

PROGRESS REPORT 1967-1973

of

THE EXTERNAL STAFF AT THE OXFORD HAEMOPHILIA CENTRE

Director of the Centre

Rosemary Biggs, MA., B.Sc., Ph.D., MD., MRCP (Part-time M.R.C.)

M.R.C. Scientific Staff

K. W. E. Denson, D.Phil. (1961-1971)

N.H.S. Medical and Scientific Staff of Oxford Haemophilia Centre

Dr. C. R. Rizza	Consultant Physician
Dr. J. M. Matthews	Medical Assistant
Dr. D. E. G. Austen	Principal Scientific Officer
Dr. R. T. Shahani	Senior House Officer (Jan. -Nov. 1967)
Dr. M. A. Gillan	Senior House Officer (Jan. 1968-Jan. 1969)
Dr. J. Eipe	Senior House Officer (Feb. 1969-Jan. 1970)
Dr. P. B. A. Kernoff	Senior House Officer (Feb. 1970-Jan. 1971)
Dr. D. Desnica	Senior House Officer (Feb. 1971-Jan. 1972)
Dr. R. McLennan	Senior House Officer (Feb. 1972-Jan. 1973)
Dr. Sophia Aroni	Senior House Officer (Feb. 1973-)

Visiting Workers

Research Workers

Dr. D. Green (From U.S.A.; Sept. 1966-Aug. 1967)
Dr. N. Akman (From Turkey; April-Oct. 1967)
Miss C. McIntyre (From U.S.A; June-Nov. 1967)
D. E. Riesenbergs (From U.S.A.; May-Sept. 1967)
Dr. J. M. Thompson (From Sweden and Birmingham University; Nov. 1967,
Feb. -March 1968, July 1968, March 1969)

Dr. A. Lurie (From South Africa; April 1968-March 1969).

Dr. S.G. Rainsford (From Lord Mayor Treloar College, Alton; Aug. 1968-)

W.E. Rousseau (From U.S.A.; June-Sept. 1968)

Dr. L. Uszynski (From Poland; Oct. 1968-March 1969)

Dr. G.W. Scott Blair (British Heart Foundation Grant Jan. 1969-March 1970;
Department of Neurology grant April 1970-March 1971)

Dr. P.N. Walsh (From U.S.A.; June 1969-June 1972)

Dr. Delfina Almagro (From Cuba; Sept. 1969-March 1970)

Dr. J. Eipe (Feb.-Oct. 1970)

Dr. F. Herynkopf (From Brazil; March-July 1970)

Dr. N.N. Sen (From India; Aug. 1970)

Dr. Linda Nahas (From Brazil; Sept.-Oct. 1970)

M.E. Haddon (From Nuffield Dept. of Obstetrics & Gynaecology; Oct. 1970-)

Dr. E.B. Crowell (From U.S.A.; Jan.-April 1971)

Dr. P.B.A. Kernoff (U.O.H. Clinical Research Fellow; Feb. 1971-)

Professor D. MacN. Surgenor (From U.S.A.; June-July 1971)

Dr. G. Casillas (From Venezuela; May 1971)

Dr. Rainer Gruson (From Germany; June 1972-June 1973)

Dr. Sophia Aroni (From Greece; July 1972-Feb. 1973)

Dr. J. Lemay (From Canada; Jan.-Sept. 1973)

Miss Prudence Nicol (Action for the Crippled Child grant; Jan. 1973-)

Dr. D. Thomopoulos (From Greece; May 1973-)

D.J. Stevens (From U.S.A.; June 1973-)

Dr. J.B. McSheffrey (From Canada; Aug. 1973-)

Dr. Judith Pool (From U.S.A.; Sept. 1973-)

Dr. A.G. Sanders (MRC Project Grant Holder; Sept. 1973-)

Professor R.G. Macfarlane (Sept. 1973-)

Medical, Scientific and Technical Visitors to Learn the Routine Laboratory
and Clinical Practice or Research Techniques Used at the Oxford Haemophilia Centre

Dr. A. Aronstam (From London; July 1967)

Miss Barbara Hatley (From Derby; July 1967)

Dr. Margaret Jenkins (From London; Nov. 1967)

K. Collins (From London; Nov. 1967)

Dr. Leila Al-Naib (From Iraq; Dec. 1967-May 1968)

L.R. Bacon (From Margate; Jan. 1968)

Miss Pamela R. Trendell (From Dorchester, Dorset; Feb. 1968)

J. Jefferey (From Kettering; Feb. 1968)

P. W. Price (From Aylesbury; Feb. 1968)

Dr. F. de Cataldo (From Italy; March 1968)

F. Allison (From Bournemouth; March-April 1968)

Dr. N. Enev (From Bulgaria; June 1968)

Dr. Audrey Dawson (From Aberdeen; Sept. 1968)

Dr. K.S. Kottas (From Greece; Oct. 1968)

Dr. Freda M. Roberts (From Liverpool; Oct. -Nov. 1968)

Miss Aileen M. Burn (From Manchester; Oct-Nov. 1968)

S. Mikelsen (From Norway; Oct. 1968)

Dr. P. M. Jones (From Newcastle; Dec. 1968)

Dr. D.H. Orrell)
) (From Warwick; Dec. 1968)

D. R. Raven)

A. Oxley (From Newcastle; March 1969)

Dr. A. Napier (From Cambridge; March 1969)

Dr. W.H.P. Lewis (From Carshalton; March 1969)

Dr. W. T. Menke (From Cambridge; March-April 1969, Dec. 1972)

Miss S. V. Mead)
) (From Reading; March-April 1969)

Miss Janis Hawkins)

J.D. Cordon (From Derby; April 1969)
G.M. Wilkinson (From Sheffield; April 1969)
Dr. Ilana Tatarsky (From Israel; April-May 1969)
Miss J.D. Cotgrove (From London; May 1969)
Dr. A.K. Saraya (May-June 1969)
Miss A. Sansom (From Alton; June 1969)
Dr. M.N. Prematilleke (From Ceylon; Aug. 1969)
Dr. Boonchai Sombotparnica (From Thailand; Aug. 1969)
Dr. V. Sanchis-Bayarri Vaillant (From Spain; Sept. 1969)
C. Morse (From Greenford, Middx.; Sept-Oct. 1969)
A. Stopforth, MRCVS (From The Animal Health Trust Equine Research Station,
Newmarket; Oct. 1969)
M. Gascoigne (From Sheffield; Dec. 1969)
Miss V. Stickley (From The Lister Institute, Elstree; Feb. 1970)
Dr. B.S. Saraladerni (From Reading; Feb. 1970)
Dr. J.M. de Pina Cabral (From Portugal; March 1970)
Dr. H. Ekert (From London; March 1970)
D.A.W. Waters (From Enfield, Middx.; April 1970)
Miss S. Strain (From Eire; April 1970)
Mlle. J. Canard (From Paris; July 1970)
Dr. C. Larrain (From Chile; Aug. 1970)
Dr. K.A. Rickard (From Australia; Aug. 1970)
Prof. M. Yamanaka (From Japan; Aug. 1970)
Dr. V. Massiah (From Trinidad; Sept. 1970)
Dr. (Mrs.) L.G. Farag (From Egypt; Sept. 1970)
Miss G.R. Myers (From South Africa; Oct. 1970)
Dr. M. Rutlant Baneres (From Spain; Oct.-Dec. 1970)

Dr. Jill Durrant (From Oxford Regional Hospital Board; Oct. 1970-1972)

Dr. J.-J. Walsh (From Nuffield Department of Obstetrics & Gynaecology;
Oct. 1970-Jan. 1971)

Dr. A. M. Holburn (From London; Nov. 1970)

Dr. M. Bronovic (From N.I.M.E.; Dec. 1970)

Mr. G. Newell (From South Africa; May 1971)

Mr. K. K. Das (From India; June-Aug. 1971)

Miss K. A. Kimber (From Basingstoke; June 1971)

Mr. T. Thomas (From Guy's Hospital, London; June 1971)

Dr. M. Venkatesan (From India; Aug. 1971)

Mr. B. Williamson (From Leics., Aug. 1971)

Dr. S. Hashimoto (From Japan; Aug.-Sept. 1971)

Miss E. Wilson (From Royal Free Hospital; Sept. 1971)

Mr. F. Mitchell (From Pulham Hospital; Sept. 1971)

Dr. G. Sitar (From Italy; Sept. 1971)

Dr. I. Temperley (From Dublin; Sept. 1971)

Dr. S. Chaudhuri (From India; Oct. 1971)

Dr. M. M. Hutton (From Glasgow Royal Infirmary; Oct. 1971)

Dr. M. McEwan (From Aberdeen; Oct. 1971)

Miss M. Allardyce (From Aberdeen; Oct. 1971)

Miss S. R. Wilson (From Leeds; Oct. 1971)

Mr. J. B. Fail (From Newcastle; Oct. 1971)

Mr. B. M. Sarup (From India; Feb. 1972)

Dr. C. H. Soto (From Spain; April 1972)

Miss Sophak Rejanasthen (From Thailand; April-July 1972)

Miss M. Bennett, SRN (From Lord Mayor Treloar College, Alton; April 1972)

Mr. C. J. Hills (From Orsett Hospital; April 1972)

Mr. M. V. Mellaerts (From Orsett Hospital; April 1972)

Miss M. A. Curran (From Reading; April 1972)

Dr. J. Tomlinson (From Alton; May 1972)

Dr. Susan Elodi (From Hungary; June 1972)

Dr. J. B. King (From South Africa; June 1972)

Miss L. S. Malpass (From Oxford Regional Blood Transfusion Centre; June 1972)

Dr. S. L. French (From Nuffield Department of Obstetrics & Gynaecology, Oxford
May 1972)

Miss M. Clatworthy (From Orsett Hospital; June 1972)

Dr. E. Rocha (From Spain; July-Aug. 1972)

Mr. Luc Noel (From France; July 1972)

Mme. Noel (From France; Aug. 1972)

Mr. J. D. Corden (From Derby Royal Infirmary; July 1972)

Dr. P. Dragos (From Roumania; Oct. 1972)

Mr. N. Porter (From Leicester Royal Infirmary; Oct. 1972)

Mr. I. Aldersley (From Banbury; Oct. 1972)

Dr. T. Watanabe (From Japan; Nov.-Dec. 1972)

Mr. J. Cassady (From Manchester; Jan. 1973)

Miss D. C. Pope (From Bournemouth; Jan. 1973)

Miss A. Cossally, SRN (From Liverpool Royal Infirmary; Jan. 1973)

Mr. P. N. Turner (From Banbury; Feb. 1973.)

Dr. D. Ramsey (From Edinburgh; Feb. 1973)

Miss S. J. Main (From Luton Hospital; Feb. 1973)

Miss R. Daly (" " " ")

Miss J. Cowan (" " " ")

Miss G. Ashby (" " " ")

Miss L. Chambers (From London; March 1973)

Miss M. Lamb (From Winchester; March 1973)

Miss I. Gore (" " ")

Miss L. Twistleton (From Daventry; April 1973)

Miss S. Honeybone (From Buckingham; May 1973)

Dr. N. Lucie (From Glasgow; May 1973)

Dr. S. Khakee (From Canada; May-June 1973)

Dr. Helen Armitage (From Brighton; June 1973)

Mr. S. Koh (From Singapore; July 1973)

Introduction

In 1967 the new Oxford Haemophilia Centre was established and the work previously undertaken by the M.R.C. Blood Coagulation Research Unit under the direction of Professor R.G. Macfarlane was reorganized. The clinical work was transferred to the N.H.S. and housed in a new building, which was completed in 1968. The fractionation work designed to develop new and better methods for producing therapeutic materials for the treatment of patients with coagulation defects was also transferred to the new building's Plasma Fractionation Laboratory under the direction of Dr. Ethel Bidwell. The Plasma Fractionation Laboratory is attached to the Blood Products Laboratory of the Lister Institute, and is the responsibility of Dr. W. d'A. Maycock. The M.R.C. has supported a Research Laboratory housed in the original building used by the Blood Coagulation Research Unit. The Research Laboratory is concerned with developing research in all aspects of haemostasis and blood coagulation and works in close cooperation with the Clinical and Fractionation Departments. The present report concerns work undertaken in the Clinical Department and in the Research Laboratory.

INTERNATIONAL COOPERATIVE STUDIES

Drs. Biggs and Denson have been involved since 1964 in the promotion of standards for use in blood coagulation research and for the control of therapy in patients. This work was carried out in association with the World Health Organization (Drs. Bangham and Outshoorn) and with the International Committee on Haemostasis and Thrombosis and has been concerned with two problems: firstly, the provision of a factor-VIII standard and secondly with the development of a method for standardising the control of anticoagulant therapy with the coumarin drugs.

Factor-VIII Standard

This standard is required for the uniform expression of results of factor-VIII assays and particularly as a means of producing therapeutic material (for haemophilic patients) with reliable activity. A collaborative study was planned by Dr. Bangham and Drs. Denson and Biggs and carried out by members of the International Committee for Haemostasis and Thrombosis during 1968. The report was circulated and corrected and in 1970 finally submitted for publication (Bangham et al. 1971). Two preparations were studied. One was a freeze-dried concentrate provided by Dr. A. Johnson (Newman et al 1971); the other was a freeze-dried pooled normal human plasma. The concentrate proved very stable and was accepted as W.H.O. standard for factor VIII in 1970. The plasma preparation was less stable but considered suitable for use as a short term reference preparation for use in Great Britain and is being distributed for this purpose.

We are studying the automation of the factor-VIII assay using a machine developed in association with scientists at A.W.R.E., Aldermaston (Steed and Trowell, 1969). The method involved the development of a new

reagent by Dr. Denson. This reagent is now commercially available and the machine has recently been modified and is still being tested.

The Control of Anticoagulant Therapy with Coumarin Drugs

In 1969 Biggs proposed a method for standardizing the one-stage prothrombin time for the control of anticoagulant therapy. Several clinical trials of this method were undertaken in association with the International Committee for Haemostasis and Thrombosis and the World Health Organisation, and a final trial was in progress in 1970 (Biggs 1965, 1967, 1969 (a & b), 1970 (a & b), 1971, 1972; Biggs and Denson 1966, 1967 (a & b); Denson 1966, 1967, 1969, 1971a). Preparations of reference thromboplastin reagent are held by Dr. Bangham and are ready for release. The method has been integrated with the Manchester Thromboplastin Scheme and the College of Pathologists to provide a British Comparative Reagent which has been calibrated with the International Reagent held by Dr. Bangham. The method is now quite widely used.

CLINICAL STUDIES

The official opening of the new clinical Oxford Haemophilia Centre was celebrated by inviting the Directors of the 36 Haemophilia Centres of the United Kingdom and holding a meeting at which research projects were planned in conjunction with the M.R.C. Cryoprecipitate Working Party.

Haemophilia Centre Directors Report

The project promoted by the Haemophilia Centre Directors concerns the incidence of jaundice in patients treated for haemophilia and Christmas disease and the occurrence of antibodies directed against factors VIII and IX in these patients. A paper on this subject is at present in press (Biggs 1974). The study has shown an annual average incidence of jaundice of 1.83 per cent. The incidence was increased to between 3 and

5 per cent per annum in patients who received substantial amounts of concentrate made from pools of plasma. The use of concentrates does not drastically increase the incidence of jaundice. The incidence of factor VIII antibodies is about 6 per cent and does not seem to be increasing.

Cryoprecipitate Working Party Report

The Cryoprecipitate Working Party (Chairman: Dr. Rosemary Biggs; Secretary: Dr. C.R. Rizza) has studied the amounts and varieties of factor VIII used in Great Britain. A comparison has been made of cryoprecipitate and freeze-dried concentrate. At present most of the available material is in the form of cryoprecipitate. The study has shown substantial advantages for the freeze-dried concentrate. A survey disclosed that very much more material is required than is at present available. It is estimated that material derived from 350,000 to 700,000 blood donations annually should be fractionated to make factor VIII.

The Effect of Epsilon-aminocaproic Acid on the Effectiveness of Factor-VIII Treatment for Dental Extraction in Haemophilic and Christmas Disease Patients

In this clinical trial patients were allocated at random to test or control groups. All patients received one large dose of factor VIII on the day of operation and no further specific treatment was given unless bleeding occurred. The test group received EACA in orange juice and patients in the control group received orange juice. The results showed a clear advantage in reduction in bleeding for those treated with EACA (Walsh *et al* 1971). The method of treatment defined in this trial has been in use for two years and the successful treatment of 94 patients has confirmed the results of the trial.

Clinical Trial of Corticosteroids on Haematuria in Haemophilia and Christmas

Disease Patients

In this trial, which is now in progress, patients at the Oxford Haemophilia Centre are allocated at random to trial and control groups. Each patient receives a standard dose of factor VIII or factor IX concentrate calculated on a weight basis. The patients also receive tablets (placebo or Prednisolone) and instructions for use according to the patient's weight. The patient records the duration and severity of haematuria 3 times a day. Further factor VIII or IX treatment is given as required. The trial is now nearly completed.

Clinical Trial of Prophylactic Treatment in Haemophilia

This trial is being conducted at the Lord Mayor Treloar College at Alton. The planning and organisation of the trial was worked out in Oxford and subsequently discussed at the Haemophilia Centre Directors Conference (October 1972) in order to perfect the plan in all its detail. The boys included in the trial have just completed the first term's treatment and many unforeseen problems have arisen. For example, the statistical plan lead to many boys being allocated to the control group in the first term. This gave the "treatment" a bad reputation with the boys.

Antibodies to Factor VIII

The development of a factor-VIII antibody is a serious hazard for haemophilic patients since the presence of an antibody interferes with specific therapy. These antibodies also occasionally arise in previously normal people. Dr. Rizza has been studying the effects of treatment of haemophilic patients with antibodies with factor-VIII concentrates from the point of view of clinical improvement, and also the incidence of inhibitors in patients who had received animal AHG (Rizza and Eipe, 1970). He has also

studied the treatment of patients with spontaneous factor-VIII antibodies (previously normal people) using immuno-suppressive drugs and factor-VIII concentrates (Rizza et al 1972). In addition Dr. Rizza has been studying the effects of treatment on antibody potency in haemophilic patients (Rizza and Biggs 1973).

EXPERIMENTAL STUDIES

Experimental studies have considered:-

1. The nature of the reactions between antibodies to factor VIII and the factor-VIII antigen.
2. The nature of the abnormality in patients with coagulation deficiencies.
3. The connection between complement and blood coagulation.
4. The role of platelets in haemostasis and blood coagulation.
5. The development of methods for the study of naturally occurring inhibitors of blood coagulation.
6. The study of the coagulant activity of snake venoms.
7. Rheology and blood coagulation.
8. A direct study of haemostasis.

1. The Nature of the Reaction between Factor VIII and Antibody

Biggs and Bidwell (1959) studied samples from six patients with factor-VIII antibodies. They studied the kinetics of the interaction and came to the conclusion that the reaction was essentially bimolecular and that the relative concentration of antibody could be measured using this assumption. Biggs and Bidwell (1959) restricted their observations to the destruction of bovine factor VIII by human antibody and their observations for this particular test system have not been invalidated. Since 1959 many more antibodies have been studied and their reactions with human factor VIII have

been observed. A detailed study of these reactions has been made (Biggs et al. 1972 (a & b). Following from these studies of kinetics a new method of measuring anti-factor VIII antibody potency has been proposed (Rizza and Biggs 1973) and a method for measuring the antibody absorbed by factor VIII has been proposed (Biggs 1974). These studies are important since the safe treatment of patients having antibodies depends on a knowledge of the potency and mode of factor VIII destruction. In addition much importance is often attached to the results of antibody absorption tests, though as usually carried out these have considerable error even when most carefully controlled (Biggs 1974).

Since September 1973 Dr. Judith Pool has been working at the Centre and the whole problem of the mode of action and stimulation of factor VIII antibodies is being studied further.

2. The Nature of the Abnormality in Patients with Coagulation Defects

Patients whose blood fails to clot normally may lack a particular coagulation factor or their blood may contain a defective protein. The distinction between the two possibilities may be made using techniques of antibody neutralization. It has been found that factor-IX deficiency may be due to lack of factor IX or to the presence of a structurally abnormal protein which retains ability to neutralize antibody. Similarly factor-VIII deficiency can be divided into two categories. Factor X deficiency is even more complex and three or four different categories may be identified (Denson et al 1968; Denson et al 1969; Denson et al 1970).

The defect caused by the Coumarin oral anticoagulants is due to deficiency of factors II, VII, IX and X. It is probable that this is not a complete deficiency in most cases but that proteins are formed which are antigenically similar to normal with reduced activity (Denson 1971b).

The human factor VIII neutralising antibodies used to distinguish two types of factor VIII deficiency do not form precipitates with factor VIII. Similar antibodies can be raised in rabbits which in addition to destroying factor VIII activity also form precipitates with a protein related to factor VIII. This protein is present in normal amounts in all haemophilic patients, including those who have antibodies against factor VIII. In normal people the concentrations of factor VIII activity and precipitable protein are correlated. Female carriers of haemophilia have normal amounts of precipitable protein. In von Willebrand's disease the levels of protein and factor VIII activity are correlated. It could be postulated that the protein is developed through an autosomal dominant gene which is defective in von Willebrand's disease. It is possible that this protein is normally important in promoting platelet adhesiveness and through adhesiveness a normal bleeding time, both of which are abnormal in von Willebrand's disease. These features are at present under study.

The actual relation of this protein to factor VIII at a molecular level is difficult to study but Dr. Austen has evidence about the aggregation of factor VIII molecules which may be relevant.

Practically the knowledge of the existence of this factor VIII related protein has lead to many lines of study at the Oxford Haemophilia Centre. Various types of protein from normal people, haemophilic patients and patients with von Willebrand's disease have been used to raise antibodies in rabbits and the cross reactions of these antibodies with various antigens are being investigated (Kernoff and Rizza 1973a). The levels of factor VIII related antigen in female carriers of haemophilia has substantially increased the probability of a reliable diagnosis of the carrier state (Kernoff and Rizza 1973b). This work is under the direction of Dr. C.R. Rizza.

Chemical Studies on the Nature of Factor VIII Activity.

Factor VIII

is destroyed by many things. It is labile on storage and destroyed by haematoporphyrin in the presence of light. The lability of the activity provides a method of studying the nature of the chemical groups involved in the activity of factor VIII. Factor VIII is destroyed by hydrogen peroxide, iodine, iodoacetamide and the sodium salt of p-chloromercuribenzoic acid. Each of these reagents is known to react with thiol groups. In the case of the mercury compound, the factor-VIII activity can be regenerated by cysteine. It thus seems likely that thiol groups are associated with factor-VIII activity. These observations are important since they open up a new approach to preventing the loss of factor-VIII activity on storage, a major difficulty in preparing therapeutic material; there is also the possibility of finding a non-toxic hapten substance which might neutralize factor-VIII antibody (Austen 1970). Further experimental work has shown that factor VIII contains a carbohydrate moiety which is an essential part of the molecule (Austen and Bidwell 1972).

Work is now in hand to study the molecular aggregates of factor VIII and it does appear from recent experiments that factor VIII could be a loose aggregate of glycoprotein molecules. In addition the ability of chemically-modified factor VIII to generate antibodies is being studied to examine the possibility of preparing new therapeutic materials for use in patients who have developed antibodies. From this new knowledge of factor VIII structure it is hoped that new isolation procedures can be formulated and that eventually that the storage stability of factor VIII can be improved.

We have also studied the effect of exercise on the plasma factor-VIII concentration in normal and splenectomized subjects. Exercise increases the concentration in normal subjects and also after splenectomy (Rizza 1971; Rizza & Eipe 1971). A special feature studied was the time scale of the increase in relation to the severity of exercise. Rather curiously the increase is very sudden in onset and unlikely to be due to release of activity from a depot (e.g. the spleen). Another feature studied was the half-life of the activity stimulated by exercise; this is much shorter than the half-life of infused material (Rizza 1971).

3. The Connection Between Complement and Blood Coagulation

Miss Prudence Nicol started work at the Oxford Haemophilia Centre in January 1973. She is studying the connection, if any, between complement and blood coagulation and assisting research work using immunological techniques. She is in receipt of a grant from the National Fund for Research into Crippling Diseases (Action for the Crippled Child).

4. The Role of Platelets in Blood Coagulation

Platelets in freshly collected platelet-rich plasma have no very marked effect on blood coagulation. On exposure to foreign surfaces or aggregation they are said to release activity. This activity is called platelet factor 3 (PF3) and is thought to be concerned in the interaction of factors II, V and X. A difficulty in the study of the release of platelet activity is that all previously described methods of platelet separation release the activity and thus the development of activity could be studied only in platelet-rich plasma. A method of platelet separation has been developed which preserves the platelets in the inactive state with respect to blood coagulation. Using these separated platelets it has been possible to study the effects of ADP, collagen and other stimulating substances. It has been found that ADP

activates factor XII adsorbed to platelets and promotes an activity which accelerates the activation of factor X by factors VIII and IX. Collagen has a similar effect but also activates factor XII in plasma and is more notably effective in releasing platelet factor 3 (Walsh 1972a, b & c).

This work is also throwing considerable light on the role of the contact phase of blood coagulation which is the earliest stage of clotting. The enigma of the contact reaction lies in the fact that patients who lack factor XII have no haemostatic defect. Thus although factor XII is the factor which initiates the chain reaction of clotting in a glass tube it seems that this factor is not required for haemostasis. It seems possible that on stimulation platelets may release an activity which bypasses the coagulation factors XI and XII (contact factors) which are concerned in the contact reaction as it is studied in vitro and with platelets replaced by phospholipid (Walsh 1972; Walsh and Biggs 1972).

Dr. Peter Walsh obtained the degree of D.Phil. in the University of Oxford for this work. He has now left the Centre. Since then we have been attempting to promote the methods that he used from the sphere of research investigation to routine methods to be used for the day-to-day study of patients. Shortly before leaving Dr. Walsh made a preliminary study of platelets in haemophilic patients. This study suggested a possible inverse relation between platelet coagulant activity and frequency and severity of bleeding. It was suggested that platelets of the more severely affected patients might have less coagulant properties than platelets from mildly affected patients (Walsh et al 1973).

We have been attempting to continue these observations on haemophilic patients. We have had considerable technical difficulties with one of the methods and find the visual record of clotting time in this technique

to be unreliable. Photoelectric record of increasing opacity (instead of measuring clotting time) does not so far confirm the results for the haemophilic patients.

5. Methods for the Study of Natural Inhibitors of Blood Clotting

Blood clotting may be defective due to the absence of clotting factors or excess of inhibitors. In addition reduction in concentration of inhibitory substances might predispose to thrombosis. We have made a study of two inhibitory substances: antithrombin III and anti-Xa and developed a method for their measurement (Biggs et al 1970). The method is being used to study variations which occur in post-operative patients (collaboration with Mr. J. Bonnar) and the effects of low dosage heparin in various classes of obstetric patients (e.g. eclampsia and failure of foetal growth).

6. The Study of the Coagulant Activity of Snake Venoms

The snake venoms coagulate mammalian plasma by activation of the coagulation mechanism in different ways. Use may be made of these venoms as reagents in the measurement of clotting factors. Thus Russell's viper venom activates factor X and can be used in the measurement of factor X. The Taipan venom activates prothrombin (factor II) and a method for the measurement of prothrombin using this venom has been developed (Denson et al 1971).

7. Rheology and Blood Coagulation

Dr. Scott Blair and Mr. Matchett (Chief Technician) have been working on the changes in viscosity which occur in normal and abnormal blood during coagulation. So far only factor-XIII deficient blood has shown a definite pattern of abnormality suggesting that the viscosity changes are related to fibrinogen polymerisation. A similar pattern has been found in some patients with disseminated sclerosis; no reason for this latter change

has been found. This work has now terminated and the results were published (Scott Blair & Matchett 1972, 1973).

8. Haemostasis

Professor R.G. Macfarlane and Dr. A.G. Sanders, with the assistance of Mr. Matchett, are devising a system for the photographic recording of platelet and blood clotting changes in flowing blood in an artificial chamber. This is a continuation of their previous work on blood platelet behaviour in the vessels of the hamster cheek pouch. It is hoped that it will be possible to make experiments with closely controlled conditions varying the types of platelets, surfaces and blood clotting factors which will promote or hinder thrombus formation.

COOPERATION WITH DR. BIDWELL

Dr. Bidwell is developing new therapeutic materials and the activity of these is being tested in the Research Laboratory. A new concentrate containing factor IX, prothrombin and factor X, and a separate concentrate containing factor VII, was developed by the Plasma Fractionation Laboratory in collaboration with the Clinical Laboratory during 1969-71 (Dike et al 1972). Dr. Bidwell is also cooperating with Dr. Austen in the study of the chemical nature of the group responsible for factor-VIII activity. Dr. Bidwell has recently appointed a graduate scientist to develop and supervise blood coagulation techniques under the general supervision of Dr. Biggs.

TEACHING

We have welcomed 112 visiting doctors, scientists, technologists, nurses and physiotherapists to learn routine and research techniques in

blood coagulation and to learn about the clinical care of patients with haemostatic defects. Much of the research work carried out in the Centre has been done by doctors and scientists visiting for periods of six months to three years.

FUTURE PLANS

1. The standardisation work on thromboplastin and factor VIII is complete. We shall continue to cooperate with Dr. Bangham on new standardisation problems which arise. At present we are helping with the standardisation of factor IX assay and assisting Dr. Bidwell to develop a reliable factor VII assay.
2. The nature of factor VIII antibodies will be studied further, with special reference to the structural relation between factor VIII activity and factor VIII related antigen.
3. The contact activity of platelets will continue to be studied in the hope of developing a reliable routine measure of this activity.
4. The studies on the chemical basis of factor VIII activity will be continued. This work is most important because a knowledge of the nature of factor VIII activity must assist in the provision of better clinical concentrates and must assist in research designed to prevent the formation of antibodies to factor VIII or provide a treatment for patients with these antibodies.
5. A major problem today concerns the organisation of haemophilia treatment in this country and the provision of adequate therapeutic materials. Factors VIII and IX can be administered to patients in the home, thus reducing the actual amount of work required in hospital. Such treatment needs close cooperation between patient, General Practitioner,

and staff of the Haemophilia Centre. We should like to make a social and medical study of the best ways that a home therapy service could be provided for the patient.

14th September, 1973.

REFERENCES

- AUSTEN, D.E.G. (1970). Thiol groups in the blood clotting action of factor VIII. *British Journal of Haematology* 4, 477.
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