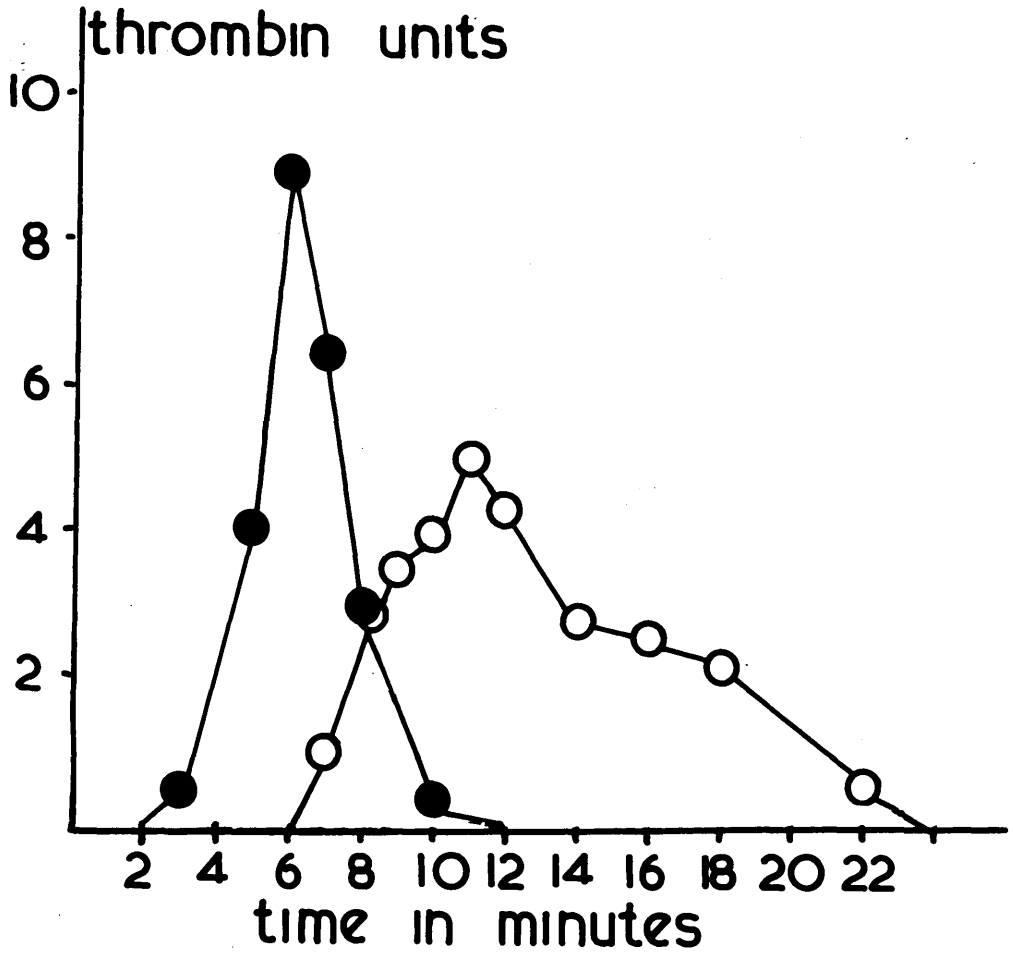
Thrombin generation in the patient with the atypical circulating thromboplastin inhibitor.

Ordinate - Thrombin units.

Abscissa - Time in minutes.

●——● normal.

O——O patient.


thrombin units
10
8
6
4
2
2 4 6 8 10 12 14 16 18 20 22
time in minutes

Experiment at bench temperature

System 0.1 plasma, 0.1 addition, 0.1 saline, 0.1 $CaCl_2$

Addition 0.1 →	0.2 plasma 0.8 saline →	Doubling dilutions in saline				
Xmas. + normal	12½	13¾	15¼	21	24	Xmas.
" + patient	17	18¾	18	19	20	alone =
" + haem.	14¾	18¾	17	18¾	23	45'
Pat. + normal	10	8	9	8	9	Pat.
" + Xmas.	10	10	10	10½	10½	alone =
" + Haem.	12	8¼	12	10	12	12
Haem. + normal	8	7	8½	10	11½	Haem.
" + pat.	11	10	10	10	8½	alone =
" + Xmas.	8	10½	7½	9¾	9	35
Norm. + normal	5½	6	6	6	6	
" + haem.	5½	5½	5½	5½	5	
" + Xmas.	5	6	6	4¾	6	
" + pat.	6	5¼	4¾	5¼	5¼	

This experiment was carried out at room temperature to produce a wider "spread" to the figures.

Protamine Titration - no evidence of excess of heparin - see appendix page 1119.

Destruction of formed thromboplastin.

As will be seen from the results in the experimental appendix page 1120 the patient's serum appeared to destroy formed thromboplastin more rapidly than the normal serum. This experiment however is difficult to interpret as the results may be due to continuing inhibition of formation.

The effect on the thromboplastin generation of normal reagents of addition of varying amounts of the patient's adsorbed plasma. (Figure /34).

In this experiment normal adsorbed plasma, normal serum, platelets and calcium were incubated together with 1cc saline as the blank (curve A in figure /34). In curve D the saline was replaced by patient's ads. plasma. In curve B 0.4 of the patient's adsorbed plasma and in curve C 0.6 of the patient's adsorbed plasma were added, the remainder of the 1 cc. being made up with saline. The inhibition of thromboplastin formation is clearly demonstrated.

Reaction to dilutions of brain thromboplastin.

With increasing dilution of the brain there is abnormal increase in clotting time relative to the normal plasma. This is a feature common to all blood thromboplastin defects.

On testing for possible destruction of brain there was no abnormality (see page ///8 of experimental appendix).

Comment on this patient.

This patient had a coagulation defect characterised by defective prothrombin consumption. There was therefore an abnormality of blood thromboplastin but the nature of this was not at first obvious. There was no deficiency of platelets, antihaemophilic globulin or the Christmas factor,

or of factors V or VII or prothrombin itself. Using re-calcification times, the patient's plasma was able to correct haemophilic and Christmas disease plasmas and did not prolong the clotting time of normal plasma. The patient's calcium clotting time was not appreciably shortened by the addition of normal plasma. The thromboplastin generation test was normal when the reagents were used at the standard dilutions. It was only when the test was modified by using the adsorbed plasma and serum undiluted that the defect was demonstrable. The abnormality was a function of both the adsorbed plasma and the serum. The likely explanation of this case is of a circulating thromboplastin inhibitor whose activity is removed by dilution. There have been no previous reports of patients of this type.

SUMMARY

(1) Patients with circulating anticoagulants can be divided into three categories.

(a) complicating haemophilia or Christmas disease.
(b) complicating pregnancy.
(c) miscellaneous - associated or not with other recognised disease.

The results on illustrative cases are described and discussed.

(2) An atypical thromboplastin inhibitor with previously undescribed features is reported. This patient had a prolonged whole blood clotting time and defective prothrombin consumption. Plasma from this patient was able to shorten the calcium clotting times of haemophilic or Christmas disease plasmas and did not prolong the recalcification time of normal plasma. The thromboplastin generation test when used as described in the original technique with the adsorbed plasma and serum diluted gave normal results. When these reagents were used undiluted the inhibition of thromboplastin formation was readily demonstrated. These results were interpreted as due to a circulating thromboplastin inhibitor the effect of which was readily removed by dilution.

REFERENCES

Beaumont, J.L. (1954)
Syndrome hémorragique acquis du à un anticoagulant circulant.
Le Sang, 25, 1.

Biggs, R. and Douglas, A.S. (1953).
The thromboplastin generation test.
J. clin. Path. 6, 23.

Biggs, R. and Macfarlane, R.G. (1953)
Human blood coagulation and its disorders.
Blackwell Scientific Publications, Oxford.

Chargaff, E. and West, R. (1946).
Biological significance of thromboplastic properties of blood.
J. biol. Chem. 162, 189.

Collins, I.S. and Ferriman, D.G. (1952).
Haemophilia-like syndrome from anticoagulants affecting the production of thromboplastin.
Lancet, 2, 712.

Conley, C.L., Rathbun, H. K., Morse, W. I., Robinson, J.E. (1948)
Circulating anticoagulant as a cause of haemorrhagic diathesis in man.
Johns Hopk. Hosp. Bull. 83, 288.

Dieter, D.G , Spooner, M. and Pohle, F.J. (1949)
Studies on an undetermined circulating anticoagulant.
Blood, 4, 120.

Douglas, A.S. (1956)
Factor V consumption during blood coagulation.
Brit. J. Haemat. 2, 153.

Douglas, A.S. (1956).
Antihaemophilic globulin consumption during blood coagulation.
Blood, 9, 423.

Douglas, A.S. and Biggs, R. (1953).
The consumption of some components involved in physiological blood coagulation.
Glasg. med. J. 34, 329.

Dreskin, H.O. and Rosenthal, N. (1950)
A haemophilia-like disease with prolonged coagulation time and a circulating anticoagulant. Report of a case in a female.
Blood, 5, 46.

Evans, D.M.D. (1955)
Haemorrhagic diathesis due to a circulating anticoagulant investigated by the thromboplastin generation test.
J. clin. Path. 8, 168.

Fantl, P. and Nance, M.H. (1946).
An acquired haemorrhagic disease in a female due to an inhibitor of blood coagulation.
Med. J. Aust. 2, 125.

Frick, P.G. (1953)
Haemophilia-like disease following pregnancy, with transplacental transfer of an acquired circulating anticoagulant.
Blood 8, 598.

Frick, P.G. (1955)
Acquired circulating anticoagulants in systemic "collagen disease". Auto-immune thromboplastin deficiency.
Blood, 10, 691.

Frommeyer, W.B., Epstein, R.D. and Taylor, F.H.L. (1950)
Refractoriness in haemophilia to coagulation-promoting agents: whole blood and plasma derivatives.
Blood, 5, 401.

Hardisty, R.M. (1954)
Acquired haemophilia-like disease.
J. clin. Path. 7, 26.

Heinle, R.W., Weisberger, A.S., Vignos, P.J. and Holden, W.B. (1949)
Haemorrhagic diathesis associated with low thromboplastic activity and a circulating anticoagulant.
J. lab. clin. Med. 34, 1606.

Hewlett, J.S. and Haden, R.L. (1949)
Haemophilia-like disease in women.
J. lab. clin. Med. 34, 151.

Hougie, C. (1953)
Pseudo-haemophilia: an acquired haemorrhagic diathesis due to a circulating anticoagulant.
J. clin. Path. 5, 30.

Hougie, C. and Fearnley, M.E. (1954)
The nature and action of circulating anticoagulants.
Acta Haemat. 12, 1-10.

Hougie, C. (1955)
Circulating anticoagulants.
Brit. med. Bull. 11, 16.

Joulis, H. and Macfarlane, R.G. (1938)
Pseudo-haemophilia in a woman.
Lancet 1, 715.

Lawrence, J.S. and Johnson, J.B. (1941)
The presence of circulating anticoagulant in a male member of a haemophilic family.
Trans. Amer. climat. (clin.) Ass. 57, 223.

Lewis, J.H. and Ferguson, J.H. (1953)
Haemorrhagic diathesis due to P.T.C. (plasma thromboplastin constituent) deficiency.
Proc. Soc. exp. Biol. N.Y. 82, 445.

Lozner, E.J., Joliffe, L.S. and Taylor (1940).
Haemorrhagic diathesis with prolonged coagulation time, associated with circulating anticoagulant.
Amer. J. med. Sci. 199, 318.

Madison, F.W. and Quick, A.J. (1945)
Haemophilia-like disease in the female with a note on the clotting time of the recalcified plasma.
Amer. J. med. Sci. 209, 443.

Pons, E.R. and de Torregrosa, M.V. (1952).
Haemorrhagic diathesis due to a circulating anticoagulant. Report of a case with laboratory observations.
Blood, 7, 20.

Quick, A.J. and Stefanini, M. (1948)
Activation of plasma thromboplastinogen and evidence of an inhibitor.
Proc. Soc. exp. Biol. N.Y. 67, 111.

Singer, K., Mond, E., Hyman, J., Levy, R.C. (1950)
Circulating anticoagulants in haemophilia and in haemophilia-like disease.
Blood, 5, 1135.

Soulier, J.P. and Larrieu, M.J. (1953)
Differentiation of haemophilia into two groups.
New Engl. J. fled. 249, 547.

Part VII. The Management of Coagulation Disorders

CHAPTER 20

DENTAL EXTRACTION IN HAEMOPHILIA AND CHRISTMAS DISEASE

CONTENTS.

CHAPTER 20

DENTAL EXTRACTION IN HAEMOPHILIA AND CHRISTMAS DISEASE

The management of the haemophiliac presents many difficulties and not least amongst these is the problem of dental extraction. The incidence of haemophilia in the population is not high, an approximate estimate for the West of Scotland being one per 30-35,000 (see Chapter 15). In contrast to the low incidence of the condition is the frequency with which these sufferers require hospital management. Amongst the commonest causes of admission of the haemophiliac to hospital is the necessity for tooth extraction.

Most haemophiliacs show great reluctance in seeking dental attention. The fear of tooth extraction is of course well justified; unfortunately this dread of dental treatment has prevented the proper care of the teeth by conservative methods and as a consequence their teeth have been neglected. We have seen on several occasions haemophiliacs of about twenty years of age, requiring complete clearance of teeth. Many of the teeth are represented merely by shells submerged in the gum margin and consequently paradontal sepsis is frequent.

It is the object of this chapter to report experiences in the management of dental extraction in patients with

haemophilia or the closely allied condition Christmas disease.

MATERIAL AND METHODS.

Twenty-two dental operations were carried out on eight patients; seven of these were haemophiliacs (lacking anti-haemophilic globulin) and the eighth was a patient with Christmas disease (lack of the Christmas factor). The number of dental operations performed on each patient varied from one to five depending on the number of teeth requiring extraction. The important features of these dental operations are summarized in Table 33. The patients were all admitted to hospital; they were warned that the measures to be adopted were unlikely to completely prevent haemorrhage but that such bleeding as would occur would not be serious in its extent.

Number of extractions at each operation.

The number of teeth extracted at each dental operation varied from one to four. The number of teeth extracted at each operation can be summarized as follows:-

12	operations	- one tooth extracted
5	"	- two teeth "
4	"	- three teeth "
1	operation	- four teeth "

The teeth were extracted by Mr. J. A. Orr in the Glasgow Dental Hospital.

Antibiotic Therapy.

Antibiotic therapy was administered routinely, starting on the day prior to the extractions. In the earlier part of the study parenteral penicillin was used but thereafter oral antibiotic therapy with chloromycetin or aureomycin. The course of the oral antibiotic was for 10 days initially but again in the later extractions, this was reduced to five days.

Acrylic resin splint.

For each patient a protective dental splint was made prior to the operation and inserted immediately after removal of the teeth. The splint was constructed in the following manner: an impression of the teeth was taken in one of the alginate impression materials such as Zelex and from this a model poured in stone plaster. From the model the tooth or teeth to be extracted were removed and over this area a thickness of dental wax was fitted. Another alginate impression was taken from the first model and a second model cast in stone. Upon this second model a layer of wax was placed, trimmed to the exact requirements of the splint and then processed in clear acrylic resin. The reason for the first layer of wax over the tooth socket was to allow sufficient space between the acrylic splint and the socket for insertion of black gutta percha. This gutta percha was well

tolerated by the soft tissues, so that at the site of operation no hard acrylic came in contact with the wound, but at the same time there was a close seal.

These splints were prepared in the Glasgow Dental Hospital. An example is illustrated in figure 150. When significant bleeding occurred there was no hesitation in removing the splint for reapplication of local haemostatic measures; the splint was then reinserted. Where insignificant bleeding occurred the splint was left in position for 10-14 days before removal; at the end of that time there was generally a healed socket when the splint was taken out.

Local haemostatic treatment.

This consisted of an absorbable dressing such as calgitex or gelatine sponge soaked in commercially available bovine thrombin or Russell's Viper Venom. Maintained local digital pressure was sometimes required.

Anaesthesia.

In a small number of the earlier operations of the series anaesthesia was regional by inferior dental injection; the great majority of the operations were undertaken with local infiltration anaesthesia.

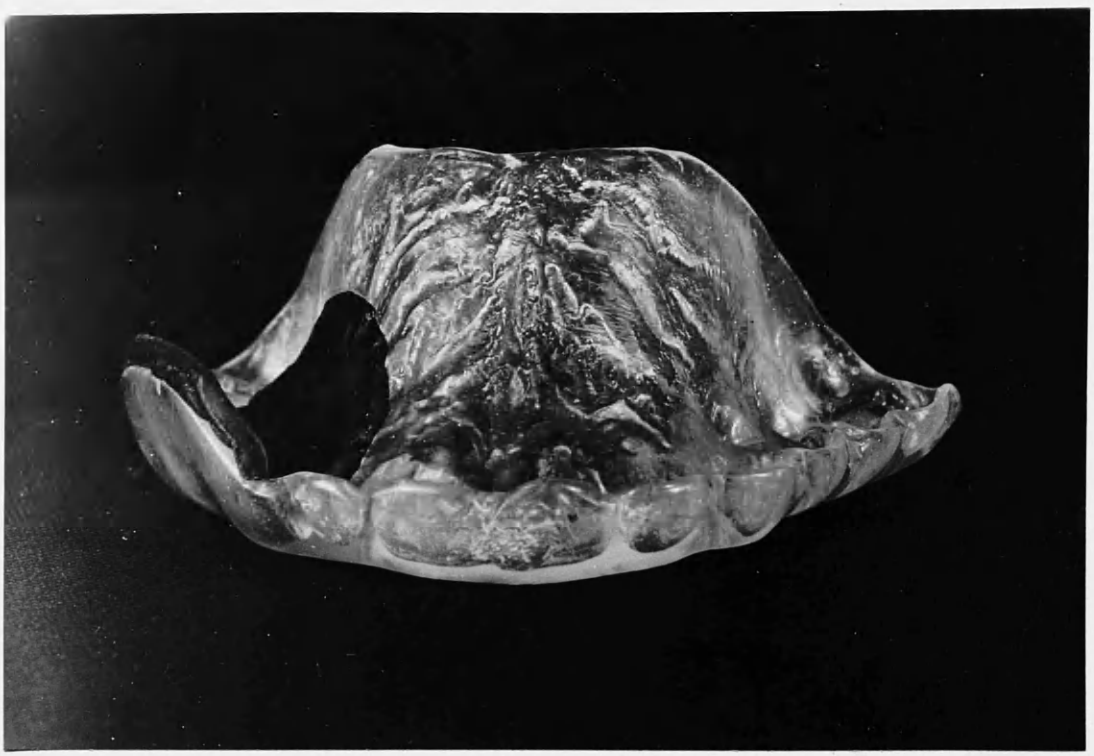
Replacement therapy.

Therapy with blood or blood products was used in an

Figure (150)

Dental splint used in the management of extractions in haemophilia and Christmas disease.

This photograph shows an acrylic resin dental splint with the black gutta percha in position.



attempt to supply the coagulation components missing in these patients. In the earlier extractions of the series bank blood was used, but later fresh frozen plasma. Fresh frozen plasma was eventually adopted as the routine form of replacement therapy for deficiency of antihaemophilic globulin. In one patient a large amount of the dried plasma fraction (Cohn I) containing antihaemophilic globulin was given.

In the later extractions whole blood was used only for raising the haemoglobin level where this had fallen significantly as a consequence of bleeding. Blood was held matched for each patient for a period 10-14 days from the time of extraction. Haemophiliacs are often the recipients of multiple transfusions and there is the danger of haemolytic transfusion reactions unless sensitive compatability tests are employed. Two of the patients were Rhesus negative and had anti-D antibodies in consequence of previous administration of blood.

RESULTS

Occurrence of haemorrhage.

In figure 61 is shown by histogram the incidence of haemorrhage, subsequent to the 20 dental operations. Even when the bleeding was minimal in extent, possibly only the staining of saliva over a short period, haemorrhage was recorded as having occurred on that particular day.

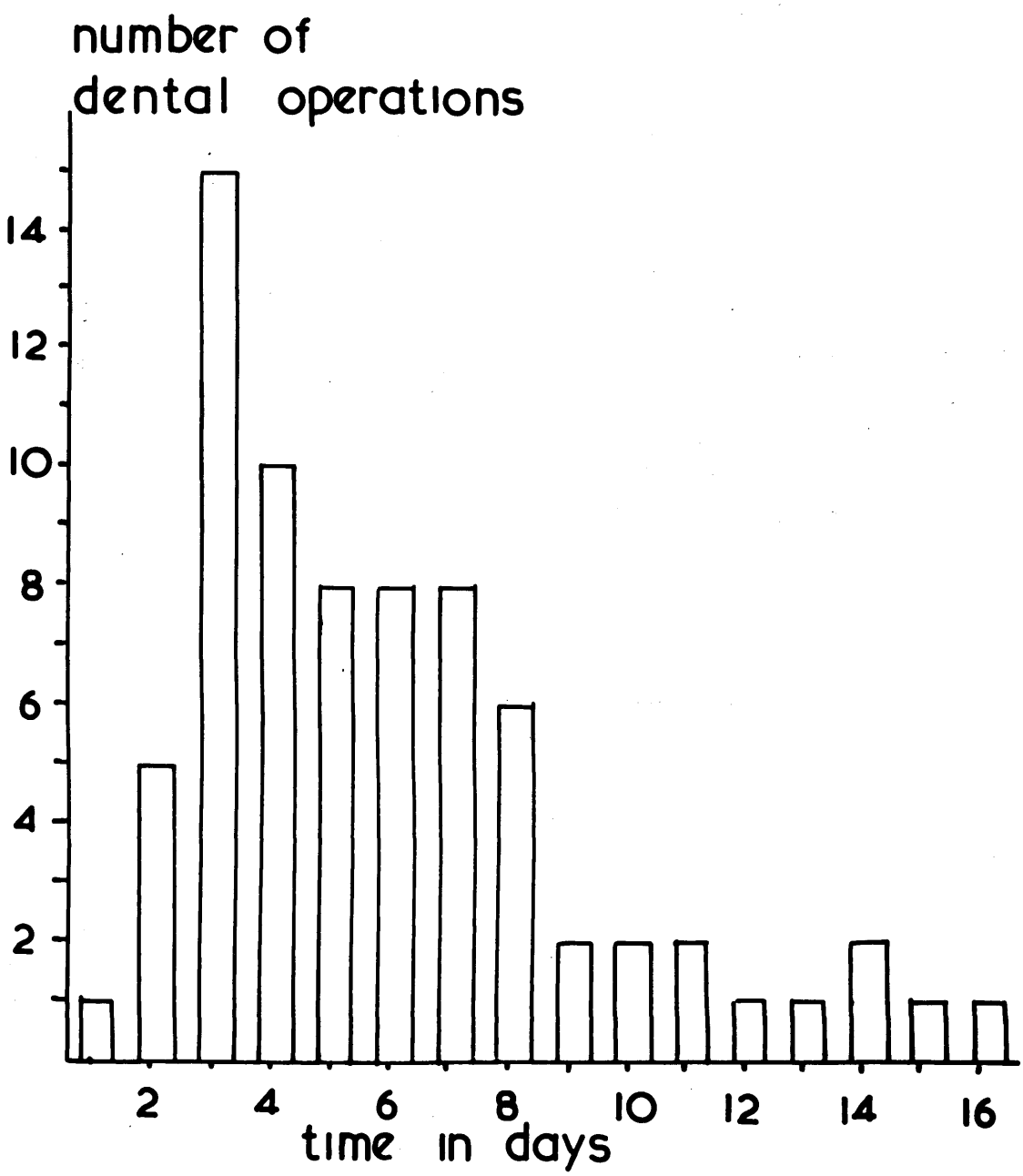
Figure (151)

Incidence of haemorrhage following tooth extraction in haemophilia and Christmas disease.

Ordinate - number of dental operations in which haemorrhage occurred.

Abscissa - time in days after extraction.

This histogram summarises the incidence of haemorrhage on the days following tooth extraction. Even when bleeding was minimal it was recorded as having occurred on that particular day.


number of
dental operations
14
12
10
8
6
4
2
2
4
6
8
10
12
14
16
time in days

Comparison with the fall in haemoglobin as recorded in Table 33 will show that the bleeding was frequently minimal in its extent. It will be seen that bleeding was rare on the first day but very common on the third day (i.e. 48 hours after extraction). There was some bleeding at this time in 15 out of the 20 operations. Haemorrhage was common up to the eighth day after extraction but unusual after that time. In one patient bleeding stopped only at the sixteenth day. The relative freedom from bleeding during the first 36-48 hours following extraction may be explained by the replacement therapy given just prior to the operation or as a result of the thromboplastic activity of the damaged tissue at the extraction site.

Blood Loss.

In an attempt to estimate the blood loss, haemoglobin levels were made daily and the lowest value recorded in the post-operative period was used as an approximate indication of the extent of haemorrhage. The mean fall in haemoglobin over all the extractions was seven per cent. per tooth removed. As would be expected the loss of haemoglobin was proportional to the number of teeth removed at each operation.

Operation	Mean fall of haemoglobin per operation in percentage.
One tooth extracted	9
Two teeth extracted	12
Three teeth extracted	22
Four teeth extracted	37

As a consequence of haemorrhage blood was occasionally swallowed in sufficient quantity to cause vomiting of coffee-ground material.

Duration of in-patient management.

This varied from 9 to 23 days with an average of 13 days per dental operation.

DISCUSSION

These experiences in dental extraction of haemophiliac patients failed to provide a complete answer to the problems of management. Nevertheless with the adoption of certain measures such haemorrhage as occurs will not be of sufficient severity as to endanger life.

Number of extractions at each operation:

As a consequence of these experiences in the management of the extractions it was decided that generally only one tooth should be extracted at any one operation. Where two adjacent teeth required to be removed it was justifiable to extract these at one operation. Where the teeth were not

adjacent it was inadvisable to extract more than one tooth at a time.

The extraction of more than two teeth resulted in much troublesome haemorrhage. These observations confirm the opinion of White and Mallett (1949). The harmorrhage which follows the extraction of large numbers of teeth carries risk to life and is demoralising to the patient. The teeth extracted were all permanent teeth; deciduous teeth do not present such a great difficulty there being less trauma involved in the extractions. It is our belief that, unless there be special circumstances, deciduous teeth should be left to shed in the normal manner.

Antibiotic Therapy.

As the result of neglect of the teeth sepsis at the site of extraction was frequently present. To combat the additional risk of bleeding which might result from socket infection all the patients were given antibiotic therapy. Initially parenteral penicillin was administered but this resulted in troublesome haematomata at the site of intramuscular injection and was discontinued. Thereafter oral antibiotic therapy was used with chloromycetin or aureomycin.

Acrylic resin splint.

The purpose of the splint was to protect the soft friable clot which fills the socket, from the trauma of food or from

disturbance by the tongue; a less important role of the dental splint was to hold haemostatic dressings in position. The splint was not used for the purpose of exerting pressure on the socket. Such pressure may be dangerous in consequence of haemorrhage occurring into tissue; such a haematoma in the neck can be of sufficient size to block the airway. Pressure on a socket also causes its enlargement and increases the area for potential haemorrhage. It was essential that the splint was made with skill; a badly fitting splint was worse than no splint at all. The splint must fit accurately on the remaining teeth and stay firmly in situ; a loose splint causes disturbance of the poorly formed clot in the socket. The splint must not cause pressure on the gingival margin at any part as such trauma will result in an additional site for haemorrhage. The application of the black gutta percha to the splint at the area of the wound was found to be of value in preventing injury of the gum by the splint. The splint enables the patient to take a soft pulped diet and he can be ambulatory in the ward.

Local haemostatic treatment.

The application of local haemostatic agents to the tooth sockets was of very limited value. No surface application of haemostatic agents can be considered highly successful in haemophilic bleeding. To be of value, a haemostatic requires

to be carried to the site of bleeding by the patient's own circulation. Thrombin, for example, when applied to a socket will produce a surface layer of fibrin from the blood filling a socket but this does not prevent bleeding occurring from the freely flowing vessels in the base of the wound.

Anaesthesia.

Local anaesthesia was eventually adopted as the method of choice. Regional anaesthesia by inferior dental injection carries the risk of haematoma into the neck and of permanent damage to the nerve by haemorrhage. Haemophiliacs not infrequently suffer peripheral nerve damage as a consequence of haemorrhage. Endotracheal intubation during general anaesthesis is potentially dangerous in virtue of the possible development of a laryngeal haematoma.

Replacement Therapy.

The problems of replacement therapy have largely been dealt with in Chapters 21 & 22 . Fresh frozen plasma was used in the management of these patients. It is probable that the administration of the large volumes of fresh frozen plasma immediately prior to the extractions had some haemostatic value at the time of the removal of the teeth. From the studies described in Chapter 22 it is clear that the haemostatic value of the infusion decreases rapidly after

the administration of the plasma so that it is negligible after 24 hours. The difficulties of maintaining haemostatic levels of antihaemophilic globulin are therefore, at the present time, very great indeed. Cohn's plasma fraction I containing antihaemophilic globulin is available as a lyophil dried preparation in ampoules. The material, contained in each ampoule provided by the transfusion services in this region, is derived from 100 ml. of plasma. Even with full recovery of A.H.G. in the ampoules, ten would be required to give the equivalent of one litre of plasma. In one patient the dried antihaemophilic fraction from fifteen pints of blood was given as a preoperative measure with satisfactory response. It is believed that at the present time the best available therapeutic agent is fresh frozen plasma and that this must be used in large quantities such as a litre at a time. Moss (1955) reports on the value of fresh frozen plasma in the management of bleeding tooth sockets in haemophilia.

There can be no reliance on bank blood as a source of antihaemophilic globulin unless it has been collected within a few hours of administration. As has been shown in Chapter 21 the Christmas factor is not only well preserved in fresh frozen plasma, but is relatively well preserved also in bank blood. Bank blood is of value as a means of raising the

haemoglobin, where this has fallen and in some measure for correction of the haemostatic defect in Christmas disease but not in haemophilia.

Occurrence of haemorrhage.

It will be observed from Figure 51 that there is a relative immunity from haemorrhage during the first 36-48 hours following extraction. This may be explained by the replacement therapy given just prior to the operation or as a result of the thromboplastic activity of the damaged tissue at the extraction site.

Blood loss.

The blood loss as estimated by haemoglobin levels is described above. It will be seen that with increasing number of extractions the fall in haemoglobin value was proportionately greater. The numbers involved in each group are small but the figures give some indication of the anticipated loss of blood from these extractions.

Duration of in-patient management.

It will be appreciated that where a complete clearance of teeth was required this involves a protracted stay in hospital. It was our policy to carry out a small number of operations, possibly two, on one hospital admission and discharge the patient for subsequent readmission. The morale of

these patients is remarkably good considering the life of chronic invalidism which they have to suffer. Nevertheless it is of benefit to them not to make their stay in hospital on any one admission too protracted.

SUMMARY

1. The results of twenty-two dental operations for tooth extraction in patients with haemophilia or Christmas disease are reported.

2. The commonest time for haemorrhage was the third day after extraction but sometimes this was still occurring as late as sixteen days after the operation.

3. It was advisable to extract only one tooth at a time; where two teeth requiring extraction were adjacent it was found justifiable to remove both at one operation.

4. Oral antibiotic therapy was used for 10 days from the time of extraction.

5. Replacement therapy consisted of one litre of fresh frozen plasma immediately prior to extraction and on the occurrence of bleeding provided this was of sufficient severity.

6. An acrylic resin dental splint was used to protect the operation site subsequent to extraction.

Table 33

Case	Dental operation	Number of teeth extracted	Details of teeth extracted	Antibiotics	Local treatment. Acrylic splint (except where noted) with	Anaesthesia
J. McI. Haemophilia	1	1	$\overline{6}$ lower 1st molar	Chloromycetin	Calgitex & thrombin Suturing later (no splint)	Regional
A.B. Haemophilia	1	3	$\overline{6\|67}$	Penicillin by injection	Calgitex. Suturing (no splint)	Regional
A.B. Haemophilia	2	2	$\lfloor$46	Penicillin 4 days. Chloromycetin	Gelatin sponge. Thrombin. Suturing.	Infiltration Anaesthesia
	3	1	R.upper molar	Chloromycetin	Gelfoam & thrombin	Infiltration Anaesthesia
	4	1	lower molar	Chloromycetin	-	Infiltration Anaesthesia
	5	4	4 upper incisors.	None	-	Infiltration Anaesthesia
D.B. Haemophilia	1	1	$\overline{6}\rfloor$	None	Gelfoam & thrombin	Infiltration Anaesthesia
	2	1	$\lceil\overline{6}$	None	Gelfoam & thrombin	Infiltration Anaesthesia
J.D. Haemophilia	1	1	L.upper	Aureomycin	-	Infiltration Anaesthesia

Transfusions			Hb Values		Days before bleeding ceased	Days before socket satisfactorily healed
Before	After Day	Amount	Before	Lowest		
2 pints blood	2nd 6th	1 pint blood 3 pints blood	92	80	7	14
None	2nd 5th	4 pints blood 3 pints blood	98	68	6	15
1 pint blood	2nd 3rd	1 pint blood 1 pint blood	98	90	6	14
1 pint blood	3rd 4th 5th	1 pint blood 1 pint blood 1 pint blood	90	84	6	16
1 pint blood	1st 2nd 7th 18th	2 pints blood 1 pint blood ½ pint fresh frozen plasma 2 pints blood	90	58	14	23
1 pint fresh frozen plasma	2nd 3rd 4th 5th 7th 9th	½ pint fresh frozen plasma " 1 " 1½ " 2 " 3 pints blood 3 pints blood	105	68	10	16
None		None	95	89	6	14
None		None	100	86	7	12
1 litre fresh frozen plasma		None	105	102	5	10

J.D. Haemophilia	2	3	R.upper	Aureomycin	-	Infiltration Anaesthesia
	3	2	Upper L. posterior	Aureomycin	-	Infiltration Anaesthesia
	4	1	-	Aureomycin	-	Infiltration Anaesthesia
	5	3	$\lfloor 6, \lfloor 7, \lfloor 8$	Aureomycin	Gelatin sponge & thrombin	Infiltration Anaesthesia
M.M. Christmas disease	1	1	Lower R. first molar	Aureomycin	Calgitex & thrombin	Infiltration Anaesthesia
	2	1	Lower L.	Aureomycin	Calgitex & thrombin	Infiltration Anaesthesia
W.Y. Haemophilia	1	2	$\overline{75}\rfloor$	Oral antibiotics caused vomiting (Penicillin Aureomycin Terramycin)	Gelatin sponge & thrombin & Russel's viper venom	Infiltration Anaesthesia
	2	2	$\overline{84}\rfloor$	-	Gelatin sponge & thrombin	Infiltration Anaesthesia
	3	3	$\lfloor\overline{578}$	-	Gelatin sponge & thrombin	Infiltration Anaesthesia
J.M. Haemophilia	1	1	-	Aureomycin	-	Infiltration Anaesthesia
	2	2	2 lower molars	Aureomycin	-	Infiltration Anaesthesia

Table 33 (contd.)

ion ia	1 litre fresh frozen plasma	5th	1 litre fresh frozen plasma 1 pint blood	104	62	6	16
ion ia	1 litre fresh frozen plasma		None	100	99	3	9
ion ia	1 litre fresh frozen plasma		None	103	94	4	10
ion ia	1 litre fresh frozen plasma		None	96	96	5	10
ion ia	¾ litre fresh frozen plasma		None	74	64	6	13
ion ia	¾ litre fresh frozen plasma		None	65	62	3	13
ion ia	1 litre fresh frozen plasma		None	105	80	6	13
ion ia	1 litre fresh frozen plasma		None	101	80	6	9
ion ia	1 litre fresh frozen plasma		None	85	69	6	10
ion ia	AHG fraction from 15 pints blood		None	101	99	No bleeding	11
ion ia	1 litre fresh frozen plasma		None	99	95	2	13

REFERENCES

Moss, R.L. (1955)
Fresh frozen plasma: use in management of a haemophiliac.
Oral Surg., Oral Med., Oral Path. 8, 27.

White, P.H. and Mallett, S.P. (1949)
Management of haemophilia in dental extractions.
J. Oral Surg. 2, 237.

CHAPTER 21

"IN VITRO" SURVIVAL OF COAGULATION COMPONENTS IN BANK BLOOD AND FRESH FROZEN PLASMA

CONTENTS.

CHAPTER 21

"IN VITRO" SURVIVAL OF COAGULATION COMPONENTS IN BANK BLOOD AND FRESH FROZEN PLASMA

In management of haemorrhage, as a result of disordered blood coagulation, therapy is directed towards the correction of the haemostatic defect. The only available source of supply of missing coagulation components is human blood, especially the plasma and plasma products. In the next chapter a study is made of the "in vivo" survival of coagulation components in particular of antihaemophilic globulin and the Christmas factor in haemophilia or Christmas disease. These studies emphasize the difficulties of successful replacement of these components. In order to obtain the optimal result from replacement therapy it is essential to know the "in vitro" preservation of these components in bank blood, fresh frozen plasma and plasma products. In this chapter this preservation has been studied with particular reference to bank blood and fresh frozen plasma. A few observations have been made on antihaemophilic globulin survival in the dried plasma fraction.

Bank Blood.

The blood loss from haemorrhage due to a coagulation

disturbance will frequently require the administration of bank blood to correct the reduced haemoglobin level.

When it is used for this purpose it is necessary to have an appreciation of the survival of the coagulation components in the bank blood. The factors studied in bank blood were prothrombin, factor V, antihaemophilic globulin, Christmas factor, factor VII platelets and fibrinogen. Blood was collected under the usual conditions for donor blood for transfusion purposes (see appendix page 122). This blood was kept in a refrigerator at 4° C. which is the standard condition of storage. On each day on which an assay of a component was made a specimen of fresh normal blood was collected in the same anticoagulant and proportions as for the bank blood. This was used as the standard containing 100% for comparison with the stored blood. The blood from the bottle was obtained by piercing the rubber diaphragm on the top of the bottle with a needle and extracting a specimen of blood with a syringe. The needle and syringe were previously sterilized using dry heat (160° C. for not less than one hour).

The details of the assay techniques for each of these components are given in the appendix where in addition are described the full results. In figures 136 - 143 the results are given as the mean of the observations on, at

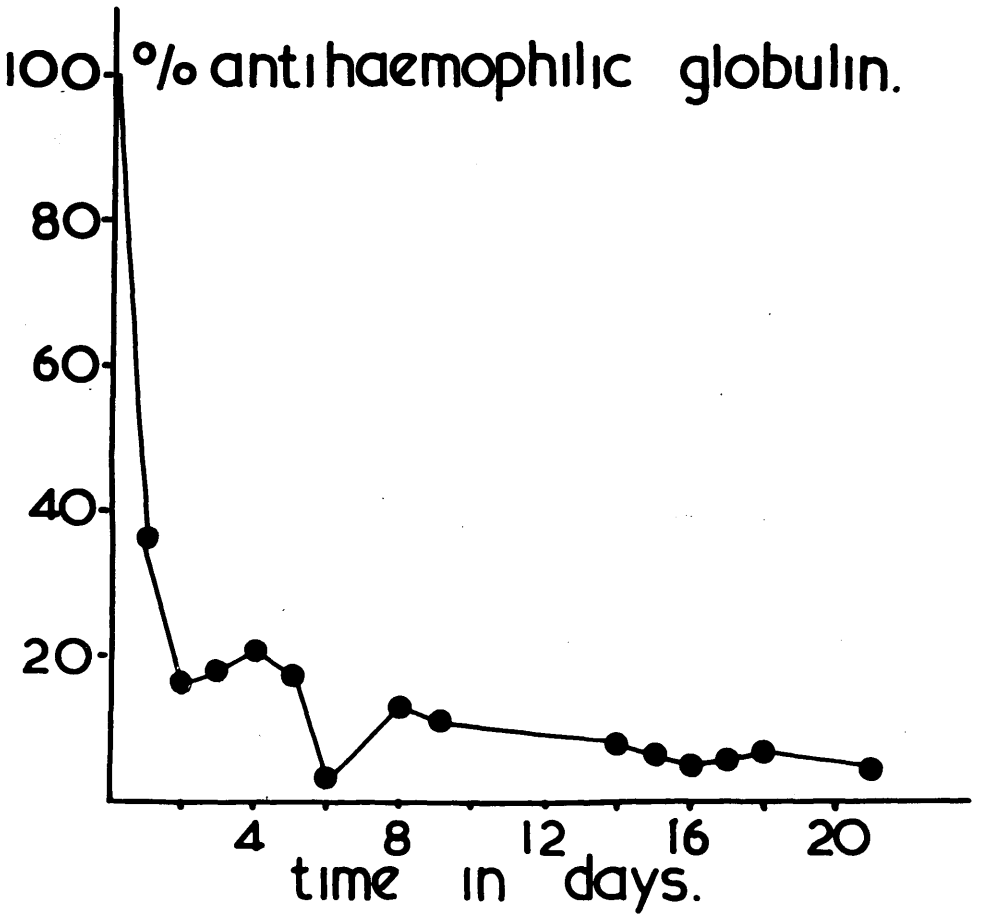
Figure (136)

Figure (illegible)

Antihaemophilic globulin survival in bank blood.

Ordinate - percentage antihaemophilic globulin.

Abscissa - time in days after collection of blood.


100
% anti haemophilic globulin.
80
60
40
20
4
8
12
16
20
time in days.

least, five bottles of blood maintained over a period of 21 days which is the standard time for storage of blood for transfusion purposes.

A small number of observations were made by removing samples of plasma from the bottles daily and storing them at -20° C. in a deep freeze. These specimens were then assayed in one experiment. It was assumed that the decay of the coagulation components in the bank blood was halted by transfer to the -20° C. deep freezing cabinet.

The factors were found to disappear at varying rates. The anti-haemophilic globulin decayed most rapidly and the prothrombin was the most stable. The order of disappearance of the plasma thromboplastin components was - A.H.G., factor V, factor VII, Christmas factor and platelets. The prothrombin was more stable than any of these. The fibrinogen was reduced by about two-fifths of its original concentration over the 21 day period.

Antihaemophilic globulin:

The results of the assays on this are shown in figure 136. The A.H.G. disappears with great rapidity so that blood which is more than 36 hours old is of no significant value as a source of A.H.G. The rate of loss is such that blood, which even a few hours old, may have a significantly lower level of A.H.G. than blood which is freshly drawn.

Figure (137)

Antihaemophilic globulin survival in bank blood - failure to correct deficient prothrombin consumption of recalcified haemophilic plasma.

Ordinate - clotting time of fibrinogen in seconds.

Abscissa - time in minutes after recalcification.

The prothrombin consumption of haemophilic plasma has been followed over one hour. Similar specimens have been studied for prothrombin consumption after additions of plasma from 21 day old bank blood and of fresh normal plasma.

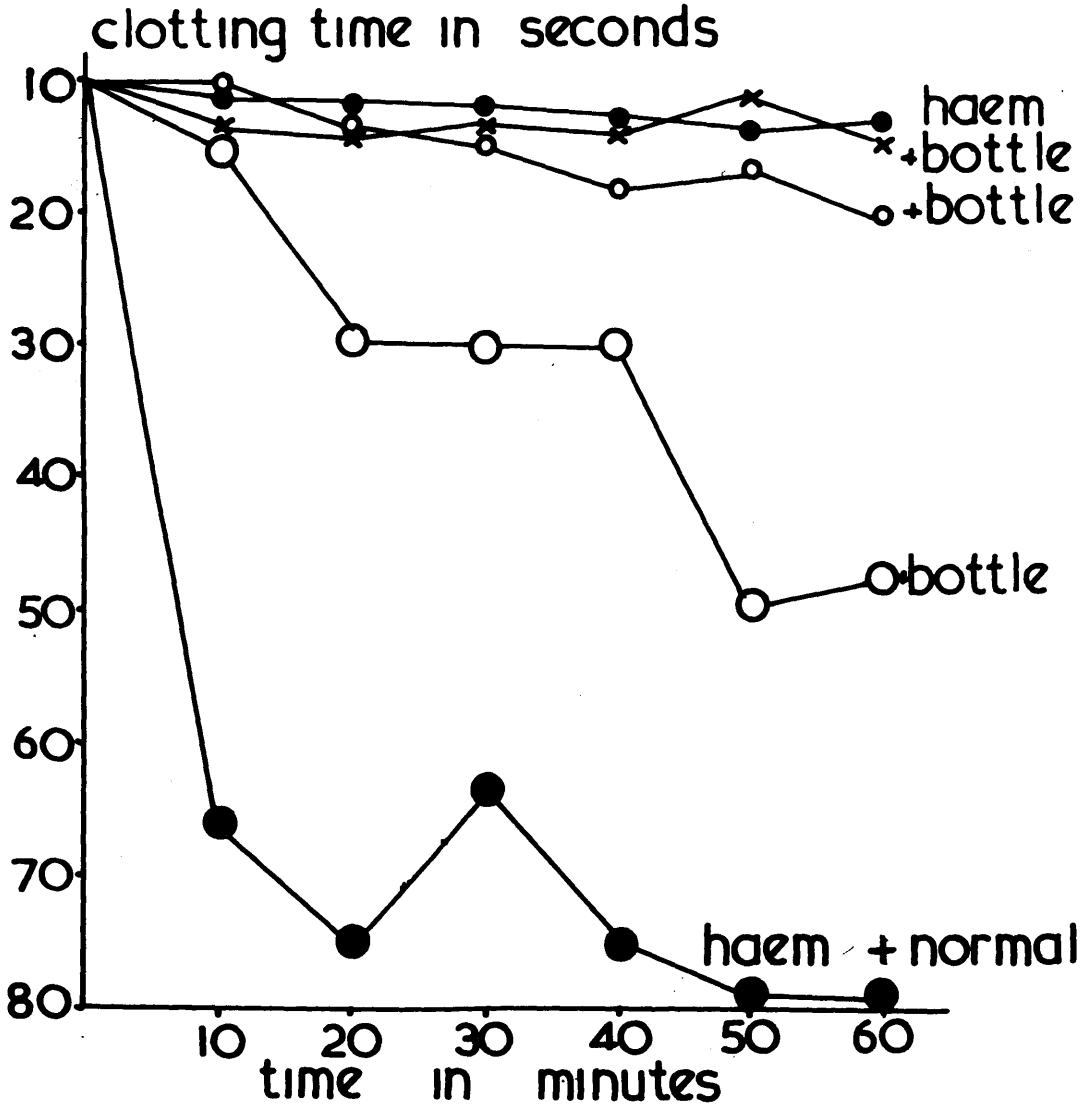
●——● haemophilic alone.

o——o haemophilic+ plasma from bottle.

x——x haemophilic+ plasma from bottle.

O——O haemophilic + plasma from bottle.

●——● haemophilic+ fresh normal.


clotting time in seconds
10
20
30
40
50
60
70
80
haem
+bottle
+bottle
bottle
haem + normal
10
20
30
40
50
60
time in minutes

It is unfortunate that this is the one component of all the clotting factors which in practice is most frequently required for purposes of replacement and it disappears with the greatest rapidity.

The assay of the A.H.G. is made on alumina-adsorbed plasma, this adsorption removing any platelets. By experiments to be described elsewhere, I have shown that platelets, prepared from low spun normal plasma which has stood overnight at 4° C., have developed considerable A.H.G. activity. In a limited number of observations it has been shown that the loss of A.H.G. from high spun plasma is almost as rapid as from low spun plasma. Pitney (1955) in a personal communication has also found this.

In order to confirm the A.H.G. deterioration of bank blood by a separate technique, its ability to correct the defective prothrombin consumption of haemophilic plasma was tested. In figure /37 the results of such an experiment are shown. This was carried out in blood from three bottles at the end of the 21 days of storage. Plasma from two of the bottles produced no correction of the rate of prothrombin consumption and the third bottle produced much less than that from fresh normal plasma. Serial estimations on the A.H.G. content of the bottles by a prothrombin consumption technique gave results essentially similar to those by the

Figure (138)

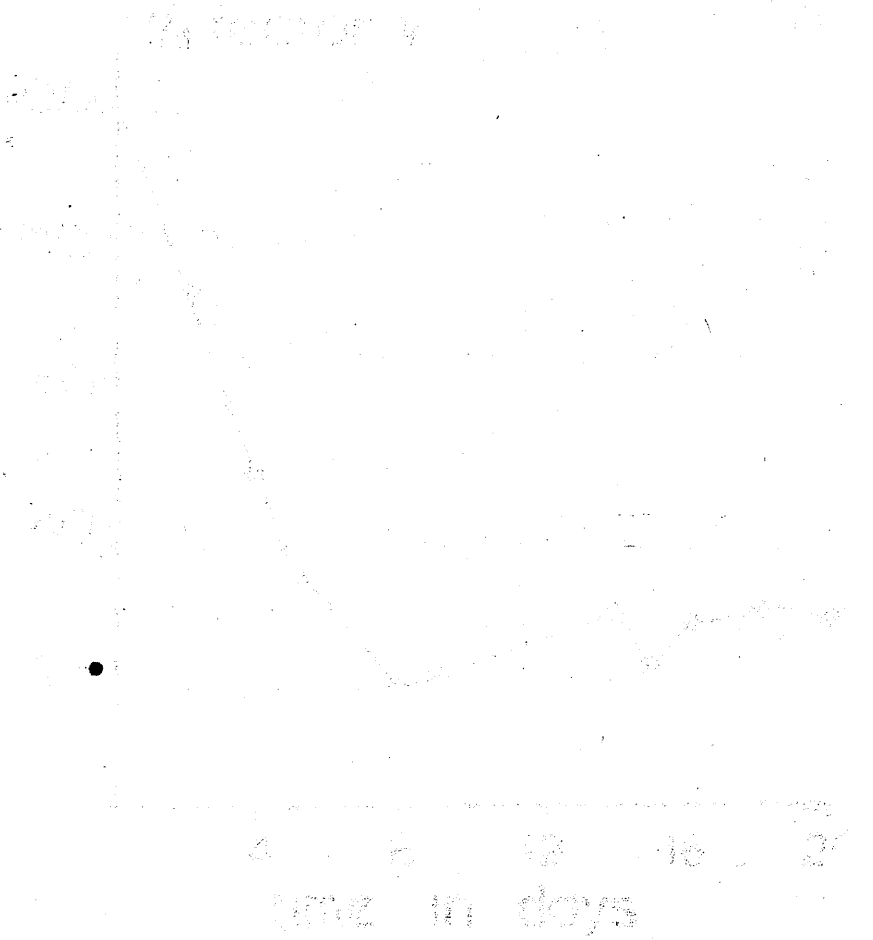


Figure (LLC)

Factor V survival in bank blood.

Ordinate - percentage factor V.

Abscissa - time in days after collection of blood.

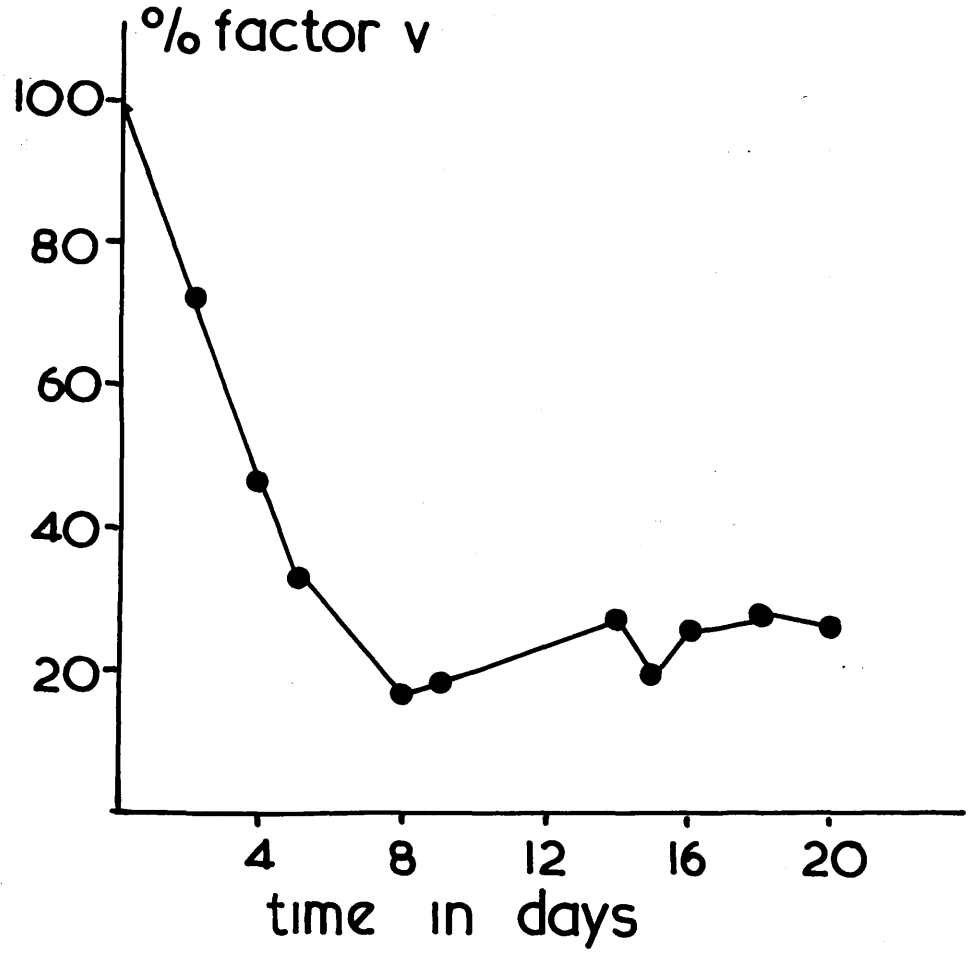

% factor v
100
80
60
40
20
4
8
12
16
20
time in days

Figure (139)

Figure (139)

Prothrombin survival in bank blood.

Ordinate - percentage prothrombin.

Abscissa - time in days after collection of blood.

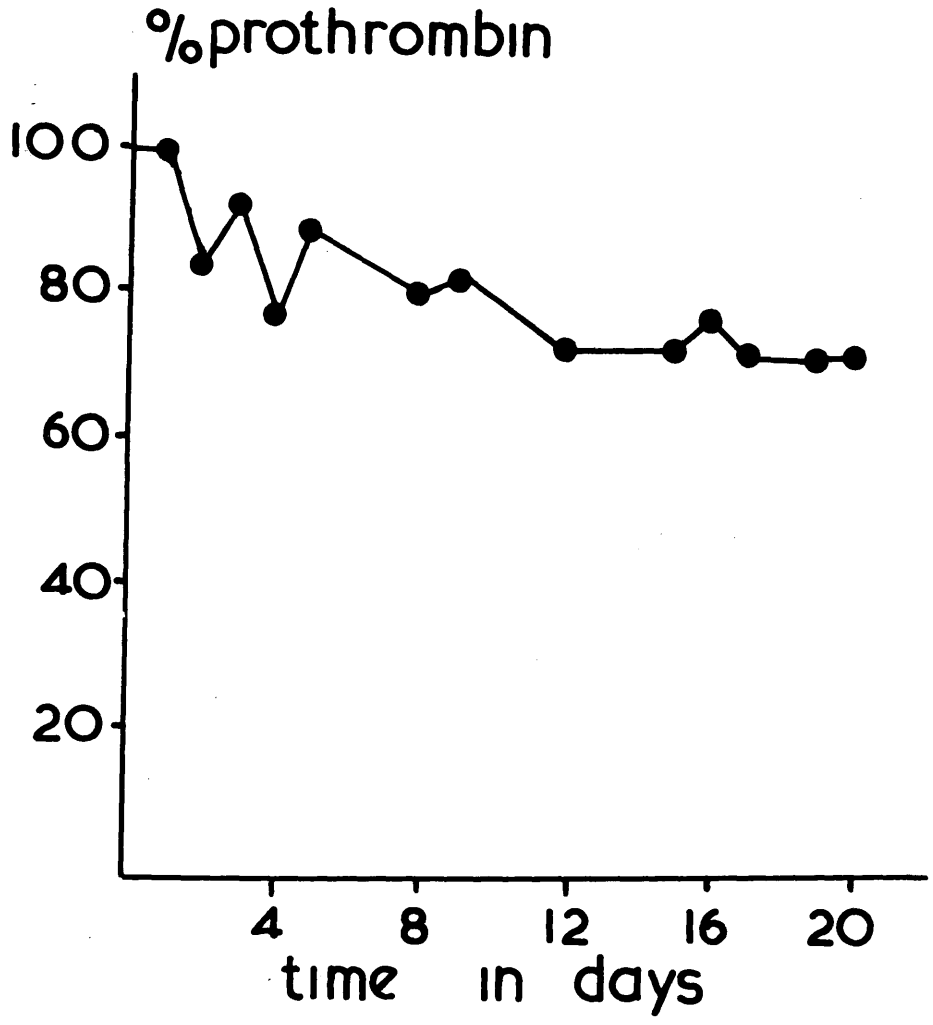

%prothrombin
100
80
60
40
20
4
8
12
16
20
time in days

Figure (140)

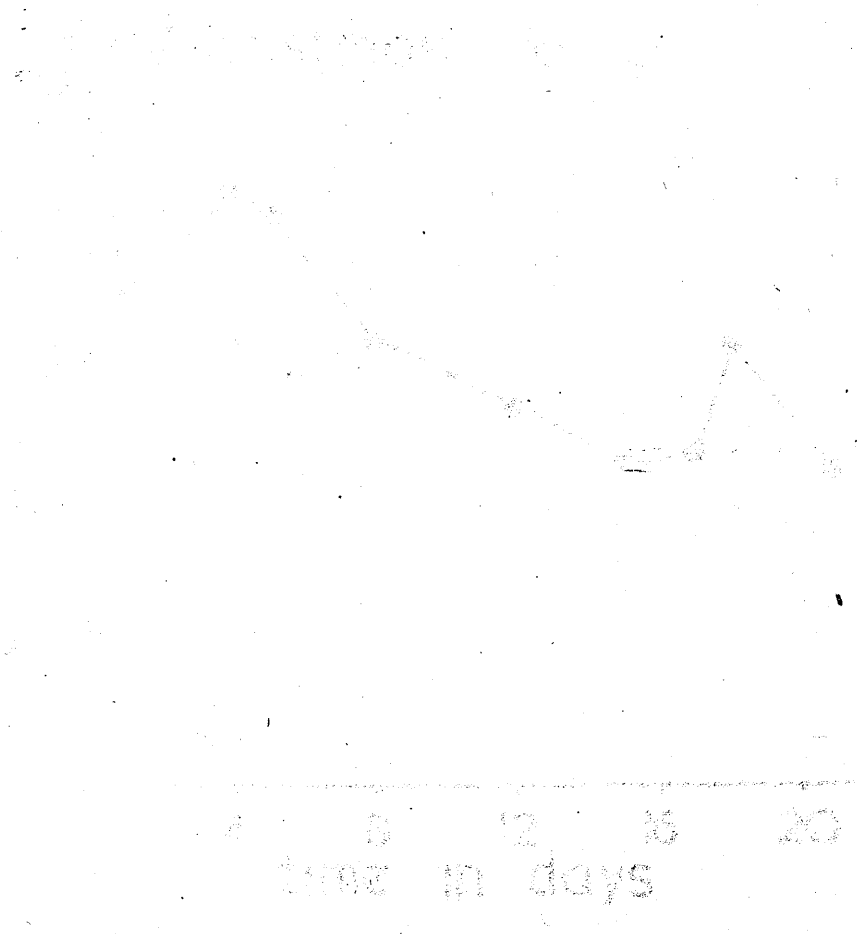


Figure (1.c)

Christmas factor survival in bank blood.

Ordinate - percentage Christmas factor.

Abscissa - time in days after collection of blood.

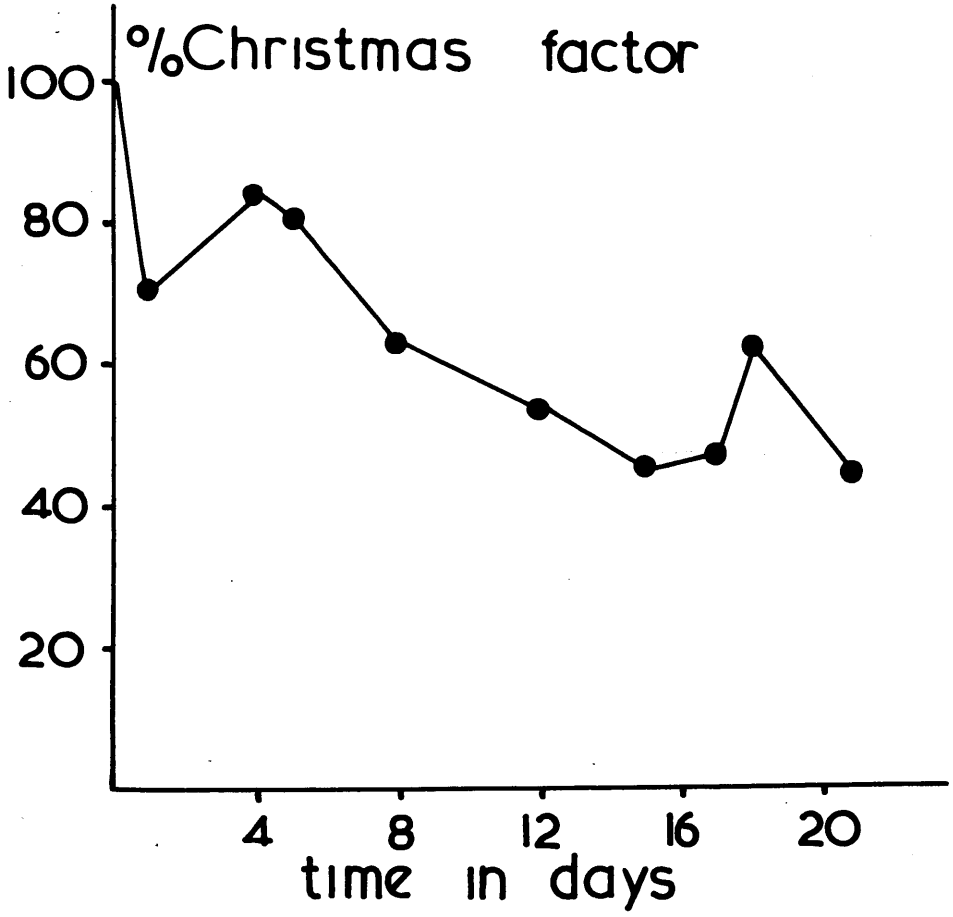

%Christmas factor
100
80
60
40
20
4
8
12
16
20
time in days

Figure (141)

Figure (III)

Factor VII survival in bank blood.

Ordinate - percentage factor VII.

Abscissa - time in days after collection of blood.

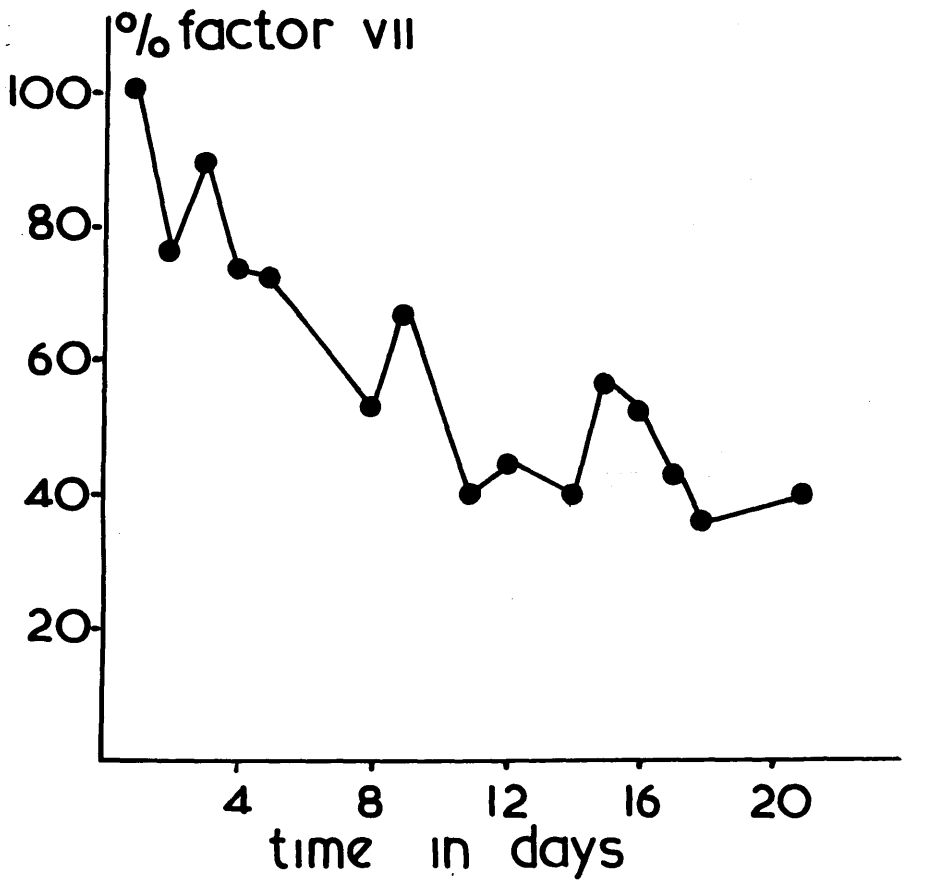
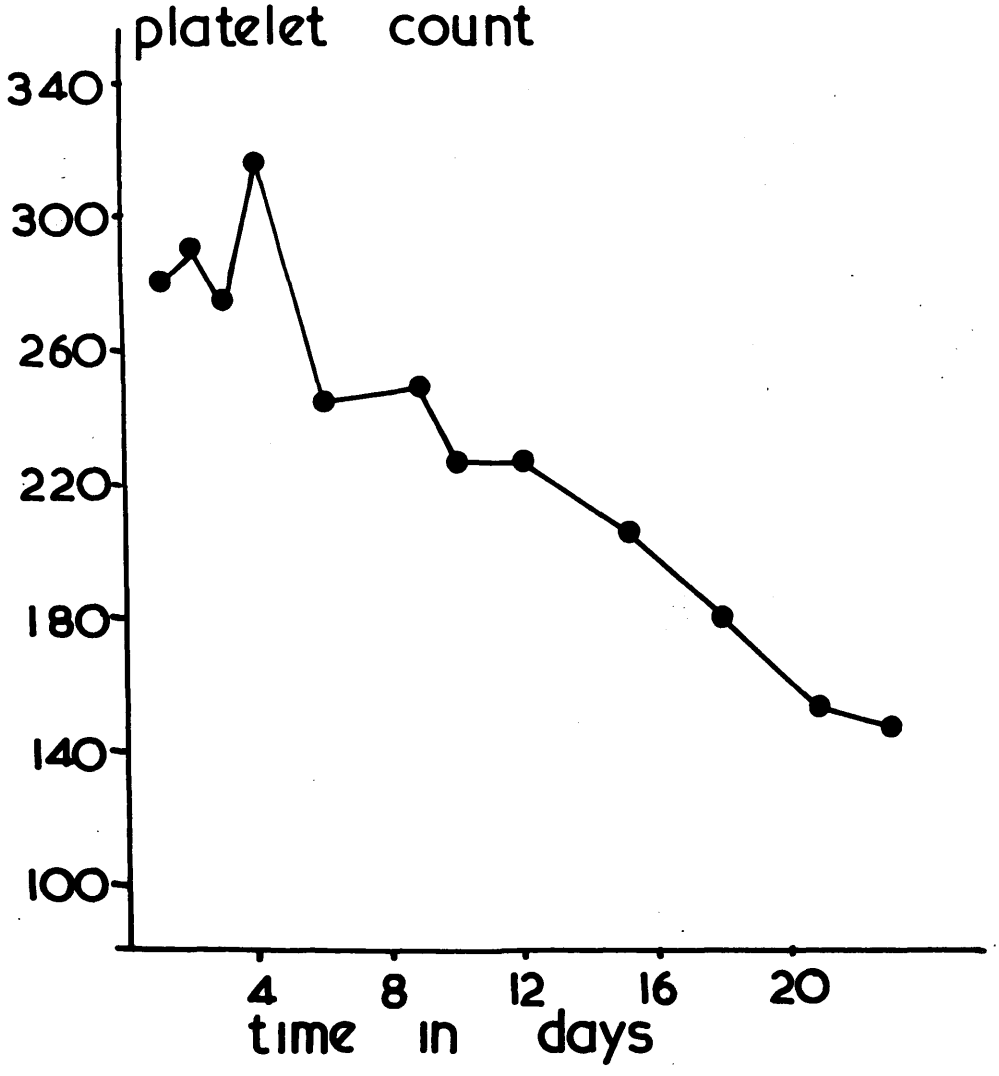

% factor VII
100
80
60
40
20
4
8
12
16
20
time in days

Figure (142)

Platelet survival in bank blood.

Ordinate - platelet count.

Abscissa - time in days after collection of blood.


platelet count
340
300
260
220
180
140
100
4
8
12
16
20
time in days

thromboplastin generation method (see page 1145 of appendix).

Factor V:

After A.H.G. this is the plasma factor which disappears most rapidly. This can be seen in Figure 138. Factor V deficiency is so seldom encountered in clinical practice that therapeutic replacement is only rarely required.

Prothrombin, factor VII and Christmas factor.

From figures 139, 140 and 141 it will be seen that these factors are much more stable than either A.H.G. or factor V. The prothrombin is very stable but the factor VII and Christmas factor are not quite so well preserved losing about half their concentration over the 21 day period.

Since these components are relatively stable, bank blood is of value for the correction of the haemostatic abnormality in coumarin drug therapy or in Christmas disease.

Platelets.

There is a gradual loss of platelets on storage of bank blood, to about 50% of the original number over the period of three weeks - see figure 142. This is in agreement with the findings of Mustard (1956). Bell, on the other hand, reports much more rapid loss of platelets from bank blood (Bell 1953). The platelets which remain at the end of the 21 day period are still capable of forming blood thromboplastin

Figure (143)

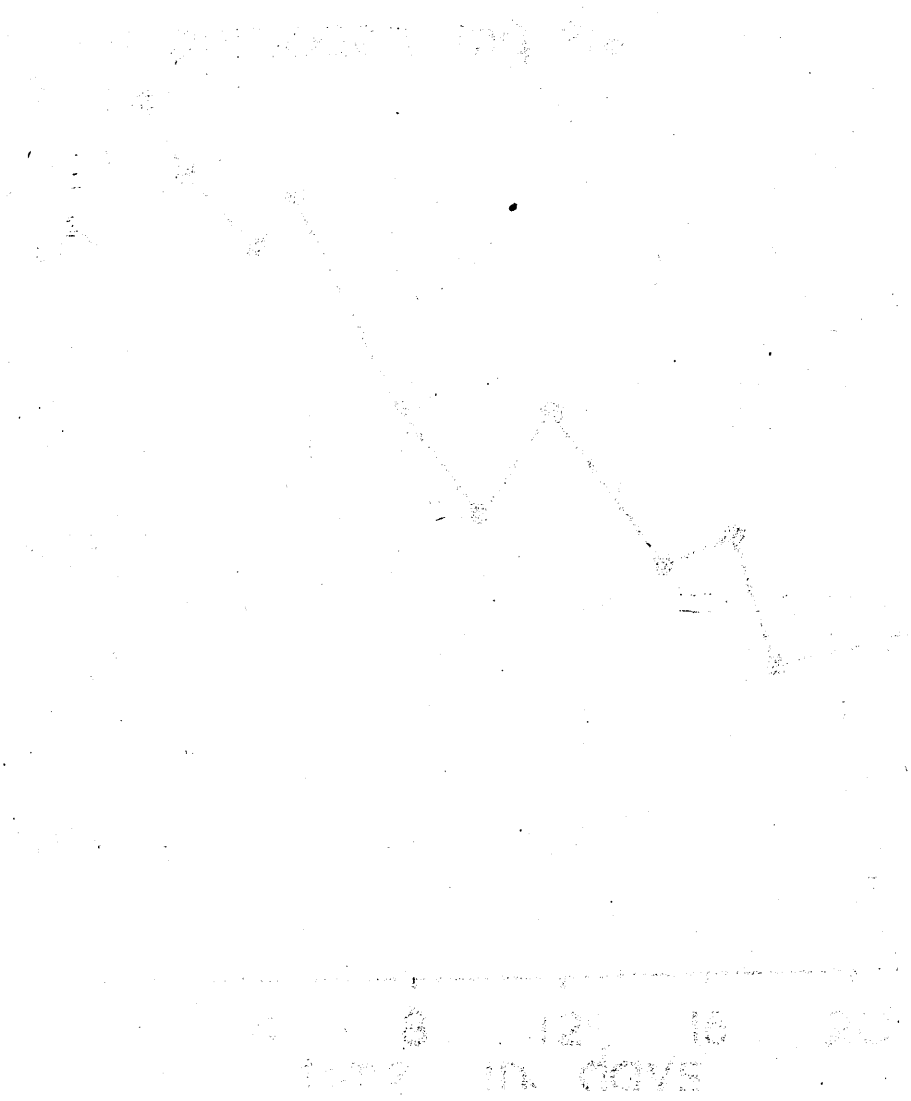
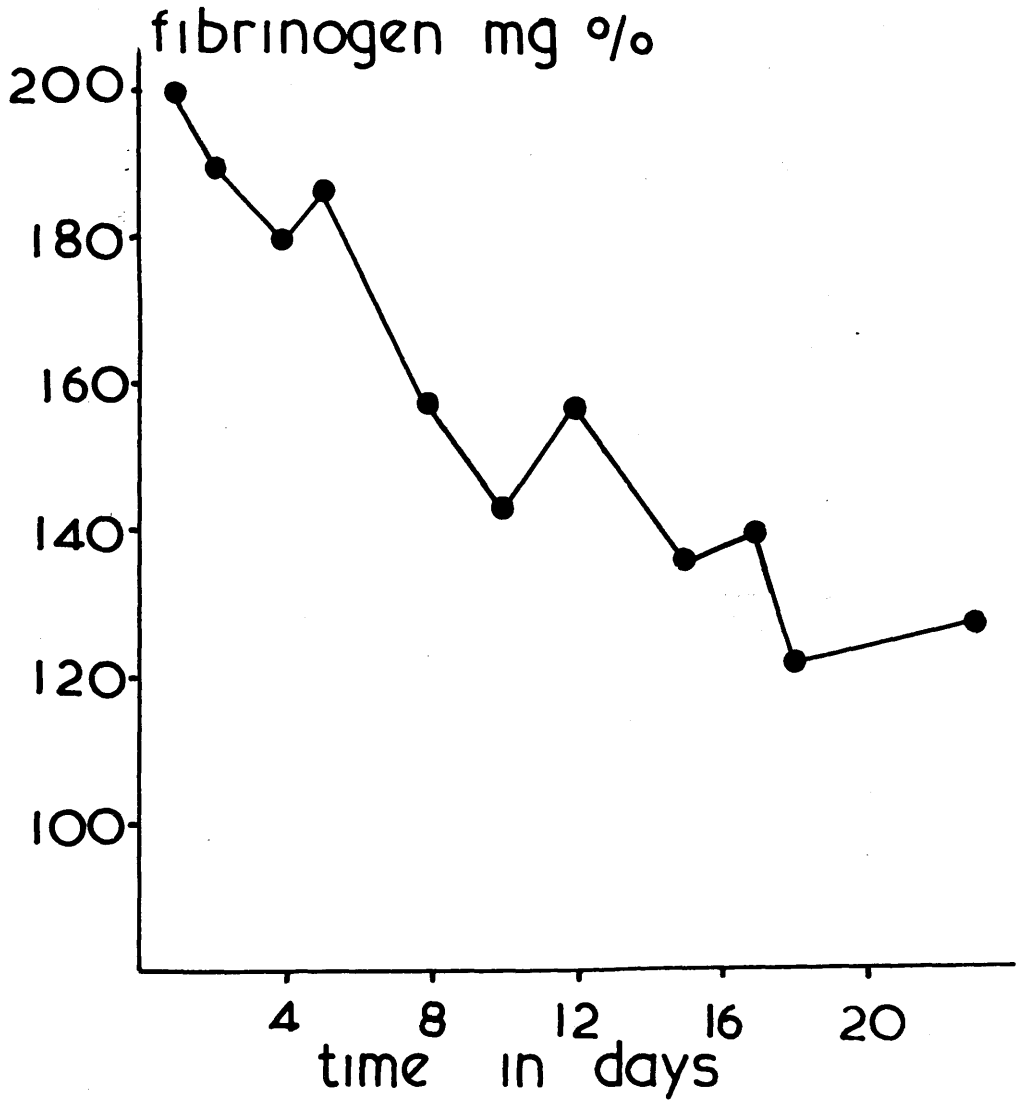


Figure (142)

Fibrinogen survival in bank blood.

Ordinate - fibrinogen in mg. %

Abscissa - time in days after collection of blood.


fibrinogen mg %
200
180
160
140
120
100
4
8
12
16
20
time in days

(see appendix page 1126). Whether these platelets will still function normally "in vivo" is, however, problematical.

Fibrinogen.

Approximately three fifths of the original concentration of fibrinogen remain at the end of the three weeks' storage period (see Figure 143). This information is of value in relation to the management of the acute fibrinogen deficiencies of pregnancy.

Discussion.

Large volumes of bank blood may be required in the management of haemorrhage, for example in the bleeding from a duodenal ulcer. Such patients at the start of haemorrhage are likely to have a normal haemostatic mechanism. It is a theoretical possibility that the administration of considerable volumes of bank blood deficient in A.H.G. and factor V could cause a temporary coagulation defect. In a limited study of this theoretical possibility no such abnormality has been detected (see page 1241 of the appendix). It is likely that the normal person is capable of producing large quantities of these components very rapidly.

Fresh Frozen Plasma:

The principle of the fresh frozen plasma is that of preservation of the coagulation components, when the plasma

is maintained at-20° C. from the time of collection. The investigations described here were designed to test the validity of this. The plasma was prepared by the Glasgow and West of Scotland Blood Transfusion Service. The emergency reserve of this plasma is taken from donors of group A or AB. Donors with a high titre anti-B agglutinin or with anti-B haemolysin were excluded. 400 ml. of blood were collected into the 100 ml. of preservative anticoagulant solution. Only donors giving blood at the Regional Transfusion Centre were used so that the plasma could be separated immediately and frozen. Centrifugation of the blood was carried out - at 1800 r.p.m. for 30 minutes. The plasma was separated with aseptic and antiseptic precautions and stored frozen at -20°C.

Three bottles of plasma were kept frozen at -20° C. for six months. Each bottle contained the plasma from one pint of blood collected under the conditions mentioned above - these being the standard conditions for the supply of fresh frozen plasma in this region. The time of six months far exceeds the turn-over time of this product in the transfusion service.

Antihaemophilic globulin:

The assay on two of the bottles gave levels of A.H.G. as good as the fresh normal whereas the third bottle was at least 50% of normal.

Factor V.

There was no deterioration of this in the fresh frozen plasma. The factor V assay was as good as the fresh normal.

Prothrombin, factor VII, Christmas factor:

These were all well preserved there being no appreciable deterioration at the end of the six months.

Platelets:

Some platelets remained in the plasma after the centrifugation involved in the preparation. After being maintained frozen for six months, the platelets and platelet fragments collected by high speed centrifugation of the plasma were still capable of acting as the platelet component of thromboplastin (Experiment p9 (20)-appendix).

Lyophil Dried Fibrinogen Fraction.

Survival of antihaemophilic globulin. The plasma fractionation section of the Edinburgh and East of Scotland Blood Transfusion Service provided me with lyophil dried fibrinogen fraction which had been stored for varying lengths of time at 4° C. Specimens stored up to two years were assayed and the results suggest that there was almost complete loss over that two year period. Any final conclusions, however, are difficult to make as the A.H.G. content of each

of the ampoules was not known at the time of preparation.

The results are shown in the appendix.

SUMMARY

(1) The preservation of coagulation components has been studied in bank blood and fresh frozen plasma.

(2) Bank Blood. A.H.G. concentration falls very rapidly over the first few days of storage so that after 36 hrs. the blood has no appreciable value as a therapeutic source of A.H.G. Factor V is also very labile on storage though it does not disappear quite so rapidly as the A.H.G. Factor VII and the Christmas factor are relatively stable losing only 50% of their concentration during the storage period. Prothrombin is even more stable losing only 25% of its original concentration. Platelets disappear gradually over the 21 days being about half as numerous at the end of the period as they were at the beginning. The fibrinogen concentration also deteriorates by about a half.

(3) Fresh frozen plasma. Prothrombin and all the plasma thromboplastin components are well preserved.

REFERENCES

Bell, W.N. (1953).
The clinical use of a coagulogram.
Med. clin. N. Amer. 37, 1843.

Mustard, J.F. (1956).
Platelets in stored blood.
Brit. J. Haemat. 2, 17.

Pitney, W.R. (1955)
Personal communication.

CHAPTER 22

"IN VIVO" SURVIVAL OF ANTIHAEMOPHILIC GLOBULIN AND THE CHRISTMAS FACTOR.

ANTIHAEMOPHILIC GLOBULIN SURVIVAL IN HAEMOPHILIACS.

Preparation of plasma.

Administration of plasma.

Collection of specimens.

Results.

Antihaemophilic globulin assay

Whole blood clotting times

Prothrombin consumption

Thrombin generation

Estimation of the haemostatic level of antihaemophilic globulin.

CHRISTMAS FACTOR SURVIVAL IN CHRISTMAS DISEASE.

CHAPTER 22

"IN VIVO" SURVIVAL OF ANTIHAEMOPHILIC GLOBULIN AND THE CHRISTMAS FACTOR

It was the purpose of the investigations described in this chapter to assay the antihaemophilic globulin (A.H.G.) in haemophilia and the Christmas factor (C.F.) in Christmas disease following plasma infusions given to patients suffering from these conditions. In the practical management of haemorrhage in haemophilia or Christmas disease it is of value to appreciate the results of plasma infusions on the A.H.G. or C.F. level of the patient's blood and the duration of this effect. It is also of importance to have a correlation between the A.H.G. level and the haemostatic efficiency. It was the purpose of the observations described here to study these aspects in patients with haemophilia or Christmas disease, following the administration of plasma.

ANTIHAEMOPHILIC GLOBULIN SURVIVAL IN HAEMOPHILIACS.

The therapeutic substances which are available for the management of the haemostatic defect in haemophilia are whole blood, fresh frozen plasma and the lyophil-dried plasma fraction I (Cohn). As a source of supply of A.H.G. whole blood is unsatisfactory. The blood requires to be fresh

because assay of the A.H.G. concentration of stored blood reveals that there are only trace amounts left after 36 hours (see Chapter 21). Furthermore, haemophiliacs are often the recipients of multiple transfusions, and there is the danger of haemolytic transfusion reactions unless sensitive compatability tests are employed; this may be impossible in an emergency. Fresh frozen plasma is readily prepared; blood is collected as for transfusion purposes, the red cells separated, and the plasma kept at -20°C. Under these circumstances the A.H.G. survives in almost normal concentration up to six months. (see Chapter 21). The lyophil dried A.H.G. fraction is relatively concentrated but is expensive to prepare, and has no clear advantage over the fresh frozen plasma which is simpler to prepare. In this study the A.H.G. was administered as plasma, generally fresh frozen plasma.

The improvement in the clotting efficiency of haemophilic blood in consequence of the therapeutic administration of plasma or plasma fractions can be assessed by a variety of *in vitro* techniques. In the past these have included the whole blood clotting time, the prothrombin consumption test (Graham *et al.*, 1951), the thrombin generation test (Pitney & Dacie, 1953) and the partial thromboplastin time test (Langdell *et al.*, 1954). The whole blood clotting time and the prothrombin consumption test are insensitive indices of

clotting efficiency, because small amounts of antihaemophilic globulin will produce disproportionately large correction (Langdell et al., 1954). The thrombin generation test and the partial thromboplastin time tests are more sensitive as they are not corrected until 12-15% of normal plasma has been added to haemophilic plasma in the test systems. (Fantl & Sawers, 1954: Langdell et al., 1954). The most sensitive available assay procedure of antihaemophilic globulin activity is based on the thromboplastin generation test of Biggs and Douglas (1953). This technique has been used already in the observations described in the previous chapter and is given in detail in the appendix. The assessment of the haemostatic level of A.H.G. is more difficult. An attempt has been made to correlate the level of antihaemophilic globulin with the occurrence of haemorrhage following tooth extraction and the cessation of haematuria in haemophilic patients.

Preparation of Plasma:- The plasma was prepared by the Glasgow and West of Scotland Blood Transfusion Service. For each observation blood was collected from four donors. Generally donors of the same blood group as the recipient were used for the preparation of the fresh frozen plasma, but for an emergency reserve donors of group A or AB were used. Donors with a high titre anti-B agglutinin or with anti-B

haemolysin were excluded. The preservative - anticoagulant solution used was:

Disodium hydrogen citrate		2 gms.
Dextrose		3 gms.
Water	to	100 ml.

440 ml. of blood were collected into the 100 ml. of the preservative anticoagulant solution.

Only donors giving blood at the Regional Transfusion Centre were used so that the plasma could be separated immediately and frozen. Centrifugation of the blood was carried out at 1800 r.p.m. for 30 minutes. The plasma was separated with aseptic and antiseptic precautions and stored frozen at -20° C.

In observation No. 2 the plasma infused had not been frozen. It was collected as described above and administered as soon as collected.

Administration of Plasma:- The plasma was thawed immediately before use by standing the bottle in a basin of warm water; thawing at room temperature requires some hours to be complete, and was avoided as such delay might result in fall of A.H.G. concentration. The plasma was given to the haemophiliac by moderately rapid intravenous infusion, the time taken to administer varying from 45 to 60 minutes.

The plasma was administered to haemophilic patients

generally for therapeutic purposes, for haematuria, haematoma formation or for tooth extraction. In one observation there was no known site of haemorrhage.

Collection of Specimens:- Blood was collected by clean venepuncture from the patients before the administration of the plasma, immediately after, and at 6, 12, 24, 48 and 72 hours from the time of starting the infusion. Standard sized 20 ml. syringes and wide bore needles of 18 S.W. gauge were used. In order to avoid frothing of the blood the syringes were allowed to fill without traction being applied to the plunger. Before delivery of the blood to the tubes the needle was removed. Using a graduated centrifuge tube 9 ml. of blood were mixed with 1 ml. of 3.8% sodium citrate. These specimens were used for the assay of anti-haemophilic globulin by the thromboplastin generation test. The specimens were immediately centrifuged at 20,000 revs. for 5 minutes in a high-speed centrifuge attachment and the plasma separated and stored at -20° C. until all the specimens had been collected. The antihaemophilic globulin content was then assayed in these specimens in one experiment.

In three of the observations the whole blood clotting time was estimated on specimens collected simultaneously with those used for the assay of antihaemophilic globulin. The whole blood clotting time was performed by the technique

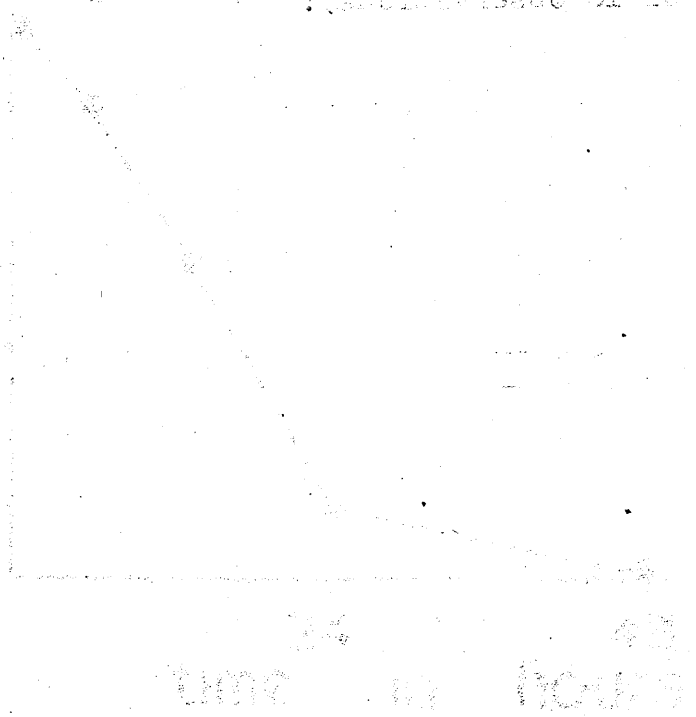
(2) described in the appendix.

Results.

Twenty-three observations were made on 8 haemophiliacs (see page 1212 of the appendix). The first thirteen of these were regarded as trial observations. From them it was determined the times at which specimens were required in order to follow the survival of the A.H.G. Subsequently ten observations were made. The specimens were collected before, immediately after, 6 hrs. 12 hrs., 24 hrs., 48 hrs. and 72 hrs. later. All but one of the observations were on adult haemophiliacs who were given almost a litre of plasma. The one exception was one of the trial observations on a boy of 10 years who was given half a litre of plasma (page 1217 page of the appendix). The time taken to administer the plasma varied from 45 to 60 minutes.

Antihaemophilic globulin assay:- The A.H.G. level in each of the 10 observations is shown in table 34 . The results are expressed as a percentage of the A.H.G. content of normal plasma. The normal plasma is said to contain 100 per cent A.H.G.; the haemophilic plasma before the infusion is assumed not to contain any A.H.G. By making dilutions of the normal in the haemophilic plasma a series of specimens were obtained containing known amounts of A.H.G. The

Figure (144)



"In vivo" antihaemophilic globulin (A.H.G.) survival.

Ordinate - percentage antihaemophilic globulin.

Abscissa - time in hours after administration of plasma.

●——● - the graph of A.H.G. disappearance obtained by assays at the time intervals as shown. (Mean of 10 observations).

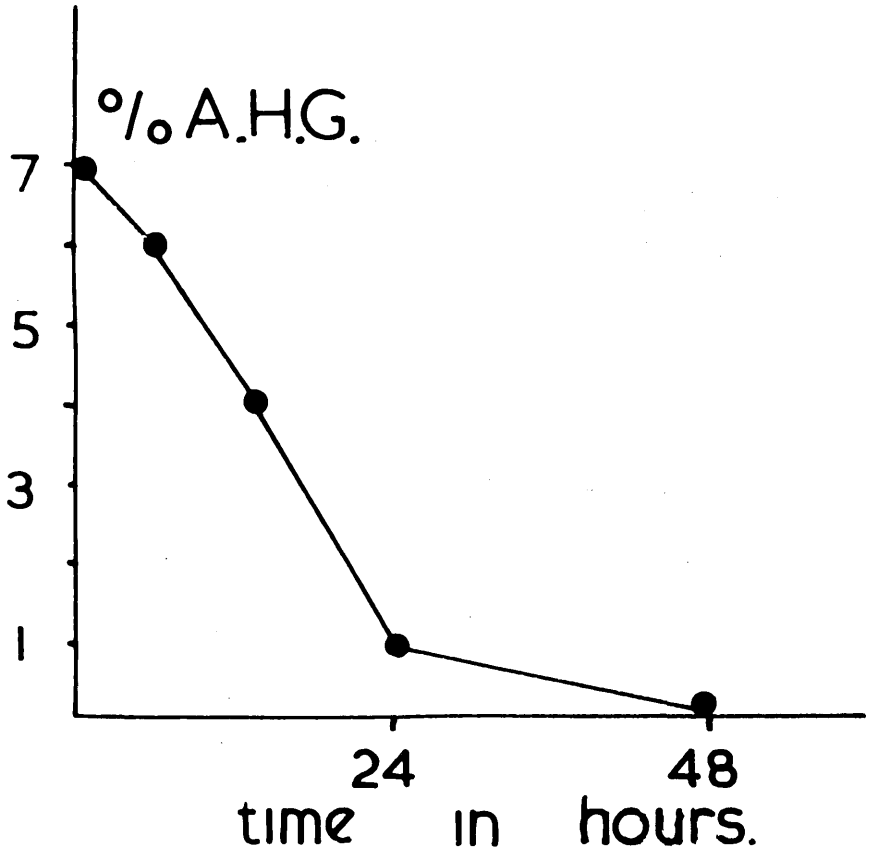

% A.H.G.
7
5
3
1
24
48
time in hours.

Figure (145)

Figure (145)

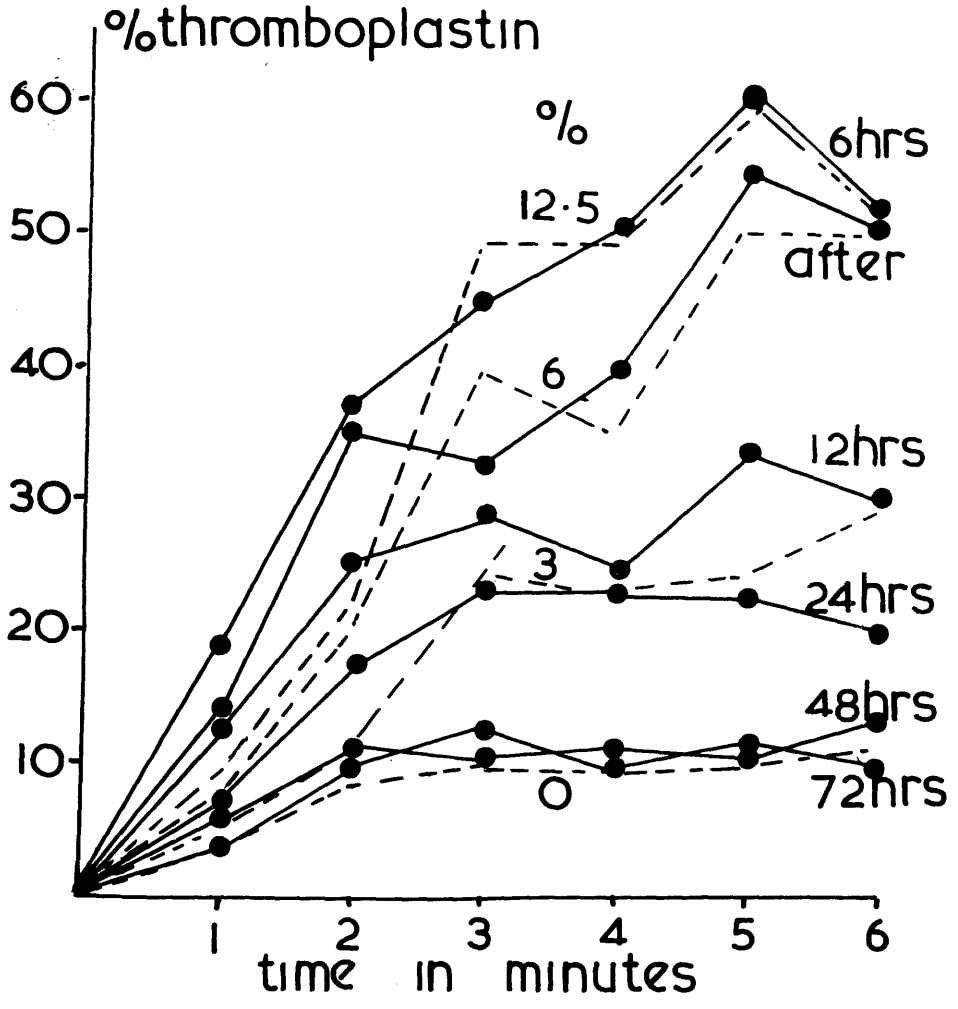
Thromboplastin generation technique from a series of adsorbed plasmas collected after the administration of a litre of plasma to a haemophiliac.

Ordinate - percentage of thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation technique with normal serum and platelets constant.

The adsorbed plasmas from the specimens collected at the intervals specified, are shown as continuous lines. The discontinuous lines represent the dilutions of adsorbed normal in adsorbed haemophilic plasma.


%thromboplastin
60
50
40
30
20
10
%
12·5
6
3
0
6hrs
after
12hrs
24hrs
48hrs
72hrs
1
2
3
4
5
6
time in minutes

thromboplastin generation technique was applied to those specimens with known A.H.G. content and to the test specimen; the assay was made by comparison of the results on the test plasma with those of known A.H.G. content. Figure 145 illustrates an example of one observation. It shows the pattern of thromboplastin generation from the specimens collected before starting the infusion (containing 0 per cent A.H.G.) and from those specimens collected at intervals after the infusion. The figure shows also the thromboplastin generation from the specimen containing 12.5 per cent A.H.G. obtained by mixing normal plasma with the haemophilic plasma. Many mixtures were required in the range from 15 per cent A.H.G. to 0 per cent A.H.G. in order to obtain sufficient curves for the assays to be made; for the sake of the clarity of the diagram all these curves of thromboplastin generation cannot be illustrated. Figure 146 illustrates a series of curves obtained by making mixtures of the adsorbed normal plasma in the adsorbed haemophilic plasma.

In some of the later observations the technique was modified in so far as only the six minute point was recorded.

Figure (146)

Series of curves of thromboplastin generation from mixtures of adsorbed normal plasma and adsorbed haemophilic plasma.

Ordinate - percentage of thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation technique with normal serum and platelets constant. The adsorbed plasma consisted of doubling dilutions of normal in haemophilic.

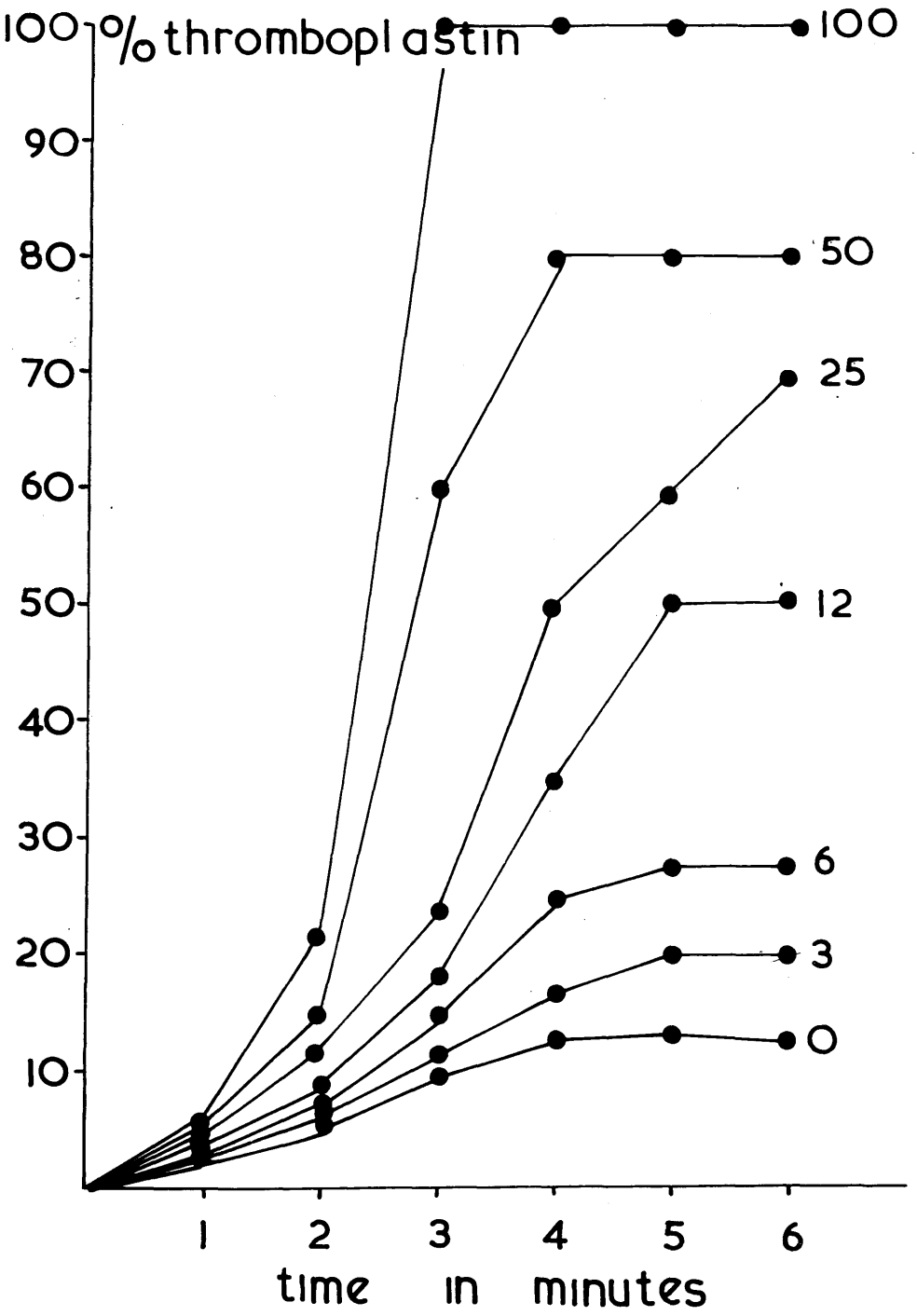

100
% thromboplastin
90
80
70
60
50
40
30
20
10
100
50
25
12
6
3
0
1
2
3
4
5
6
time in minutes

TABLE 34

Antihaemophilic globulin (A.H.G.) assay percentage as compared with normal plasma									
(Observations 14-23 of series - see page of appendix	Immediately after	6 hrs.	12 hrs.	24 hrs.	48 hrs.	72 hrs.	Volume of plasma administered.	Time taken to administer in minutes	Details of patients
1 J.D.	7	6	5	1	0	0	980	50	J.D. was a long clotting time haemophiliac aged 23 - clotting time by method (2) was generally over 2 hrs.
2 J.D.	12	8	5	3	0	0	990	45	
3 J.D.	6	5	2	2	0	0	950	55	
4 J.D.	6	4	1	4	0	0	950	55	
5 J.D.	1	2	2	1	0	0	940	50	
6 W.Y.	12	8	8	2	0	0	970	50	W.Y. was a short clotting time haemophiliac aged 39 - clotting time 14-18 minutes
7 W.Y.	5	10	8	3	0	0	1030	50	
8 M.M.	12	12	5	4	0	0	980	55	M.M. & J.M. were brothers - long clotting time haemophiliacs aged 20 and 32 - clotting time generally over 2 hours.
9 J.M.	6	6	3	0	0	0	995	45	
10 J.M.	3	2	1	0	0	0	1025	60	

The mean of the results on the specimens collected at the specified times is shown in Figure 144. In some of the observations the specimens collected six hours after the completion of the infusion had an antihaemophilic globulin content at the same level or even slightly higher than that of the specimen collected immediately subsequent to the infusion. In Figure 144 it will be seen that the mean fall during the first six hours was considerably less than during the second six hour period. This phenomenon is possibly related to changes in plasma volume subsequent to the infusion. If the rate of utilization in the haemophiliac is comparable to the normal the physiological turnover must be of the order of 10 per cent per day.

In observations Nos. 2 and 3 the rate of disappearance of the A.H.G. was similar to that of the other observations. In observation No. 2 the plasma was administered immediately after collection, no freezing having been carried out. In observation No. 3 there was no known site of haemorrhage at the time of the infusion. These observations were made in view of the possibility that the rapid rate of utilization of A.H.G. was related either to the method of storage of the plasma by freezing or to its consumption for haemostatic purposes at a site of haemorrhage.

Whole Blood Clotting Times:- The results on the whole blood clotting times in three of the observations (6, 9, 10) are shown in Table 35 . It will be seen there there was a correction almost to normal immediately after the infusion. This marked correction of the whole blood clotting time is to be compared with the very defective correction of thromboplastin generation as demonstrated in the A.H.G. assay. As has been recognised previously, correction of the whole blood clotting time may give a false impression of the efficacy of therapy.

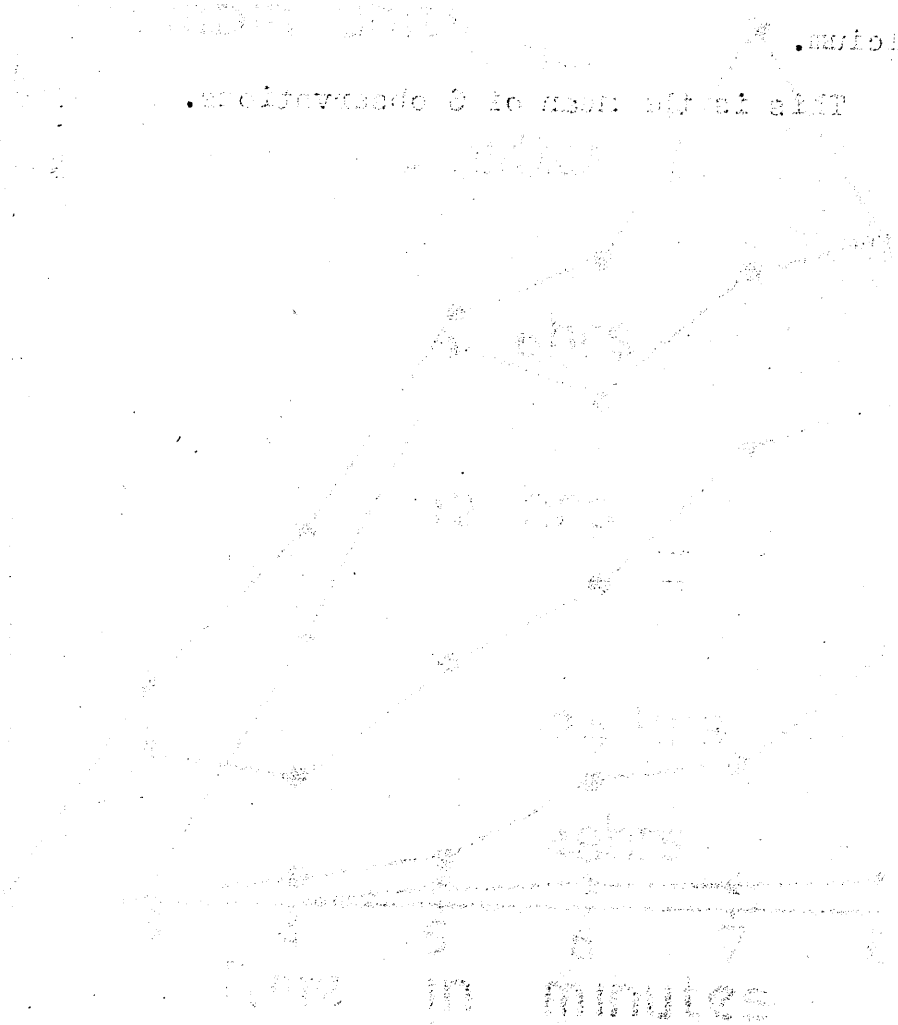
TABLE 35

WHOLE BLOOD CLOTTING TIMES IN MINUTES

Observation	Before	Immediately after	6 hrs.	12 hrs.	24 hrs.	38 hrs.	72 hrs.
6	120+	11	13	21	28	90	120+
11	120+	11	14	25	30	70	120+
12	120+	14	12	30	60	120+	120+

Prothrombin Consumption: Using Merskey's technique the effect of the plasma infusions on prothrombin consumption was studied. In Table 36 the mean result of two observations is shown. The prothrombin consumption was corrected to

Figure (147)

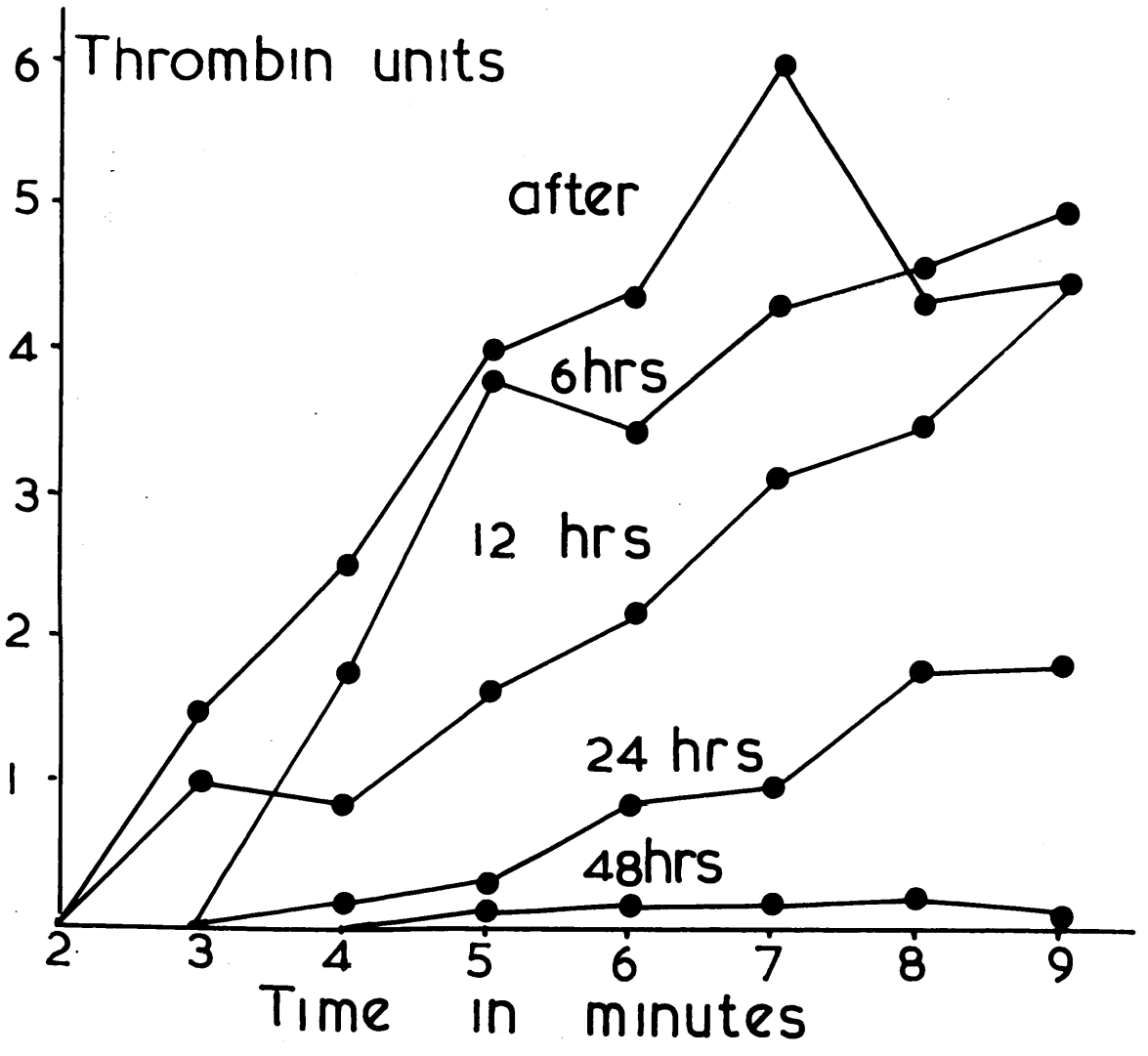


Thrombin generation from plasmas collected at intervals following infusion of a litre of plasma to haemophiliacs.

Ordinate - Thrombin units.

Abscissa - time in minutes after addition of calcium.

This is the mean of 6 observations.


Thrombin units
6
5
4
3
2
1
after
6hrs
12 hrs
24 hrs
48hrs
2
3
4
5
6
7
8
9
Time in minutes

within the normal range in the specimens immediately after and at six hours. In the 12 hour specimen the prothrombin consumption was above the normal range.

TABLE 36

Prothrombin Consumption Index (mean of 2) observations

Before	167%
After	26%
6 hrs.	29%
12 hrs.	61%
24 hrs.	72%
48 hrs.	125%
72 hrs.	168%

Thrombin generation technique. In six of the observations the thrombin generation from recalcified plasma was studied. The results are shown in figure 147. This provides confirmatory evidence that by 24 hours there are only trace amounts of A.H.G. remaining as a consequence of the infusion.

Estimation of the haemostatic level of antihaemophilic globulin: This was a more difficult aspect to estimate. In observation 8 of table 34 the patient had haematuria for one week. Prior to starting the plasma infusion he passed a specimen of urine showing gross haematuria and was given two pints of water to drink to produce diuresis. At 15 minute intervals from the time of starting the infusion further specimens of urine were collected. The 15' and 30' specimens

still contained blood; the 45' specimen was obviously clearer and the 60' specimen contained no obvious blood. To obtain haemostasis in this patient for this particular purpose an A.H.G. level of 12 per cent was effective.

In 8 observations (Nos. 1, 2, 4, 5, 6, 7, 9, 10) tooth extraction was carried out within one hour of completion of the infusion. During the 24 hours following extraction there was no bleeding. In four of the dental operations some bleeding started 24 to 48 hours after the completion of the infusion, and in a further three there was some haemorrhage between 48 and 72 hours. It is probable from this that the level of antihaemophilic globulin attained in consequence of the infusion had some haemostatic value.

DISCUSSION

This investigation demonstrates the difficulties involved in the therapeutic replacement of A.H.G. in the haemophiliac. In order to obtain a level of 7 per cent of A.H.G. in a haemophiliac, approximately a litre of plasma has to be given with a rapid infusion. This administered A.H.G. has been utilised in 24 hours. The maintenance of a level of 7 per cent A.H.G. would require the administration of a litre of plasma every 6-12 hours. This rapid utilization of A.H.G. is related neither to the method of storage of the plasma by freezing nor to its consumption for haemostatic purposes at

a site of haemorrhage.

There is suggestive evidence from this study that, from these sites of haemorrhage, the level of A.H.G. attained had some haemostatic value. It is clearly desirable if possible to produce higher levels of A.H.G. activity in the haemophiliac's plasma and this has been described by Macfarlane *et al.* (1954), Biggs *et al.* (1955) and Bidwell (1955) using preparations of bovine A.H.G. Although a level of 12 per cent A.H.G. was of some value in haematuria and in bleeding from tooth extraction, these are relatively minor tests of haemostatic efficiency. It is probable that for surgical procedures involving trauma to larger vessels a higher level of antihaemophilic globulin would be required.

Preparations of Cohn's plasma fraction I are available as a source of antihaemophilic globulin for the treatment of haemophilia. The difficulty remains, however, of the large amounts required. Even if there is full recovery of the antihaemophilic globulin from the plasma it would still need the fraction derived from a litre of plasma for administration every 6-12 hours to maintain a haemostatic effect.

Until such time as preparations of the bovine antihaemophilic globulin as described by Macfarlane *et al.* (1954) become available, human plasma must remain as the mainstay of therapy. As mentioned above A.H.G. assays on the plasma

of blood stored for transfusion purposes at 4° C., reveal that there are only trace amounts left after 2 days. In plasma maintained at -20° C. from the time of collection there is good preservation of A.H.G. This can be kept available by transfusion services to meet the emergency of bleeding in haemophilia.

The observations confirm, as has been recognised before, that the correction of the whole blood clotting time and prothrombin consumption does not mean restoration to a normal coagulation mechanism. The whole blood clotting time was reduced to normal in these observations when the A.H.G. assay revealed only 10 per cent to be present.

Whether the rapid disappearance of antihaemophilic globulin in the haemophiliac represents the physiological utilization of the factor is problematical. Langdell et al. (1954) administered plasma to haemophilic and normal dogs. The utilization in the normal dogs was as rapid as in the haemophilic dogs.

CHRISTMAS FACTOR SURVIVAL IN CHRISTMAS DISEASE.

Using similar techniques five observations were made on patients with Christmas disease. These are not quite comparable with those on haemophiliacs as only one of the patients with Christmas disease was comparable in build with the haemophiliacs. The other two were a boy of 10 years and

Figure (148)

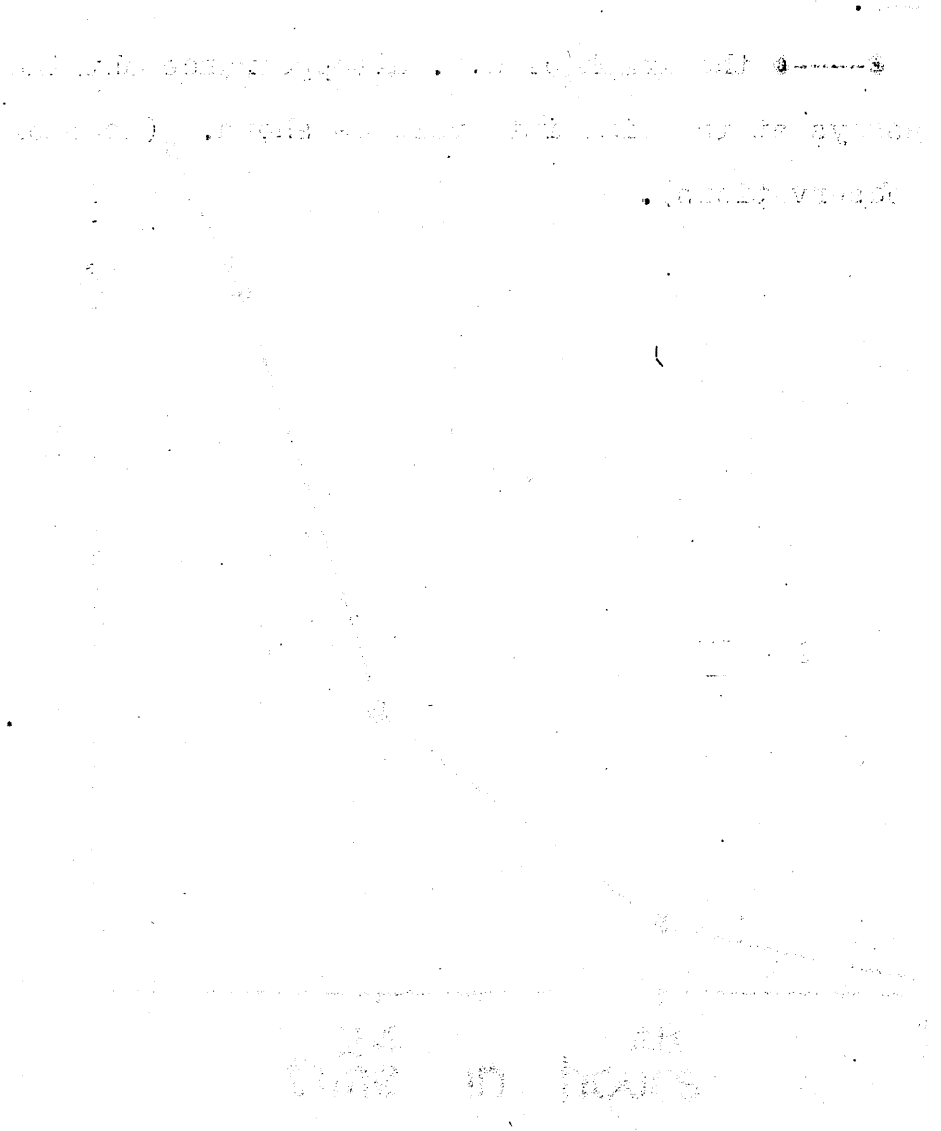


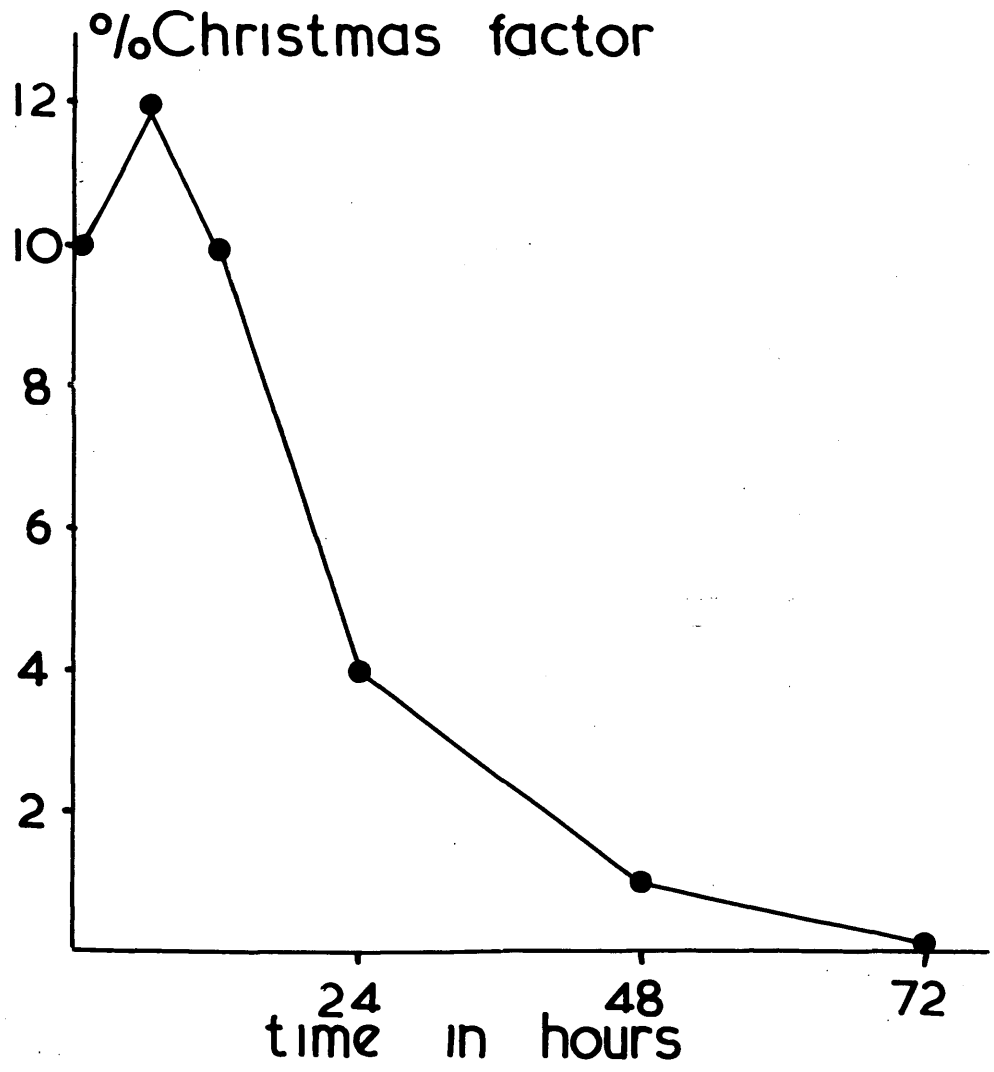
Figure (148)

"In vivo" Christmas factor (C.F.) survival.

Ordinate - percentage Christmas factor.

Abscissa - time in hours after administration of plasma.

●——● the graph of C.F. disappearance obtained by assays at the time intervals as shown. (Mean of the observations).


%Christmas factor
12
10
8
6
4
2
24
48
72
time in hours

a very under-developed youth of 17 years weighing only 5½ stones.

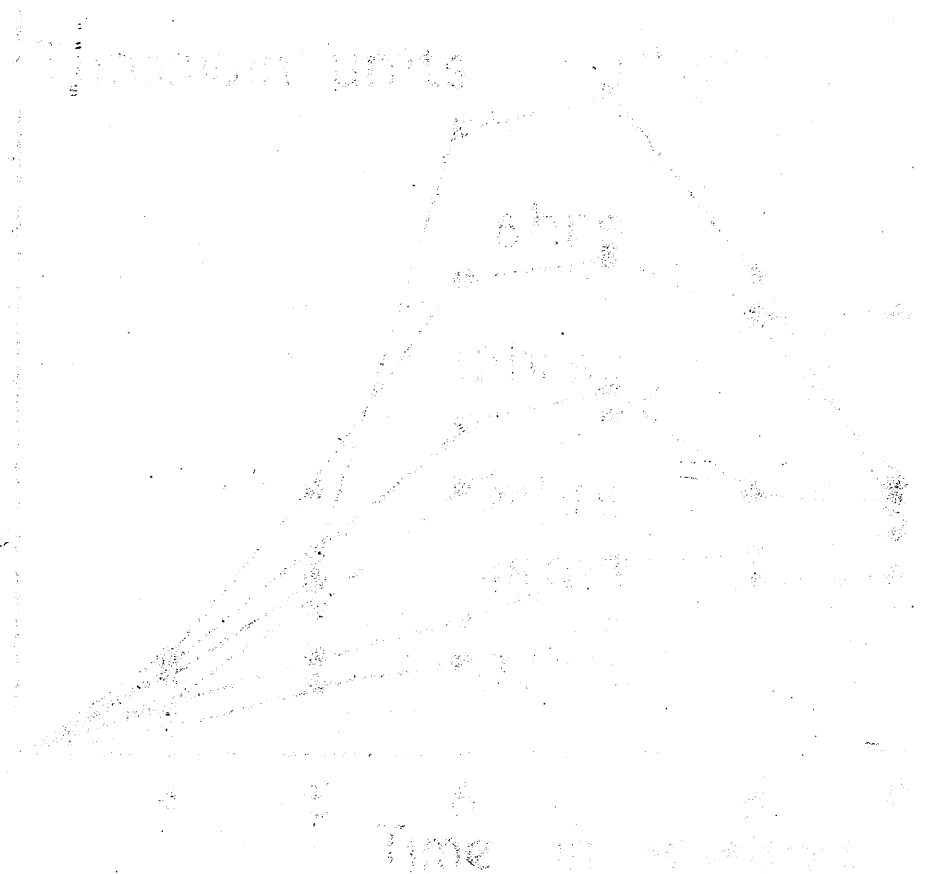
The technique of C.F. assay is similar in principle to that described for A.H.G. and is given in the appendix. Specimens of serum were collected from the patient at each of the times of assay. These sera were used in the thromboplastin generation system with normal adsorbed plasma and platelets; the assay was read by comparing these results with those from mixtures of normal serum in Christmas disease serum.

The mean result of the assays is shown in figure 148. It will be seen that the curve is very similar to that for A.H.G. survival. The results of the assays are given in table 37 together with the details of one observation on the prothrombin consumption and whole blood clotting time. These also are similar to those in haemophilia.

Aggeler and his colleagues report on the prolonged 'in vivo' survival of Christmas factor. (White et al. 1953). In their observations the whole blood clotting time had not returned to its pretransfusion level at the end of two-three weeks. This discrepancy remains unexplained. The mean of the results of C.F. assay at the chosen times after infusion was higher than the equivalent assays in the haemophiliacs. There was no question however of the C.F. surviving appreciably

longer than the A.H.G. in vivo. Any difference was a matter of hours and not days and, as pointed out above, the results are not strictly comparable as four of the observations out of the five were on patients of much smaller build than the haemophiliacs tested.

Figure (149)



Thrombin generation from plasmas collected at intervals following infusion of a litre of plasma to a patient with Christmas disease.

Ordinate - Thrombin units.

Abscissa - Time in minutes after addition of calcium.

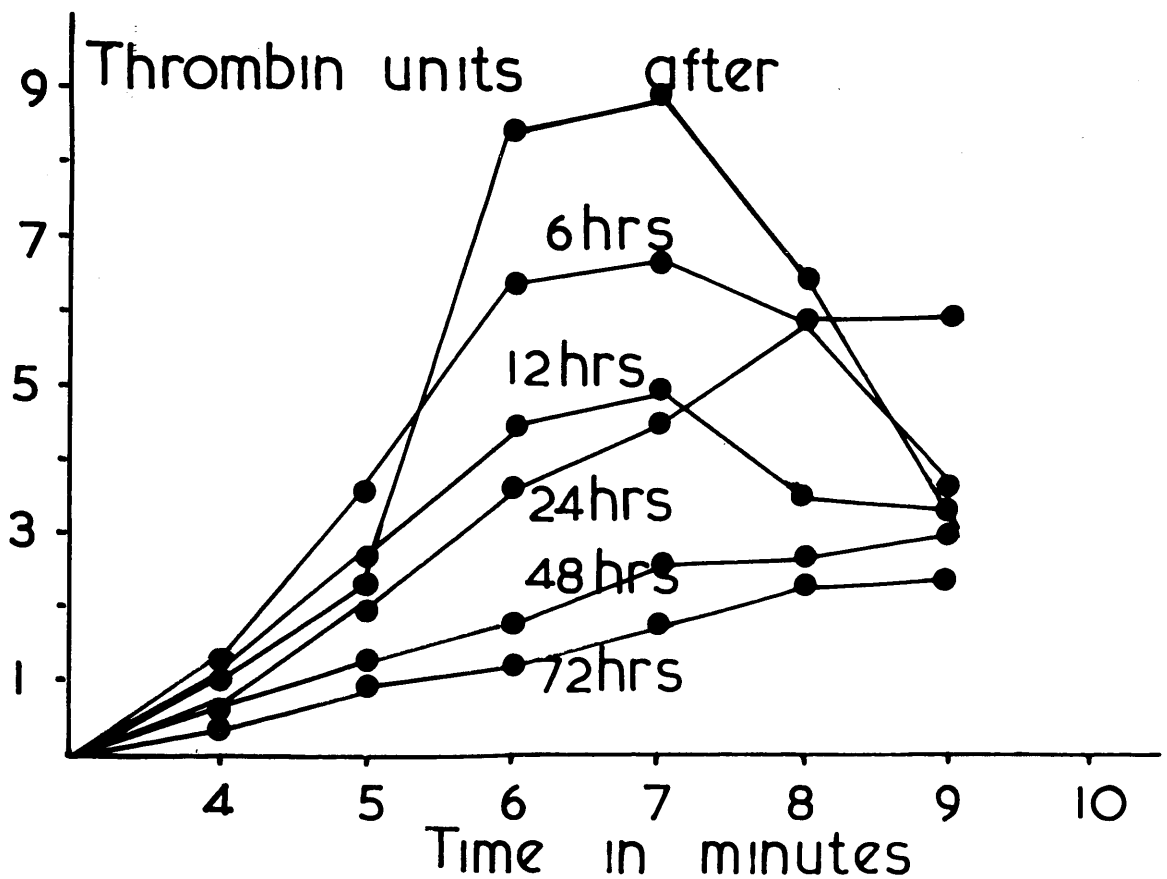

Thrombin units after
9
7
5
3
1
6hrs
12hrs
24hrs
48hrs
72hrs
4
5
6
7
8
9
10
Time in minutes

TABLE 37

		Before	After	6 hrs.	12 hrs.	24 hrs.	48 hrs.	72 hrs.	Time to administer	
M. McG.	C.F. assay		12	5	4	1	0	0	2 hrs.	750 ml. plasma
M. McG.	C.F. assay		15	12	6	3	0	0	2 hrs.	750 ml. plasma
W. B.	C.F. assay		3	2	1	0	0	0	12 hrs.	1000 ml. plasma ½ pint blood
R. S.	C.F. assay		After plasma 6 After Blood 12	-	-	3	1	0	75 mins. ---- 12 hrs.	750 ml. plasma + 2 pints blood
M. McG.	C.F. assay									
	Proth.Cons. Index.	205	46	19	33	63	200	180	90 mins.	1000 ml. plasma
	Whole blood clotting time - technique (2)	60'+	14	15	18	43	35	60'+		

SUMMARY

(1) A.H.G. has been assayed in haemophiliacs after the administration of a litre of plasma. The rise in A.H.G. expressed as a mean of the observations was 7% and this disappeared over the next 24 hours.

(2) The A.H.G. assay was dependent on the thromboplastin generation technique. The failure in correction of thromboplastin generation was compared with the correction of the prothrombin consumption and the whole blood clotting time.

(3) An attempt was made to correlate A.H.G. level with haemostatic efficiency. A level of 12% was sufficient to stop haematuria in one patient. The other levels obtained were probably of some value in haemostasis after tooth extraction.

(4) A small number of similar observations have been made on patients with Christmas disease. The rate of disappearance of the administered Christmas factor in Christmas disease was similar to that of the A.H.G. in haemophilia.

REFERENCES

Bidwell, E. (1955).
The purification of bovine antihaemophilic globulin.
Brit. J. Haemat. 1, 35.

Biggs, R. and Douglas, A.S. (1953).
The thromboplastin generation test.
J. clin. Path. 6, 23.

Biggs, R., Eveling, J. and Richards, G. (1955)
The assay of antihaemophilic globulin activity.
Brit. J. Haemat. 1, 20.

Fantl, P. and Sawers, R.J. (1954)
"Haemophilia", a syndrome under review: Investigations of alpha and beta prothromboplastin deficiencies.
Aust. Ann. Med. 3, 245.

Graham, J.B. & Penick, G.D., Brinkhous, K.M. (1951).
Utilization of the antihaemophilic factor during clotting of canine blood and plasma.
Amer. J. Physiol. 164, 710.

Langdell, R.D., Wagner, R.H. and Brinkhous, K.M. (1954).
Changes in levels of antihaemophilic globulin (A.H.F.) of normal and haemophilic dogs after transfusions.
Fed. Proc. 13, 436.

Macfarlane, R.G., Biggs, R., and Bidwell, E. (1954).
Bovine antihaemophilic globulin in the treatment of haemophilia.
Lancet, 1, 1316.

Pitney, W.R. and Dacie, J.V. (1953).
A simple method of studying the generation of thrombin in recalcified plasma.
J. clin. Path. 6, 9.

White, S.G., Aggeler, P.M. and Glendining, M.B. (1953).
Plasma thromboplastin component (P.T.C.). A hitherto unrecognised coagulation factor.
Blood, 8, 101.

CHAPTER 23

THE ACTION OF VITAMIN K PREPARATIONS ON THE HYPOPROTHROMBINOPENIC EFFECT OF DICUMAROL AND TROMEXAN

CONTENTS.

CHAPTER 23

THE ACTION OF VITAMIN K PREPARATIONS ON THE HYPOPROTHROMBINOPENIC EFFECT OF DICUMAROL AND TROMEXAN

The administration of dicumarol (3,3'-methylene-bis-(4-hydroxycoumarin)) or "tromexan" (bis-3,3'-(4-oxycoumarinyl)-ethyl acetate) in the treatment of thromboembolic states may lead to severe or even fatal haemorrhage. Although careful control of treatment by repeated estimation of the one-stage clotting time reduces this risk, a reliable and convenient antidote against such potent therapeutic substances would be of great value. Both dicumarol and tromexan are anticoagulants by virtue of their causing "hypoprothrombinaemia" and the two measures recommended to reverse this effect are the administration of preparations possessing vitamin-K activity and the transfusion of blood or plasma. There is no doubt that the latter treatment gives immediate and significant results, but for it to be most effective the blood must be in sufficient quantity. It is not always easy to meet this requirement because of possible circulatory disturbance, and reliance may be placed on the use of vitamin-K preparations such as are commercially available.

There is a measure of belief, based on experimental observations carried out during the past ten years, that these vitamin-K preparations are capable of reversing dicumarol-

induced hypoprothrombinaemia. From the available evidence at hand there can be no doubt that substances with vitamin-K activity do possess such powers, but there is still much confusion regarding the practical value of the various preparations, & this required to be assessed.

It is hoped that the experiments described below will provide more conclusive evidence concerning the action and therapeutic value of these preparations.

Method of study.

Either dicumarol or tromexan was administered in therapeutic doses to patients in whom there was no obvious potential source of haemorrhage or evidence of hepatic, alimentary, or renal dysfunction. A test dose of the anti-coagulant drug was given, and the effect on the "prothrombin" levels was observed at least daily. When the "prothrombin" levels had returned to normal, and had remained normal for two to three days, the same dose of the same drug was repeated, and a selected dose of a vitamin-K preparation was given. Plasma "prothrombin" levels continued to be estimated at least daily. In a few instances the technique was varied in respect of time of administration and of the number of the doses of vitamin K. The variations are indicated with the results in Table 39.

The hypoprothrombinaemia expected from the anticoagulant therapy was not usually beyond customary therapeutic levels.

Vitamin-K Preparations.

The vitamin-K substances which were tested, and their routes of administration, are shown in Table 38. At the time of this study, with the exception of vitamin K_1 these preparations were readily available commercially, and the doses of those which were given parenterally were limited only by the occurrence of untoward reactions. In the case of vitamin K_1, which is fat-soluble and which was received as an oily concentrate, a preparation suitable for intravenous administration was made as described by Davidson and MacDonald (1943). The oily fluid was dissolved in absolute alcohol (200 mg. K_1 in 4 ml. alcohol), and the solution was then added to 5% glucose (200-400 mg. K_1 in 500 ml.). The preparation was then autoclaved. Suspension of the vitamin in the glucose solution was assisted by injecting the alcoholic solution forcibly through a long fine needle into the centre of the bottle of glucose solution, but in spite of this the preparation tended to be unstable and had to be used almost at once. This preparation of vitamin K_1 was administered by intravenous infusion over a period of half to five hours without delay, otherwise the vitamin K_1 tended to settle out and adhere to the glass, with consequent loss of potency. No untoward reaction of any kind was encountered.

TABLE 38

Details of the Vitamin-K Preparations Tested

Substance	Other Designation	Route of administration	Dosage
2-methyl-3-phytyl-1:4-naphthoquinone	Vitamin K_1	Intravenously	200-340 mg. single doses
2-methyl-1:4-naphthoquinone	"Prokayvit"(B.D.H.); menaphthone; menadione	Intramuscularly	15-30 mg. in 2-3 days
2-methyl-1:4-naphthohydroquinone diacetate	Acetomenaphthone; oral Prokayvit (B.D.H.)	Orally	90 mg. in 3 days.
2-methyl-1:4-naphthohydroquinone carboxymethoxine	"Kapilon" (Glaxo)	Intramuscularly	60 mg.in 3 days.
2-methyl-1:4-naphthohydroquinone diphosphate	"Synkavit" (Roche)	Intravenously	300-1,600 mg. in 3-4 days
Dipotassium 2-methyl-1:4-naphthylene bisulphate	Water-soluble K analogue (Boots)	Intravenously	200 mg.i.v. single dose.

Since this work vitamin K_1 has become available commercially in ampoules of 50 mg. in 1 ml. alcohol. This is drawn up into a syringe partly filled with saline and readily forms an emulsion for intravenous administration. No reactions have been found during a now extensive experience, with this preparation.

One-stage clotting time

The same batch of rabbit-brain thromboplastin was used in each case, for the control and the test periods. The estimations were carried out on 100% and on 12.5% plasma. Only the figures obtained with 100% plasma are recorded here.

At the time of this study (1949-1950) the thromboplastin in use was a phenol-saline extract of rabbit brain; 0.2 ml. amounts of plasma, brain thromboplastin and calcium chloride were used. The end point was recorded using a glass rod with the end drawn out into a hook to catch the first of the fibrin formed. (see appendix page 569).

Results

The results of the various therapeutic trials of the vitamin-K analogues against the hypoprothrombinaemic effect of dicumarol and tromexan are presented in Table 39. In the case of vitamin K_1 the results of single tests are tabulated. In the case of the other preparations the average results from groups of patients are recorded: a more detailed analysis of these results is given below.

Vitamin K_1:

Vitamin K_1 was tested against a single dose of tromexan in three patients. In the first case (Table 39) 900 mg. of tromexan resulted in a prothrombin time approximately four

times normal. The same dose of tromexan was given on the first day of the test period, and on the following day, when the "prothrombin" time had begun to rise, 200 mg. of vitamin K_1 was given intravenously. Within twenty-four hours the "prothrombin" time was 19 seconds, as compared with 60 seconds at this stage in the control period. In Case 2, 200 mg. of vitamin K_1 given at the same time as 900 mg. of tromexan appeared to act as a complete antidote. In Case 3 the effect of 900 mg. of tromexan was likewise blocked by simultaneous administration of 340 mg. of vitamin K_1. As an additional check on the method of testing, this patient was given a further dose of tromexan without vitamin K_1. Hypo-prothrombinaemia resulted.

Vitamin K_1 was tested against a single dose of dicumarol in two patients. In the first of these (Case 4) the administration of 200 mg. of vitamin K_1 on the third day of the test period was followed by return of the "prothrombin" time to pre-treatment levels within twenty-four hours. In Case 5 the simultaneous administration of 300 mg. of vitamin K_1 was almost completely effective in blocking the action of 400 mg. of dicumarol.

As a further test of the action of vitamin K_1 (Case 6) 900 mg. of tromexan was given over two days. On the third day, when the prothrombin time was 40 seconds, 200 mg. of

TABLE 39

Effect of Dicumarol and Tromexan on Plasma Prothrombin Time and the Modification of this Effect by Vitamin-K Analogues

	Days of Control and Test Period					Remarks
	1	2	3	4	5	
	sec.	sec.	sec.	sec.		
Case 1	16(T)	32	60	25	--	Control. T=900 mg. tromexan.
	15(T)	28(K)	19	16	-	K=200 mg. vitamin K_1 i.v.
" 2	20(T)	30	22	18	-	Control. T=900mg. tromexan.
	19(TK)	18	20	20	-	K=200 mg. vitamin K_1 i.v.
" 3	17(T)	36	26	-	-	Control. T=900 mg. tromexan.
	21(TK)	20	19(T)	38	-	K=340 mg. vitamin K_1 i.v.
" 4	17(D)	19	28	33	25	Control. D=400 mg. dicumarol.
	20(D)	22	28(K)	19	19	K=200 mg. vitamin K_1 i.v.
" 5	19(D)	19	28	33	25	Control. D=400 mg. dicumarol.
	16(DK)	19	22	19	19	K=300 mg. vitamin K_1 i.v.
" 6	17(T)	22(T)	40(K)	12	-	T=600+300 mg. tromexan. K=300 mg. vitamin K_1 i.v.
" 7	-	75(T)	75(TK)	to 21 in 6 hrs.		T=Maintenance 300 mg. tromexan/day. K=300mg.vit.K_1 i.v.
Group 8 (5 cases)	16(T)	25	28	-	-	Control. T=900 mg. tromexan.
	16(TM)	21(M)	23	-	-	M=10 mg. menaphthone i.m.
Group 9 (5 cases)	16(T)	24	28	21	-	Control. T=900 mg. tromexan.
	18(TAM)	26(AM)	21(AM)	16	-	AM=300 mg.acetomenaphthone
Group 10 (2 cases)	16(D)	20	22	21	-	Control. D=200 mg.dicumarol.
	18(DAM)	22(AM)	22(AM)	21	-	AM=30 mg.acetomenaphthone.
Group 11 (5 cases)	16(T)	25	22	18	-	Control. T=900 mg. tromexan.
	18(TK)	22(K)	22(K)	18	-	K=20 mg. kapilon i.m.
Group 12 (3 cases)	15(D)	19	26	29	-	Control. D=200-400mg.dicumarol.
	16(DK)	24	22	20	-	K=20 mg. kapilon i.m.
Group 13 (6 cases)	15(T)	24	28	12	-	Control. T=600 mg. tromexan.
	16 (TS)	24(S)	20(S)	11	-	S=100 mg. synkavit i.v.
Group 14 (5 cases)	15(D)	20	28	25	-	Control. D=200 mg. dicumarol.
	15(DS)	16(S)	28(S)	20	-	S=100 mg. synkavit i.v.
Group 15 (1 case)	18	18(T)	31	23	18	Control. T=1,200 mg. tromexan.
	18(S)	18(TS)	29(S)	22(S)	19	S=400 mg. synkavit i.v.
Group 16 (1 case)	16(T)	42	34	17	-	Control. T=1,200 mg. tromexan.
	17(TB)	40	29	18	-	B=200 mg. water-soluble analogue (Boots)

vitamin K_1 was given intravenously. The prothrombin time fell to 30 seconds in two hours, and to normal within twenty-

four hours. Case 7 illustrates the effect of 300 mg. of vitamin K_1 on a persistently high "prothrombin" time (75 seconds) occurring in a patient who was given 300 mg. of tromexan daily for several weeks for therapeutic purposes. In spite of the administration of the usual daily dose of tromexan, vitamin K_1 produced a rapid fall in the "prothrombin" time to 21 seconds within six hours.

From these investigations it is evident that, when 200-340 mg. of vitamin K_1 was given at the same time as a reasonable therapeutic dose of tromexan or dicoumarol the action of the anticoagulant drug on the plasma "prothrombin" levels was completely, or almost completely, inhibited. When the vitamin was given at any time after hypoprothrombinaemia had been induced, the prothrombin levels were restored to normal in 24 hours. Furthermore, significant reduction of the "prothrombin" time occurred within an even shorter period, and safe values were achieved within six to nine hours even when previously the hypoprothrombinaemia had been more severe than is usually considered necessary for therapeutic purposes.

Menaphthone and Acetomenaphthone.

The average results of the intramuscular administration of menaphthone (Group 8, Table 39) suggest that this substance had an incomplete inhibitory effect on mild hypoprothrombinaemia induced by tromexan. In this group the

individual test results and the control results were practically identical.

Acetomenaphthone was given orally to seven patients, to five who had received tromexan, and to two who had received dicumarol. The average figures for those given tromexan (Group 9) suggest that the response to the anti-coagulant was modified slightly by the acetomenaphthone. The individual results indicated that the effect of this vitamin-K analogue was slight and inconstant. Acetomenaphthone was tested against dicumarol in two patients (Group 10). In each the test curve and the control curve were identical. Although larger doses of menaphthone and acetomenaphthone might well have been given by mouth, it was felt that oral treatment was unsuited to this purpose and no further observations were made with acetomenaphthone. Dosage of menaphthone was limited by pain which followed the intramuscular injection.

Kapilon, Synkavit and Water-soluble K Analogue (Boots).

Kapilon was tested against tromexan in five patients (Group 11, Table 39). In no case could it be said that kapilon given intramuscularly in doses of 20 mg. daily for the first three days significantly affected the response to tromexan. Kapilon was tested against dicumarol, which was given in doses of 300 mg. During the test period kapilon was given in doses of 20 mg. on the first and third days.

In all three patients slight but significant modification of the "prothrombin" response occurred. In no case, however, did the prothrombin time return to normal within three days of administration of the first dose of kapilon.

Synkavit, given intravenously in doses of 100 mg., was tested against tromexan in six patients (Group 13). In five of these the control and the test curves were practically identical. In the sixth case of this group slight modification of the response occurred during the test period. Synkavit was tested against doses of dicumarol, which produced prothrombin times of 30-40 seconds, in five patients (Group 14). Each patient was given 100 mg. of synkavit daily for the first three days of the test period, and in no case was there any demonstrable effect on the prothrombin-time curve. As an additional test of the action of synkavit, even larger doses were given (400 mg. intravenously), beginning on the day before the administration of tromexan (Group 15) and continuing for a further three days. No modification of the action of the tromexan was demonstrated: the control curve and the test curve were identical.

Water-soluble K analogue (Boots) was tested against tromexan in one patient. Given intravenously in a dose of 200 mg. it slightly modified the effect of the anticoagulant (Group 16) but failed to reduce the prothrombin time to normal

within 24 hours.

The various water-soluble vitamin-K analogues have thus been shown to be relatively inactive against the hypo-prothrombinaemic effect of tromexan and dicumarol. Although in some instances - notably the tests of kapilon against dicumarol - an effect was demonstrated, this effect was insufficient to prevent a fall in plasma "prothrombin" concentrations as a result of the administration of a single relatively small dose of the anticoagulant. That the lack of effect is not merely a matter of dosage of the vitamin-K analogue is indicated by the complete failure of 1,600 mg. of synkavit given over a period of four days to modify the normal therapeutic effect of a single dose of tromexan.

DISCUSSION

Among all the vitamin-K analogues tested against dicumarol and tromexan in this investigation the only substance which was uniformly significantly active was vitamin K_1. This material was given intravenously in doses of 200-340 mg. and it was almost invariably completely effective in blocking or in reversing the action of the anticoagulant under the circumstances of the test. The other substances tested did occasionally modify the effect of the anticoagulant, but the effect was inconstant and slight. Comparable doses were not

always given, but, from the practical point of view, oral administration of vitamin K is unlikely to be satisfactory as an emergency treatment, and given by other routes, the doses of menaphthone and synkavit were limited by local or general reactions.

In the present investigation no attempt was made to give doses of anticoagulants which would result in dangerously low prothrombin levels. It might therefore be considered that the tests do not give information directly applicable to the treatment of the hypoprothrombinaemic emergency. On the other hand, this would merely point to some doubt about the value of vitamin K_1 under more exacting conditions, and the immediate result of administering it to a patient with a prothrombin time (100% plasma) about four times normal (Case 7, Table 39) without stopping the tromexan administration is good evidence of the potency of this substance even in very adverse conditions. As regards the other analogues, the relatively small dosage of the anticoagulant employed serves to emphasize the inefficiency of these vitamin-K preparations.

During the past 10 years considerable interest has been shown in the effect of vitamin-K analogues on dicumarol-induced hypoprothrombinaemia, and numerous experiments have been carried out both in animals and in the human subject. It cannot be said, however, that there is general agreement about either

the relative effects of the various vitamin-K preparations or the form of vitamin-K activity which is most suitable for the treatment of hypoprothrombinaemic bleeding occurring as the result of the administration of dicumarol or tromexan.

This lack of agreement has probably been due to two variable factors. In some experimental trials water-soluble vitamin-K preparations were regarded as effective when the anticoagulant was given in minimum active quantities. There is good evidence that this is so (Shapiro et al., 1943), but little that they are effective against therapeutic doses of dicumarol. The second variable factor has been the expected response to administration of vitamin K. Many writers have considered adequate a response demonstrable in the course of days and have been content to advise the use of the water-soluble preparations. Our own findings on the inadequacy of these preparations and the greater reliability of vitamin K_1, together with evidence presented by others on the use of vitamin K_1 or its oxide, seem to justify the conclusion that there are only two known effective means of treating hypoprothrombinaemia induced by dicumarol or tromexan. These are by transfusion of blood or by administration of vitamin K_1 or its oxide.

In view of the fact that transfusion, to be effective, involves the use of blood or of frozen fresh plasma from

possibly more than a single donor, and that thereby delay may often occur in beginning adequate treatment, there is some justification for regarding a suitable preparation of vitamin K_1 or its oxide as an essential material in any department in which treatment by dicumarol or tromexan is being carried out. No reliance should be placed on the use of larger doses of the synthetic water-soluble vitamin-K analogues. The action of vitamin-K preparations against the hypoprothrombinaemic effect of dicumarol or tromexan is not simply a matter of dosage. There is a difference in the action of water-soluble and oil-soluble preparations which has not so far been explained except that it has been related to the presence of the "phytyl" group in the molecule of vitamin K_1 (Miller et al., 1950).

S U M M A R Y

1. The effect of vitamin K preparations in modifying the action of dicumarol and tromexan has been studied. The preparations of vitamin K were vitamin K_1, menaphthone, acetomenaphthone, kapilon, synkavit and water-soluble K analogue (Boots).

2. Apart from vitamin K_1 these substances have been shown to possess a minor and inconstant ability to modify the action of dicumarol and tromexan.

3. Vitamin K_1, on the other hand, is a reliable and efficient antidote. It can block the effect of these drugs on the one-stage clotting time and reverse their effect once established, within a few hours.

4. Since these differences between the vitamin-K preparations are not merely a question of dosage, of all the substances tested here only vitamin K_1 can be regarded as a reliable antidote for a hypoprothrombinaemic emergency.

REFERENCES

Davidson, C.S. and MacDonald, H. (1943). Effect of vitamin K_1 oxide on hypoprothrombinaemia induced by dicumarol. New Engl. J. Med. 229, 353.

Miller, R., Harvey, W.P. and Finch, C.A. (1950). Antagonism of dicumarol by vitamin K preparations. New Engl. J. Med. 242, 211.

Shapiro, S., Redish, M.H. and Campbell, H.A.(1943). Prothrombin studies. III. Effect of vitamin K upon hypoprothrombinemia induced by dicumarol in man. Proc. Soc. Exper. Biol. & Med. 52, 12.

CHAPTER 24

ACTION OF VITAMIN K_1

CONTENTS.

The response of prothrombin, factor VII and serum thromboplastic activity to vitamin K_1, in

Coumarin drug therapy

Vitamin K deficiency

Salicylate therapy.

CHAPTER 24

ACTION OF VITAMIN K_1

In the previous chapter the effect of vitamin K in modifying the action of the coumarin drugs was investigated in the human subject. It was shown that apart from vitamin K_1, other vitamin K preparations have a minor and inconstant ability to modify the action of these anticoagulants. Vitamin K_1 on the other hand was found to be a reliable and efficient antidote capable of completely blocking the action of therapeutic doses of dicumarol and tromexan, and capable of restoring to safe levels within a few hours excessive "hypoprothrombinaemia" due to these drugs. At the time of this work (1949-50) the nature of the coagulation defect in coumarin therapy had not been studied. It was only after subsequent experiments (see Chapter 7) that it was appreciated that the prolongation of the one-stage clotting time in coumarin therapy was largely attributable to deficiency of the recently recognized coagulation component - factor VII. It was then also realised that although there was some measure of prothrombin deficiency the degree of this was much less than of factor VII. Furthermore the serum from patients under the influence of the coumarin drugs was unable to form

blood thromboplastin, when incubated with normal adsorbed plasma, platelets and calcium.

Previously (Chapter /3) investigations were described on the action of vitamin K on the coagulation disturbance in the newborn. The objects of the studies to be described in this chapter were to assess the response of the factor VII, prothrombin and serum thromboplastic activity to the administration of vitamin K_1 :-

(a) in coumarin drug therapy and to compare this with the response to cessation of therapy without administration of vitamin K_1.

(b) in vitamin K deficiency.

(c) in salicylate therapy.

The techniques used have been described and discussed previously and can be found in the appendix. The measurement of prothrombin was by the method of Douglas and Biggs (1953). The measurement of factor VII was dependent on a comparison of the activity of the test plasma to correct the one-stage clotting time of coumarin plasma, when compared with a normal plasma in this respect. Mixtures were made of one part of the test or normal plasma with nine parts of a sample of coumarin plasma with a long one-stage clotting time. The one-stage clotting times of these mixtures were then determined. Further dilutions of the normal plasma in

the coumarin plasma were made and the one-stage clotting times estimated. From these latter a curve was prepared by which the factor VII content of the test plasma was read (see Chapter /3).

The following observations were made.

(a) Coumarin drug therapy.

(1) Three patients, who had been maintained for 28 days on therapeutic doses of phenylindanedione were given 50 mg. of vitamin K_1 intravenously. The last dose (50 mg.) of phenylindanedione was given just prior to the vitamin K_1.

(2) Three patients, who had been maintained for 28 days, on therapeutic doses of phenylindanedione were studied following cessation of therapy without administration of vitamin K_1. The last dose of 50 mg. was given just prior to the start of the investigation.

(b) Vitamin K deficiency.

(3) Three patients with vitamin K deficiency were given 50 mg. of vitamin K_1 intravenously. These patients were vitamin K deficient in consequence of failure of entry of bile into the intestinal track. One was subsequent to surgical ligation of the bile duct, one to carcinoma of the common bile duct and the third to a carcinoma of the head of the pancreas.

Specimens of plasma and serum were collected at the start of the study and subsequently at intervals of 3 hrs., 6 hrs., 9 hrs., 12 hrs., 24 hours and if required at 30 hrs., 36 hrs., 48 hrs., 72 hrs., and 96 hrs. - Table 40 refers. Quick's one-stage test and the factor VII estimation were performed on the specimens as they were collected. The sera were kept in a 4° C. refrigerator until all were collected and then tested in one experiment on the thromboplastin generation technique.

(c) Salicylate therapy.

(4) One patient on salicylate therapy who had "hypoprothrombinaemia" was given 50 mg. vitamin K_1 and maintained on the same dosages of salicylate. Blood was collected before giving the K_1 and 24 hours later the prothrombin, factor VII and serum thromboplastin activity were assessed in these specimens.

Figure (152)

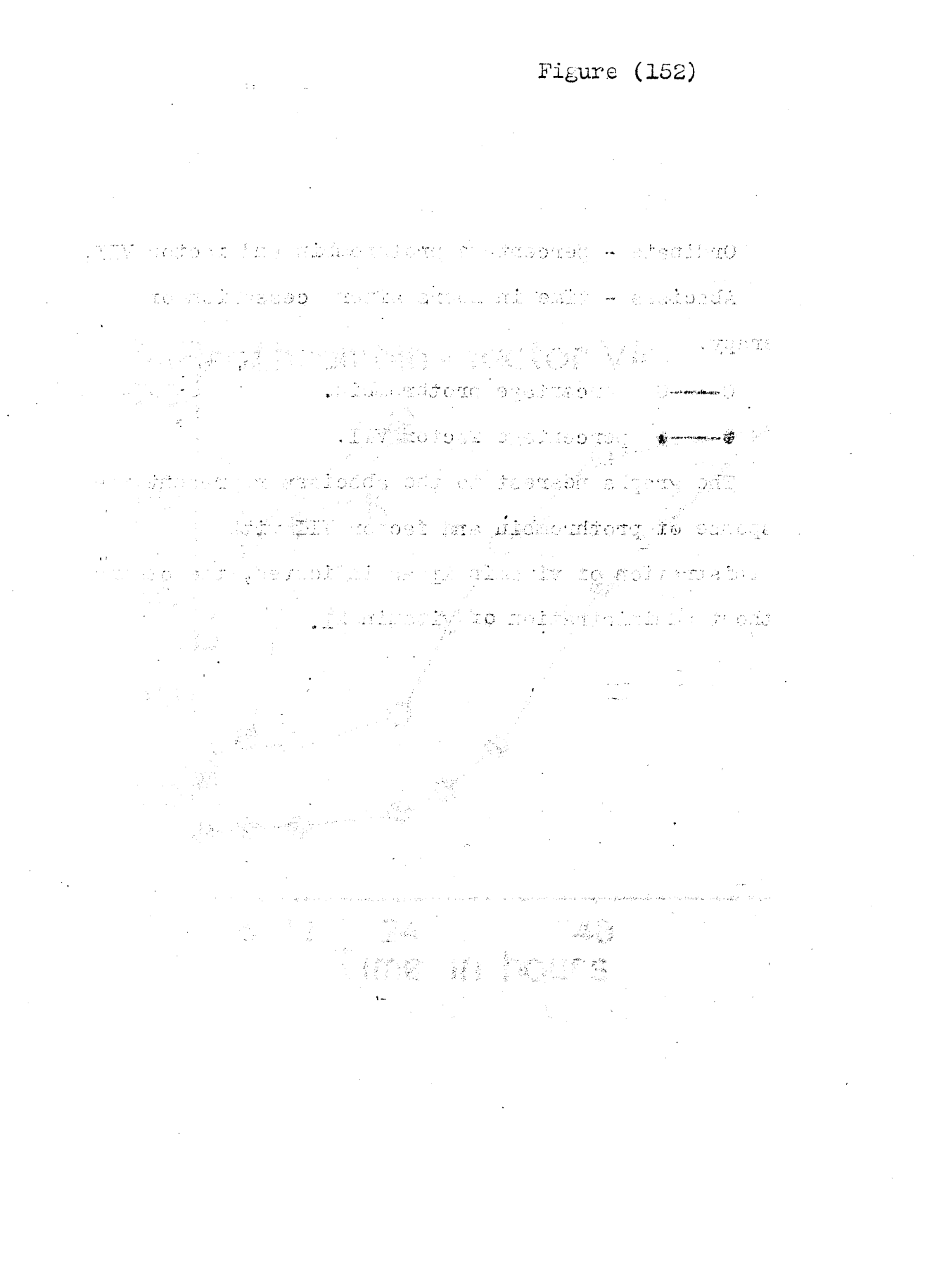


Figure (182)

Response of prothrombin and factor VII to cessation of coumarin therapy with and without administration of vitamin K_1.

Ordinate - percentage prothrombin and factor VII.

Abscissa - time in hours after cessation of therapy.

O——O percentage prothrombin.

●——● percentage factor VII.

The graphs nearest to the abscissa represent the response of prothrombin and factor VII with administration of vitamin K_1 as indicated, the others without administration of vitamin K_1.

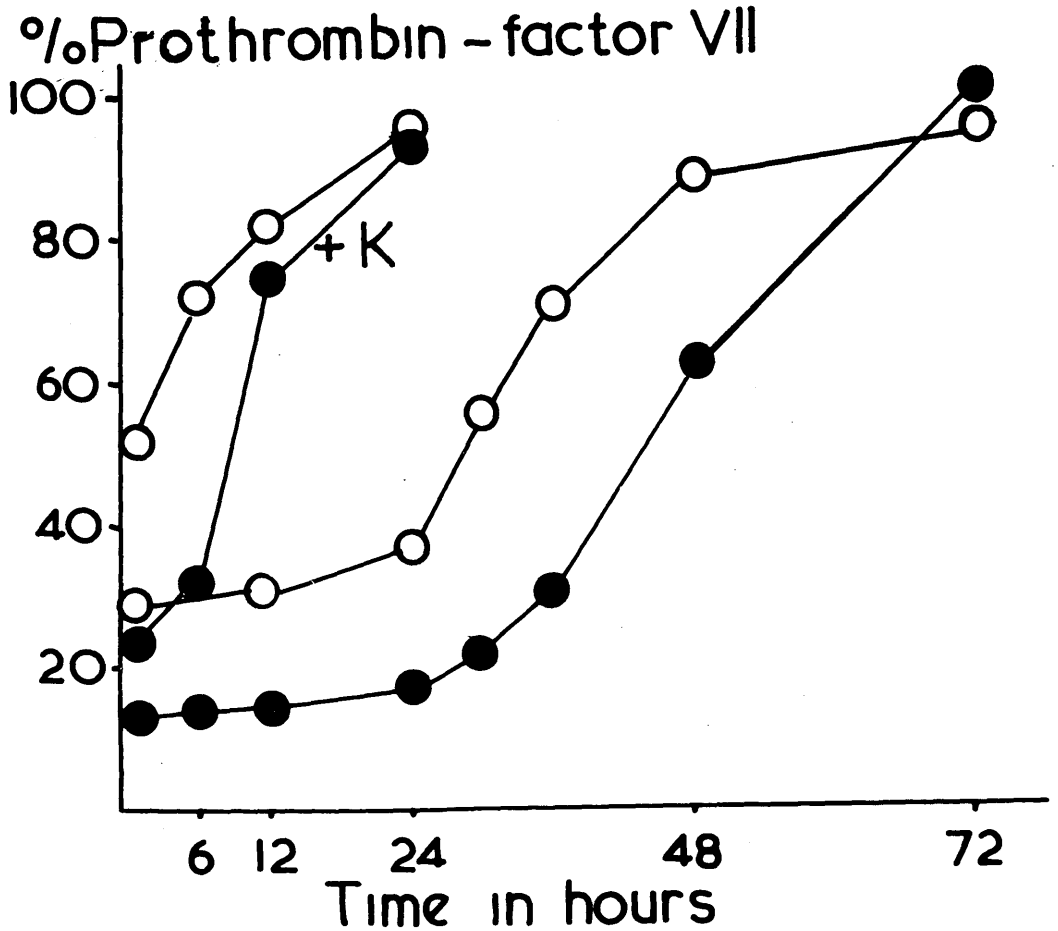

%Prothrombin - factor VII
100
80
60
40
20
+K
6
12
24
48
72
Time in hours

TABLE 40

SUMMARY OF RESULTS OF ONE-STAGE CLOTTING TIMES

(mean of 3 observations)

Hrs.	Coumarin therapy No K.	Coumarin therapy +K.	Vit. K Deficiency
0	40	31	31
6	41	23	20
12	42	16	18
24	39	15	16
30	31		
36	24		
48	18		
72	15		
96	15		
Control	15	15	15

RESULTS

(see pages 1242 -1257 of the appendix)

(a) Coumarin drug therapy.

(1) Patients on coumarin therapy given vitamin K_1. The response of the prothrombin and factor VII to vitamin K_1 is shown in figure 152. The factor VII is at a lower value

Figure (153)

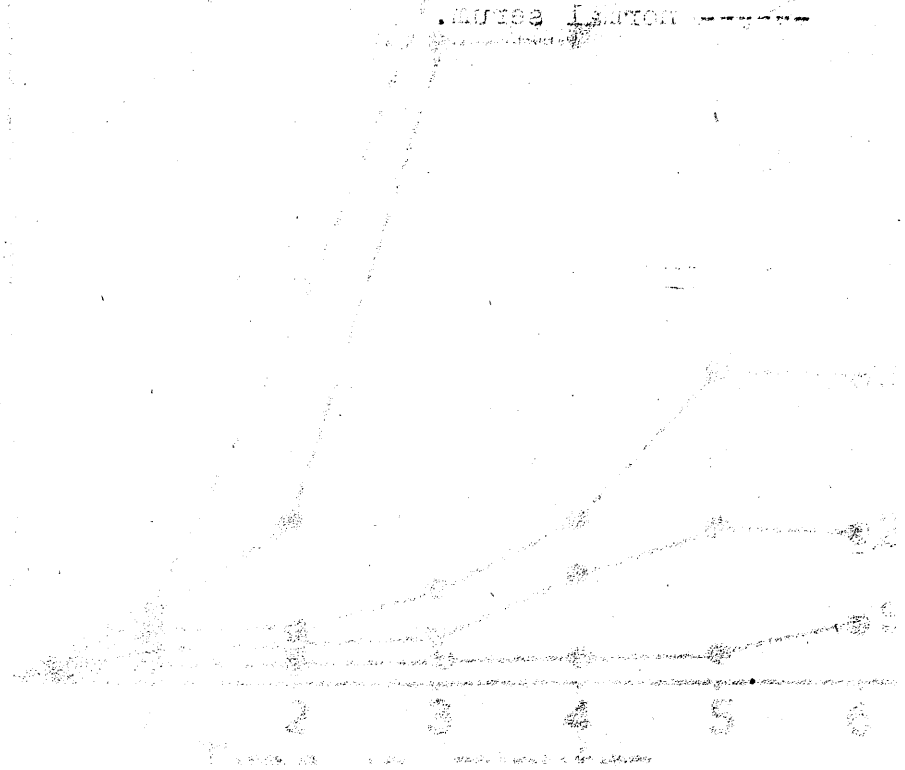


Figure (158)

Response of serum thromboplastic activity to cessation of coumarin therapy.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation technique with adsorbed normal plasma and platelets constant.

●——● Sera from patient at time intervals as stated after last dose of dindevan.

--•----- normal serum.

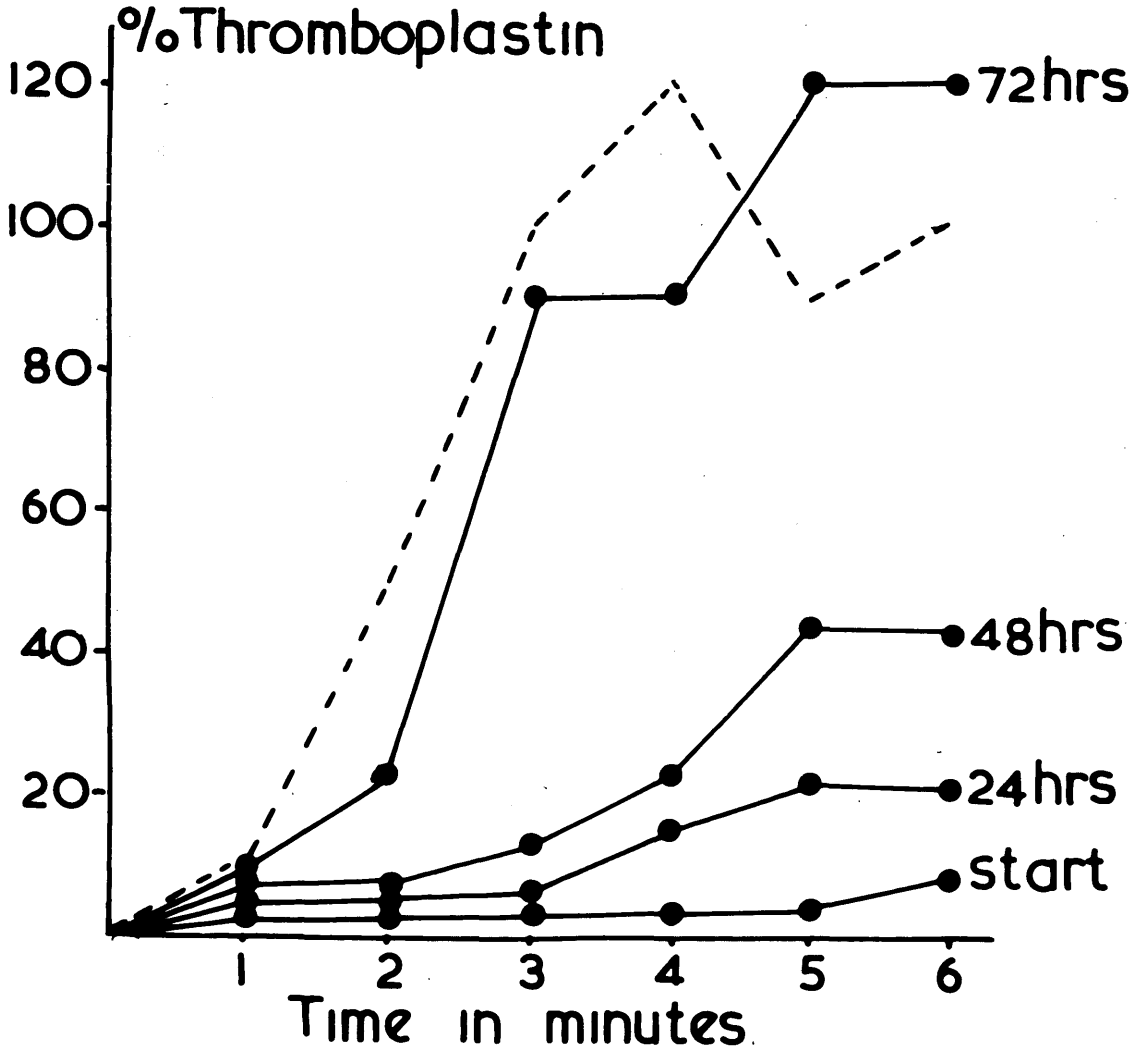
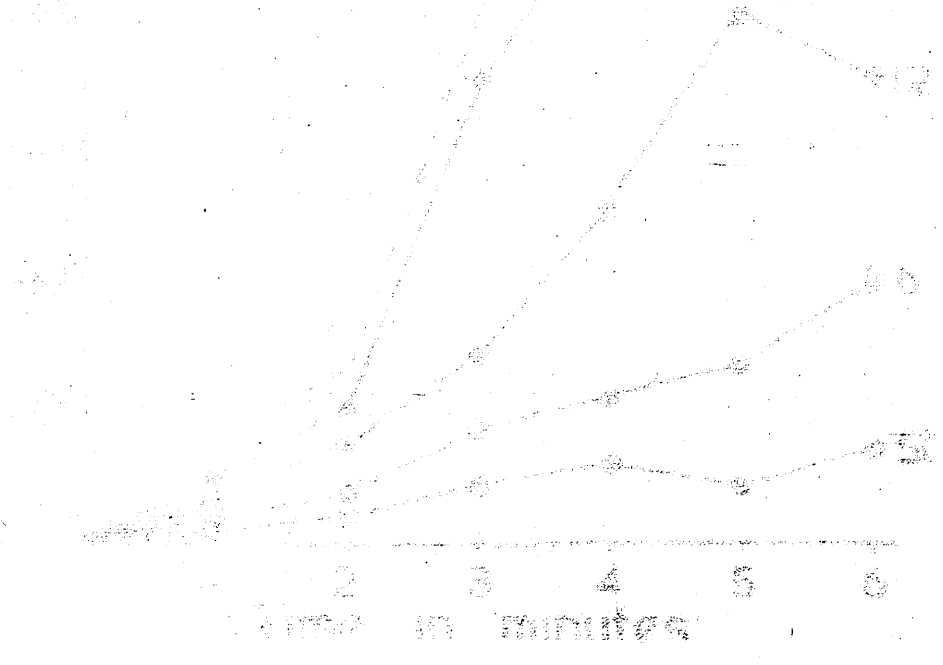

%Thromboplastin
120
100
80
60
40
20
72hrs
48hrs
24hrs
start
1
2
3
4
5
6
Time in minutes

Figure (154)



Response of serum thromboplastic activity in coumarin therapy to the administration of vitamin K_1.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation technique with adsorbed normal plasma and platelets constant.

●——● Sera from patient at time intervals as stated after last dose of dindevan and intravenous injection of 50 mg. vitamin K_1.

------ normal serum.

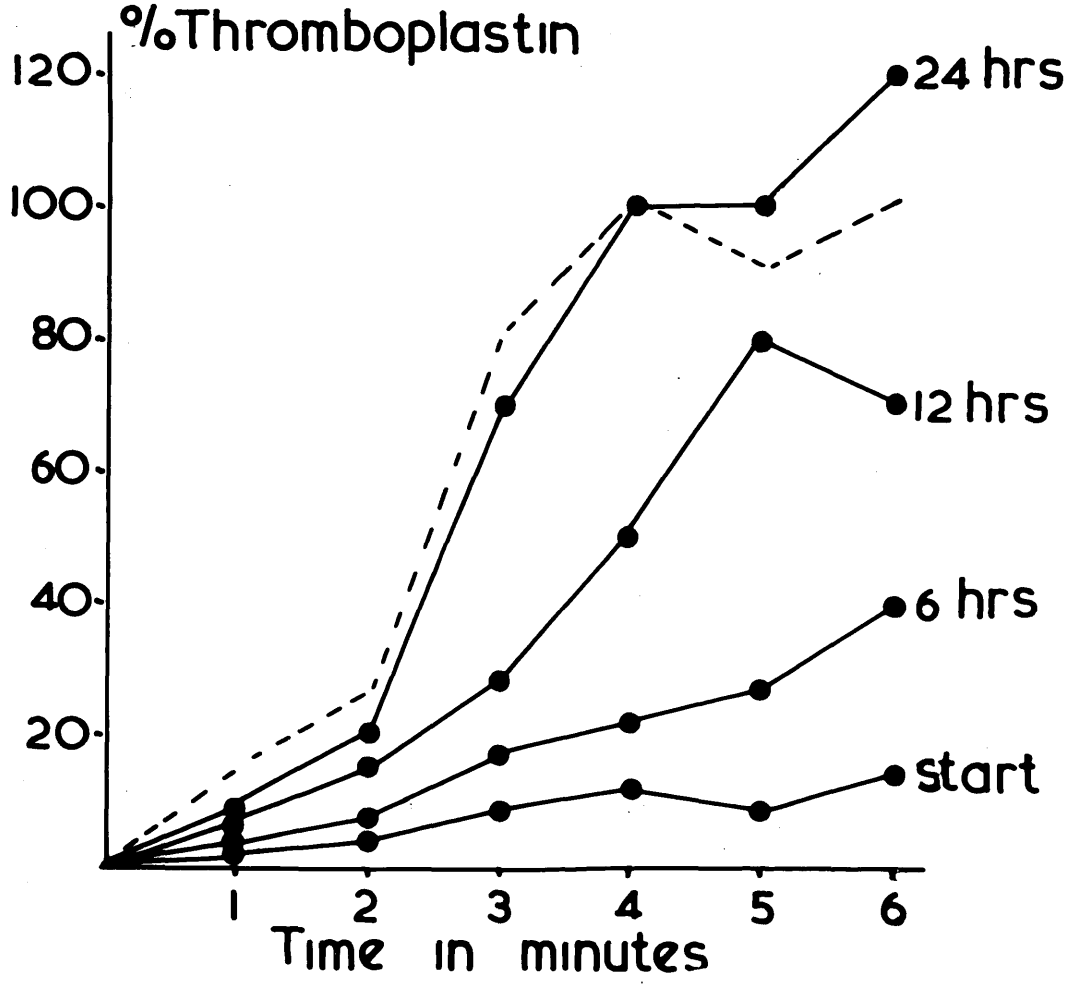
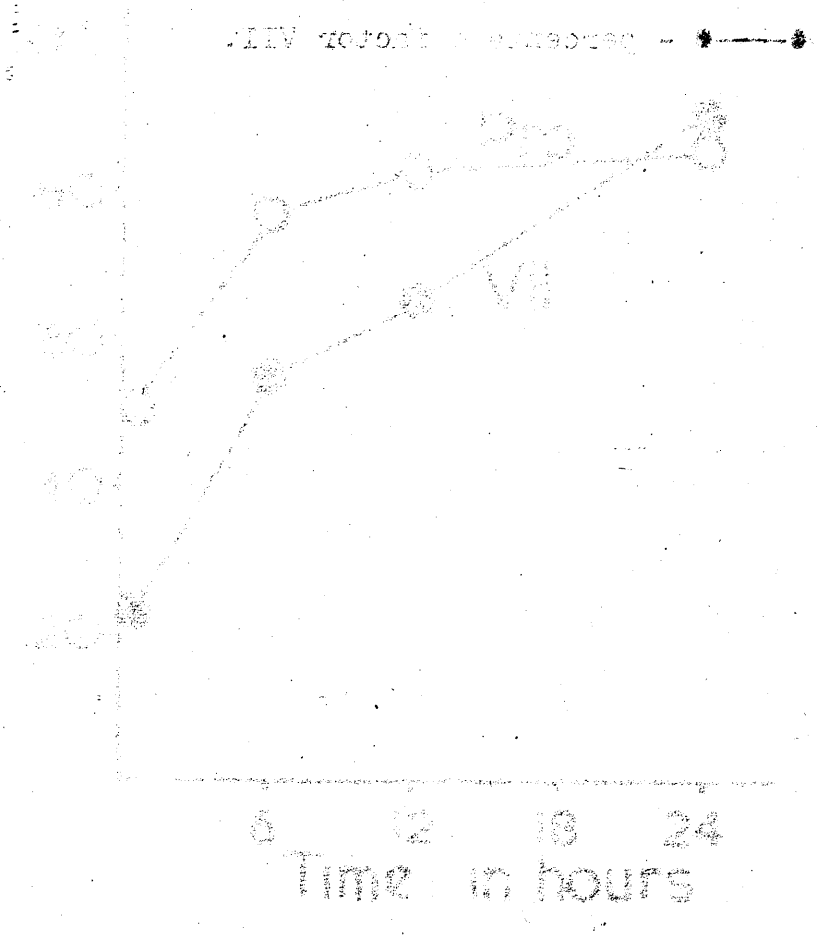

%Thromboplastin
120
100
80
60
40
20
24 hrs
12 hrs
6 hrs
start
1
2
3
4
5
6
Time in minutes

Figure (155)



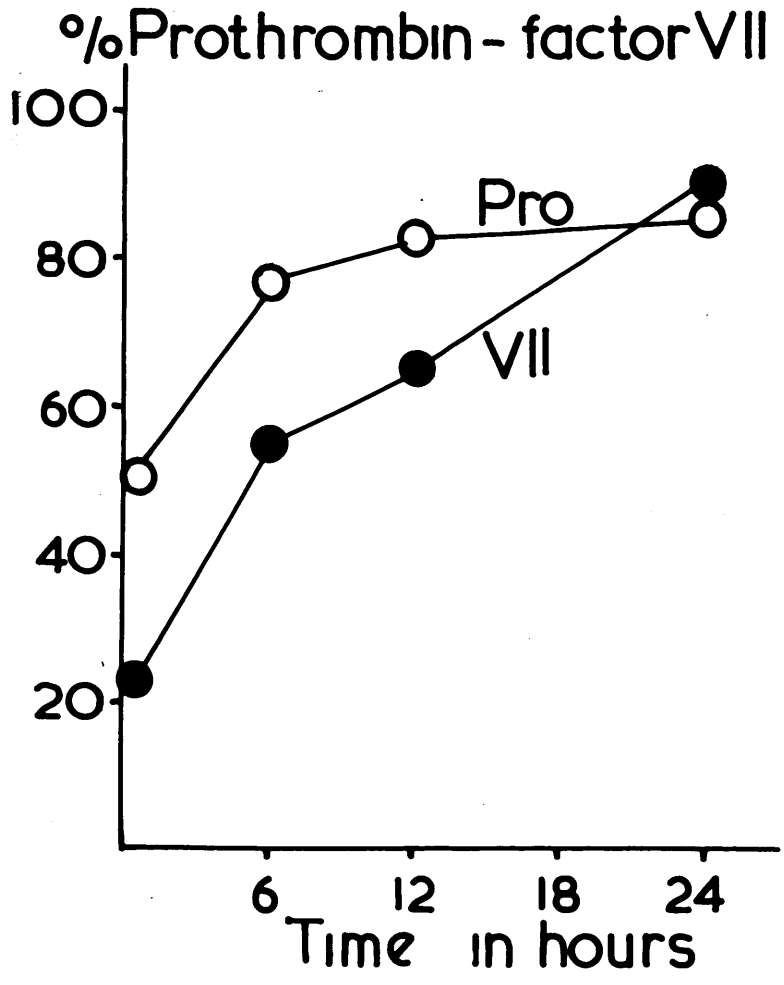
Vitamin K deficiency - response of prothrombin and factor VII to the administration of vitamin K_1.

Ordinate - percentage thromboplastin.

Abscissa - time in hours after intravenous administration of vitamin K_1.

O——O - percentage prothrombin.

●——● - percentage factor VII.


%Prothrombin - factor VII
100
80
60
40
20
Pro
VII
6
12
18
24
Time in hours

than the prothrombin at the start of the observations but by 24 hours, both are restored almost to normal. The rapid improvement in the thromboplastin generation of the coumarin serum is shown in figure 154 . These results are the mean of three observations.

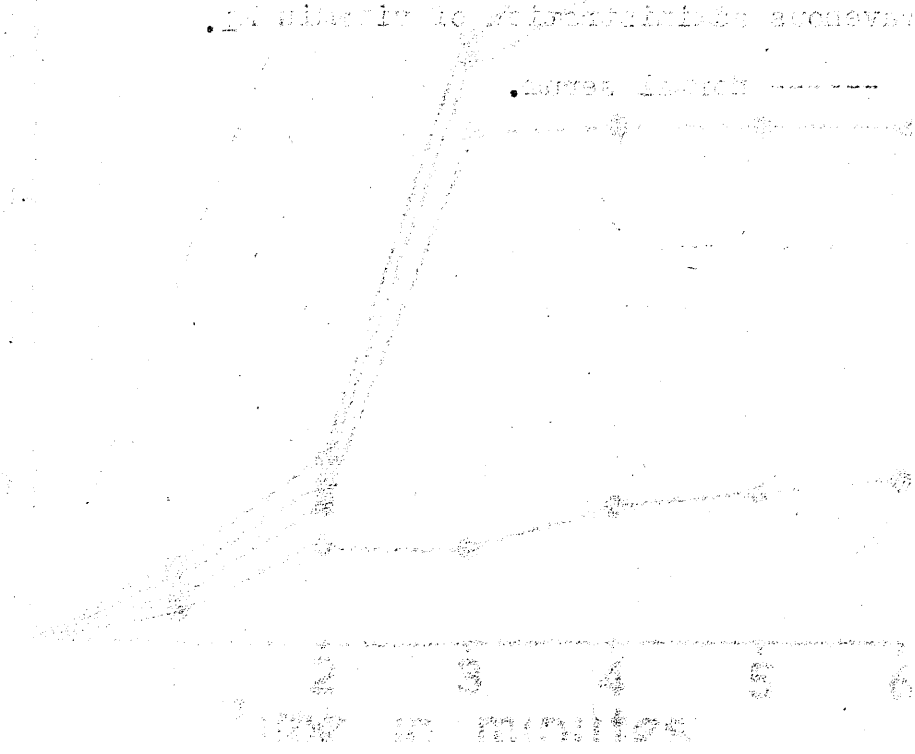
(2) Patients on coumarin therapy not given vitamin K_1. The response of the prothrombin and factor VII to cessation of therapy without administration of vitamin K is shown in figure 152 and in thromboplastin generation in figure 153. It will be observed that the return of these aspects towards normal is much less rapid. The figures represent the mean of the three observations.

(b) Vitamin K Deficiency.

(3) The coagulation defect in vitamin K deficiency is the same as in coumarin drug therapy (see Chapter 13). There is deficiency of factor VII and to a lesser extent of prothrombin and an inability of the serum to form blood thromboplastin. The response to vitamin K_1 is similar in character and in speed of response to that in coumarin drug therapy. (See figure 155).

In one patient with vitamin K deficiency the serial specimens of sera collected, were tested not only for their ability to form blood thromboplastin but also for their

Figure (156)



Vitamin K deficiency - thromboplastin generation of sera, following intravenous administration of vitamin K_1.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation technique with adsorbed normal plasma and platelets constant.

●——● Sera from patient at stated intervals after intravenous administration of vitamin K_1.

------ normal serum.

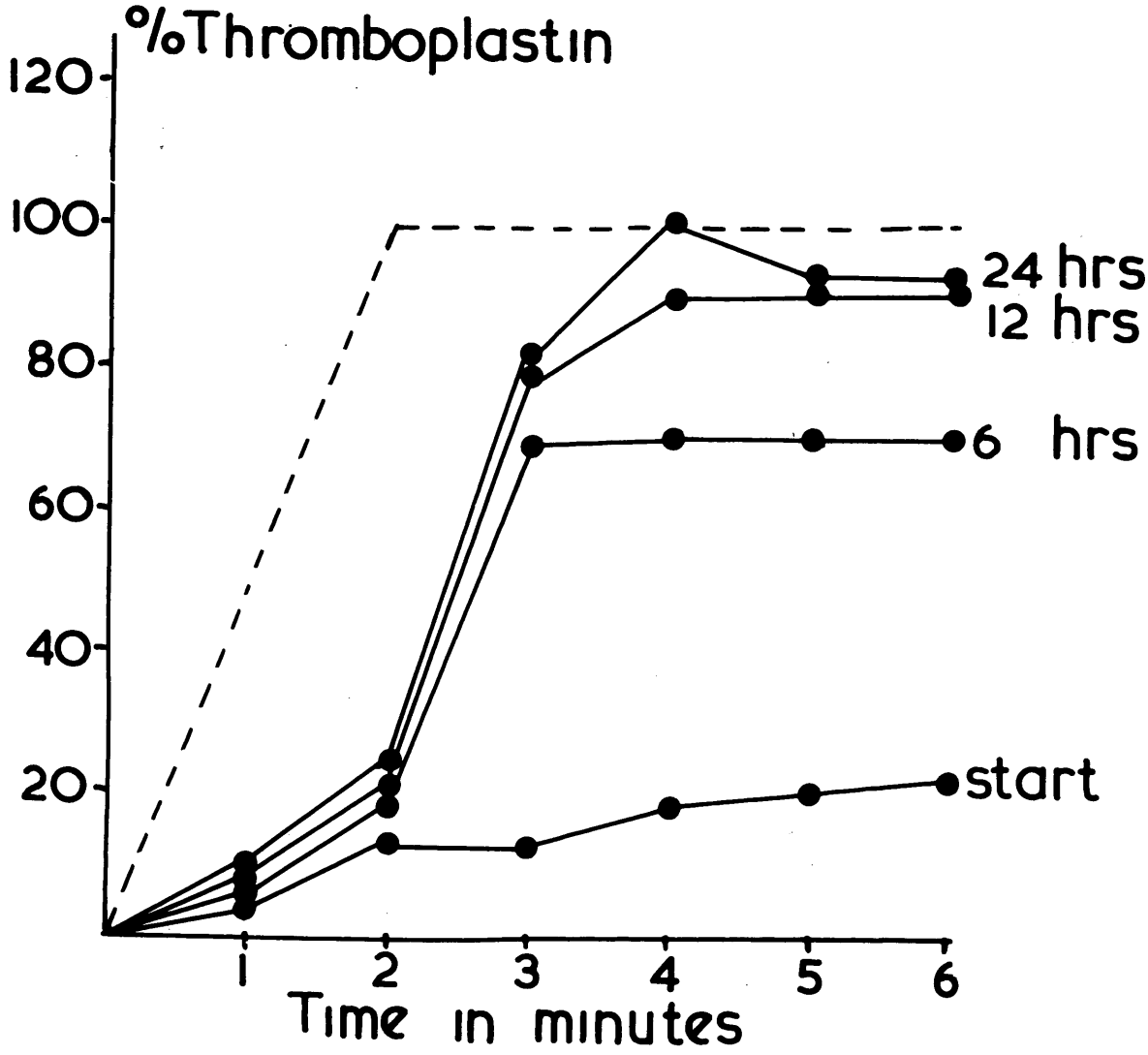
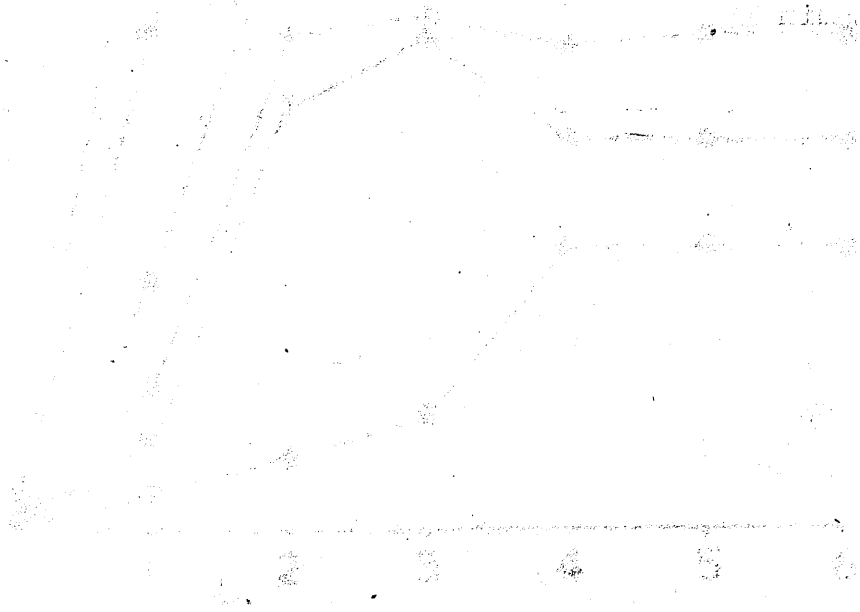

%Thromboplastin
120
100
80
60
40
20
24 hrs
12 hrs
6 hrs
start
1
2
3
4
5
6
Time in minutes

Figure (157)



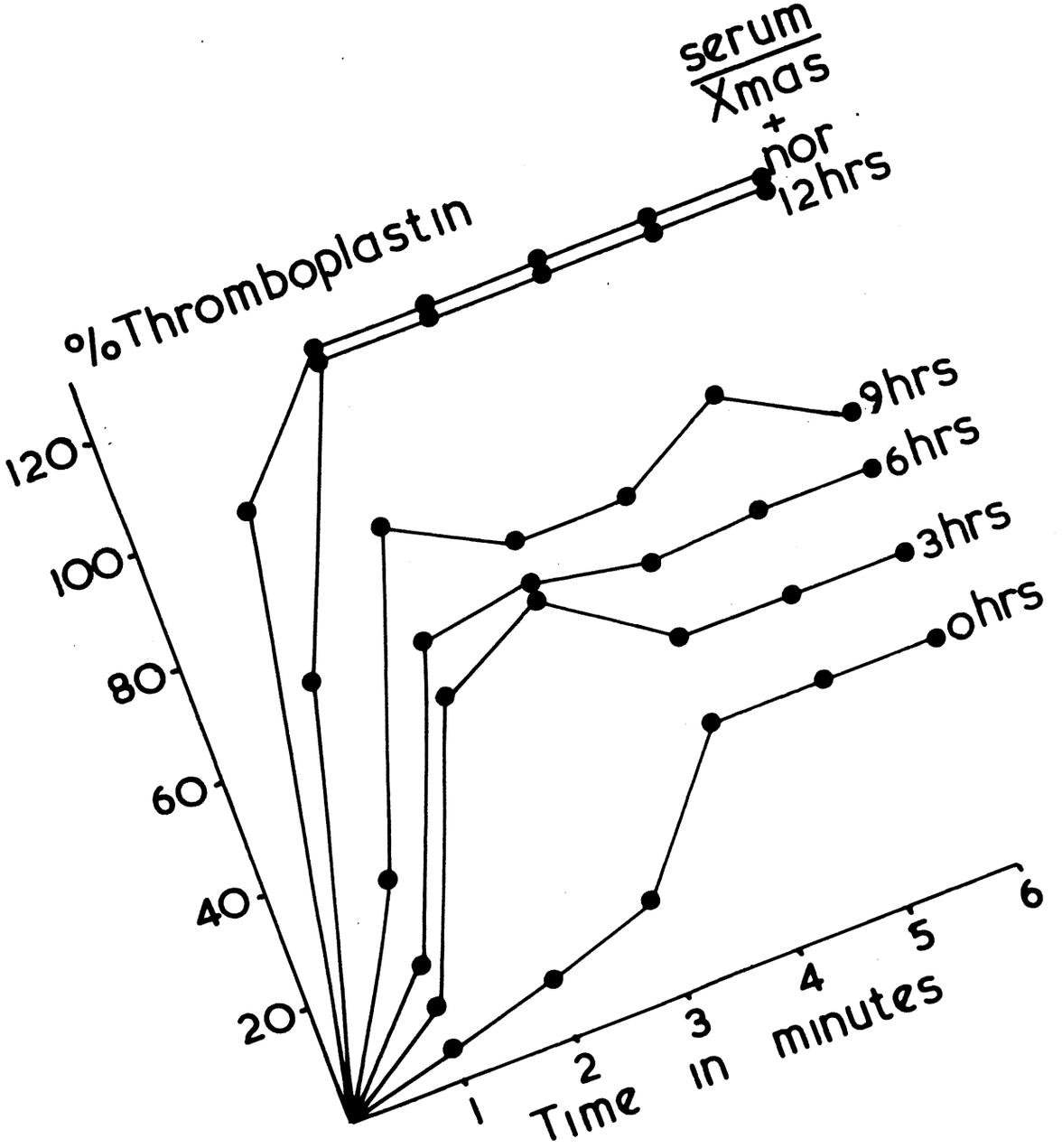
Vitamin K deficiency - thromboplastin generation of sera, following intravenous administration of vitamin K_1 - their ability to correct Christmas disease serum.

Ordinate - percentage thromboplastin.

Abscissa - time in minutes after addition of calcium.

Thromboplastin generation technique with adsorbed normal plasma and platelets constant.

●——● Mixtures of serum - Christmas disease serum with normal serum and serum collected from the patient at stated intervals after administration of vitamin K_1.


serum
Xmas
+
nor
12hrs
%Thromboplastin
120
100
80
60
40
20
9hrs
6hrs
3hrs
ohrs
1
2
3
4
5
6
Time in minutes

ability to correct the serum thromboplastin deficiency of Christmas disease serum. The results of this experiment are shown in figures 156 - 157. It will be seen that there is initially failure to correct the Christmas factor defect but following the administration of the vitamin K the serial specimens of serum show progressive ability to rectify the thromboplastin deficiency in Christmas disease serum. This is supportive evidence that there is a deficiency of the Christmas factor in the coagulation abnormality from vitamin K deficiency. (see also Chapter 13).

(c) Salicylate therapy.

(4) In the patient on salicylates the administration of the vitamin K_1 caused restoration to normal concentration of the prothrombin, factor VII and serum thromboplastic activity.
