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HISTORICAL SKETCH

Oxford, the Mecca for blood coagulation research in the 1950s and 1960s

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Gwyn Macfarlane was undoubtedly the instigator of blood coagulation research at Oxford during the last century. From his earliest paper in 1931 on a survey of hemophilia [1], he engendered an interest in the diagnosis and treatment of hemophilia. Joined by Rosemary Biggs in the mid 1940s, together they embarked on blood coagulation research. The fascination that blood coagulation had for its devotees is best described in the words of Biggs [2]: 'To begin with the spontaneous transformation of fluid blood to a solid clot has a stimulating effect on the curiosity like that produced by a well executed conjuring trick. One feels that so simple and striking a phenomenon must have a simple and striking explanation, and the urge to discover it is immediate. Next, there is the deceptive ease with which the work can be started. All that is needed is a few glass tubes and a watch, a supply of blood and ingenuity'. They augmented their coagulation research by pioneering replacement therapy for hemophilia and set the stage for the Mecca for blood coagulation research that Oxford was to become in the 1950s and 1960s. Naturally, hemophilics flocked to the Oxford area like moths to a light, to be near to where they could obtain the best available treatment in the UK, so that by 1960 half the total number of hemophiliacs in the UK were treated at Oxford. For their part they provided a very large database of hemophilia A and B patients. More and more patients with unusual hemostatic defects were also referred to Oxford, the plasma samples from whom provided useful tools in subsequent research.

The greatest problem in the early 1950s, because of the large number of local hemophiliacs to be treated, was the supply of sufficient therapeutic material which at that time was simply whole blood or fresh frozen plasma with all the attendant problems of circulatory overload. Because of the shortage of human plasma, it was Macfarlane (and not without criticism) who first suggested the use of concentrates from bovine and porcine blood despite their antigenicity. To this end Ethel Bidwell, a biochemist, joined the team in the early 1950s charged with this task and the subsequent preparation of concentrates from human plasma. Despite the risk of allergic reactions, the use of these animal concentrates saved the lives of many hemophiliacs and with effective replacement therapy allowed the use of major surgery. Although factor (F)VIII inhibitors did develop in many of these patients, they cross-reacted to a much lower titer with human concentrates subsequently prepared, and it was possible to switch the species of concentrate. Just before Langdell Wagner and Brinkhous published their landmark paper on the Partial Thromboplastin Time [3], the major breakthrough in hemophilia research came with the publication of the first seven cases of Christmas disease which appeared in the post-Christmas issue of the *BMJ* in 1952 [4]. Everybody looked at the paper because they thought it was something to do with overeating at Christmas (R. Biggs, personal communication). Some readers complained at the use of a religious festival for the name of a disease. The authors pointed out that the disease was named after the first patient, Stephen Christmas, but noted that if they discovered the precursor protein they would resist the urge to call it the Christmas Eve factor. Earlier observations had noted that the blood of some patients with hemophilia of classical X-linked recessive inheritance appeared to correct the defect in others. Biggs and her colleagues showed that the defect in classical hemophilia was corrected by antihaemophilic globulin in the fibrinogen fraction of normal plasma and by the plasma from patients with Christmas disease but not by serum fractions. The defect in Christmas disease was corrected by plasma or serum from normal patients or patients with classical hemophilia but not after the plasma or serum was absorbed with aluminum hydroxide. Both had abnormal formation of intrinsic thromboplastin, classical hemophilia in the absorbed plasma component, and Christmas disease in the serum component.

The thromboplastin generation test (TGT), a measure of intrinsic thromboplastin formation, was then published in 1953 by Biggs and Douglas [5] and this led directly to the development of assays for FVIII and factor (F)IX [6]. It also

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Fig. 1. From left to right. Ethel Bidwell, Gwyn Macfarlane and Rosemary Biggs outside the gardeners hut (the original Blood Coagulation Research Unit) in 1959.

Fig. 2. From left to right. Nripen N. Sen, Ken Denson, Jean Spooner, Charles Rizza outside the improved and extended gardeners hut in 1967.

allowed us to distinguish between factor (F)VII and factor (F)X and was widely used in coagulation research. All this work was done in cramped premises at the Radcliffe Infirmary Oxford. The early work of Macfarlane and Biggs and the publication of their first book on Human Blood Coagulation in 1953 had put Oxford on the map as the center of excellence for coagulation research in the UK. Because of this the Medical Research Council of the UK established and funded the Blood Coagulation Research Unit (BCRU) in 1959 and invited Macfarlane to be its Director. The premises initially were a converted gardeners hut at the Churchill Hospital which was rather appropriate because, before she received her MD, Biggs obtained a PhD in botany at Toronto University. The premises gradually expanded to provide a new laboratory for me and a new fractionation laboratory for Bidwell.

During the 1960s we developed the ratio method for the standardization of the prothrombin time (PT) for the control of oral anticoagulant therapy which led to the establishment of the International Calibrated Ratio and subsequently the International Normalized Ratio. Amongst many other projects we characterized the mode of action of FVIII inhibitors, antithrombin III, anti-FXa, heparin, and the coagulant action of snake venoms.

In the early 1960s, we realized that several reactions involved the conversion of pro-enzymes to active enzymes; for example, FX could be activated to an activated form by Russell's viper

venom which would then activate the pro-enzyme prothrombin to produce thrombin. It was Macfarlane [7] who visualized the 'enzyme cascade' in intrinsic clotting, in which pro-enzymes were activated to enzymes sequentially and with amplification, from the activation of factor (F)XII to FXIIa to the formation of a fibrin clot. Davie and Ratnoff published a similar mechanism independently at the same time which they called the 'Waterfall sequence' for intrinsic blood clotting [8], and these schemes remain to this day as the basis of our understanding of intrinsic and extrinsic blood coagulation. Visitors came from all over the world to learn and to copy, and thus Oxford became the Mecca for blood coagulation research and the treatment of patients with hemorrhagic diatheses.

Fresh from my studies on the characterization of FX, I joined the BCRU in 1960 and my brief was to supervise the routine assays, teach short-term visitors methods in coagulation, and to do collaborative work with long-term visitors. I thought I might miss clinical pathology in which the disciplines of bacteriology, clinical chemistry and hematology were practised in one laboratory at a small cottage hospital in London. My research was done mainly in the evenings and weekends and many of the small items of equipment were provided out of my own pocket. I can remember when I moved to Oxford, the sheer joy of being paid to do research with funding for equipment readily available. I had never known such luxury. In parallel with my other duties I commenced work on a DPhil thesis in the medical faculty at the University of Oxford [9]. I chose as subject 'The Use of Antibodies in the Study of Blood Coagulation'. It proved to be a useful topic which encompassed the purification of clotting factors, the preparation of polyclonal antibodies to the purified proteins, characterization of the antibodies and their use in kinetic experiments. The work had its lighter and darker moments. I shunned the use of fraction collectors, preferring to collect and test the chromatographic fractions immediately, and one darker moment concerned the use of such a fiendish invention. I had worked from morning to night for 4 days over an Easter holiday to produce the purest preparation (at that time) of FIX known to man. At midnight on Easter Monday I embarked on the final chromatographic separation, when a colleague who shall be nameless, returning slightly inebriated from the social club, suggested I use the fraction collector. I weakened at the thought of another 3 h of work and spent an hour connecting the contraption in the cold room. I watched the first two fractions through, the mercury switch made contact and the turntable moved round and I went home and slept like a baby. The next morning I discovered the fraction collector had jammed after the second fraction and deposited my factor IX all over the floor. I would gladly have retrieved it from the floor but the same colleague had been responsible for installing a drain in the floor and my pure FIX had gone straight to the sewer.

The use of antibodies answered many questions. At the time the proponents of the classical theory postulated that FVII, FIX and FX were required for the activation of prothrombin, based on the study of patients with various defects. This theory was challenged by W. H. Seegers, who on the basis of laboratory experiments without recourse to patients, hypothesized that prothrombin was autocatalyzed to a number of derivatives, autoprothrombin I (FVII), autoprothrombin II (FIX) and autoprothrombin C (FXa) in 25% citrate solution. He published hundreds of papers and held on to this theory doggedly for more than two decades, but the use of specific antibodies to the various clotting factors showed that the results of his experiments were due to contamination of prothrombin with trace amounts of other clotting factors, and in particular to the presence of FX.

Another useful application of the antisera was in the detection of genetic variants of clotting factors. During this period we thought that clotting defects were simple deficiency states in which the patients lacked the appropriate clotting factor with different degrees of expression.

We had a child with Christmas disease referred to us in 1963 because the plasma of the child had an abnormal Thrombotest which was due to a prolonged one-stage PT with bovine thromboplastin but a relatively normal PT with human thromboplastin. We published this as a short letter in the *Lancet*. This phenomenon niggled at the back of my mind for some years until suddenly a hypothesis germinated. What if this child instead of lacking FIX had a molecular variant of FIX which lacked biological activity and in some way because of its molecular structure interfered in the reaction with bovine brain but not with the more specific human brain? A hypothesis that cannot be tested is useless, so how to test this particular one? I had an antibody to bovine FIX which cross-reacted with human FIX. If this removed the molecular variant of FIX in the plasma sample from the patient, the PT with bovine brain should become normal. Two tenuous hypotheses rolled into one; it looked as though they would both be despatched to the waste bin. However, the antibody did remove the FIX variant completely and the PT with bovine thromboplastin became normal. In collaboration with P. M. Mannucci and Biggs we studied three patients with Christmas disease with prolonged PTs with bovine thromboplastin [10]. We showed that the abnormal FIX was also removed by inhibitors to FIX in Christmas disease patients, gave a line of identity on immunodiffusion with normal FIX and interfered in the reaction between bovine thromboplastin, FVII and FX, but not in the reaction of FXa on prothrombin. We called the variant hemophilia B^{+}. At the same time, Cecil Hougie and J. J. Twomey in the USA described similar findings and they proposed the name hemophilia B_M for the FIX variant [11]. They were not able to do the definitive test with an antibody but arrived at a similar conclusion.

It seemed extremely likely that if there were genetic variants of FIX in Christmas disease, there would also be genetic variants of FVIII in classical hemophilia. I devised an antibody neutralization technique to test for this, and set one of my willing assistants to work on screening hemophilic patients. After testing 20 patient samples, she came to me with a dejected look and said 'They are all negative'. 'Keep trying and you will find one' I said. Two days later she appeared with a beaming

face and said 'I have found two'. Thus was born a further paper on genetic variants of FVIII [12]. A study on genetic variants of FX followed in collaboration with Professors Mannucci and de Cataldo and Dr Lurie [13]. We managed to obtain six different plasma samples from patients with a FX defect and showed by different tests that the six patients from five different kindreds included five distinct and separate abnormalities of FX. Further studies on genetic variants of FVII were also later undertaken [14,15]. Of course these were the early days—times have moved on and we can now identify individual variants by their amino acid substitutions.

Thus, my antibodies served science well, but my contribution was small compared with the many contributions from Oxford. I have lost count, but not the memory of the many great people who entered the portals of Oxford for short or longer periods. To name but a few of many hundreds, these included Stuart Douglas, Hymie Nossel, Charles Rizza (who succeeded Rosemary Biggs as director of the Haemophilia Center), John Bonnar, Cecil Hougie, Ed Salzman, Jacques Caen, M. J. Larrieu, Gerardo Cassillas, Ugo Barbieri, David Green, Nripen N. Sen, Peter Kernoff, Peter N. Walsh, Albert Cattan, Aubrey Lurie and one known to you all, Pier M. Mannucci.

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