

# HAEMOPHILIA I:

## DOES THE ANSWER LIE AT THE BOTTOM OF AN "ICEBERG"?

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In 1957 a total of 153 Haemophilic families in Great Britain was recorded (1). Comparing the population of Britain and Malta and assuming the same incidence ratio, one would expect to find only one family of Haemophiliacs in Malta, whereas, in fact, 18 affected families are known (2). Since the number of units comprising a family in Malta was higher than in Britain, then there is a relatively higher incidence in Malta than is suggested by the above comparison.

The greatest number of reported cases of Haemophilia occurs among the Germanic peoples of Northern Europe, Great Britain and North America. Fewer cases are reported among the Latin races, and in the Asiatic and Negro races it seems to be almost unknown (1).

### WHY STUDY SUCH A RARE DISEASE?

In comparison with Arterial Diseases, Malignant Diseases, Rheumatoid and Osteo Arthritis etc. Haemophilia affects so few people that it almost seems superfluous to spend any time contemplating what is, after all, apparently a rare disease entity. It is more usual for the mechanism of Haemostasis to prove over-sensitive in the normal populace and a clot may form in some part of the intact circulatory system.

Thrombosis is all too commonly encountered by physicians and surgeons in the Western world. By the study of such rare conditions of lack of adequate Haemostasis new light might be thrown on the common condition of Thrombosis.

To enter the field of recent study of coagulation disorders is to be overwhelmed by the sheer volume of the material written, technical terminology used, and the controversial opinions held; but also it is to be enthralled by the ingenuity and perseverance shown by the great workers in this field.

### HISTORICAL

#### WHAT IS HAEMOPHILIA?

This might be easier to answer by stating what it is not, but most standard textbooks of Medicine define Haemophilia thus:-

"Haemophilia is a Hereditary Disease, affecting males but transmitted by females and characterised by a prolonged coagulation time and by lifelong tendency to excessive haemorrhage due to a quanti-

tative deficiency of Anti Haemophilic Globulin".

The word Haemophilia is derived from the Greek, Haima meaning blood and philia meaning affection. The word was coined by Hopft in 1828.

The earliest documentation of what is assumed to be Haemophilia occurred in the 2nd Century A.D. in the Talmud. The Jews and the Arabs observed that occasionally after the traditional circumcision a young boy would bleed to his death. They recognised that this bleeding tended to recur in certain families and exempted such families from this rite. Does this represent one of man's earliest attempts at controlling Natural Selection?

The next significant step was the publication in 1820 of Nasse's Law or Rule which stated:

"Women whose fathers were bleeders transmit the trait to their children even if married to normal men. In the women themselves and, in general, in no female person is the trait ever expressed".

1911 saw the publication of Bullock and Fil-des' "Treasury of Human Inheritance" (3) which surveyed the whole of the relevant literature then available, the term Haemophilia being reserved to cover the condition in which the following criteria were to be observed:-

1. Liability to excessive bleeding which had existed from infancy and was restricted to the male sex.
2. Evidence of similarly affected males in the family and of transmission only by the apparently normal female.
3. Demonstration of prolonged clotting time and absence of any other abnormalities that may cause this bleeding.

These criteria established Haemophilia as a syndrome (a running together) of excessive Haemorrhage, occurring in males of certain families, whose blood demonstrated a greatly prolonged clotting time. These criteria at the same time eliminated the considerable amount of confusion which had existed in the latter part of the 19th Century concerning the term "Haemophilia", which had been used to cover such disease entities as Thrombocytopenic Purpura, Telangiectasia and others, even scurvy!

This classic concept of Haemophilia was held for over forty years. Research of the last twenty years indicated that this view had to be modified greatly. Haemophilia had always been

a multifaceted disease — presenting a different face to each observer, depending on the observer's viewpoint.

In 1943 the life expectancy of a Haemophiliac was no more than 16 years. Nowadays, with the advent of modern therapy, Haemophiliacs can look forward to reaching the age of 50 or 60. Men not undergoing treatment are even able to shave with a safety razor if they wish since if they cut themselves they bleed — but only for a normal length of time.

#### **WHY DO HAEMOPHILIACS HAVE A NORMAL BLEEDING TIME?**

Large injuries cease to bleed because of retraction of the blood vessels AND coagulation of the blood. Injuries perforating to a depth of less than 5 mm cease to bleed because capillary contraction reduces the size of the injured area which becomes covered with a mass of fused platelets which form thrombi.

For normal Haemostasis to occur in man three main groups of factors are required.

1. An adequate supply of NORMAL platelets.
2. Active contraction of capillaries in response to injury.
3. Coagulation of blood occurring in normal time.

The coagulation time of whole blood obtained by venipuncture in a Haemophiliac is usually greatly prolonged. (The venipuncture can be performed without danger because the elasticity of the vessel wall is sufficient to close the wound when the needle has been inserted into the vein at an angle).

From the above it thus seems likely that Haemophiliacs bleed unduly because of a defect in Blood Coagulation.

#### **WHAT IS THE NATURE OF THE CLOTTING DEFECT IN HAEMOPHILIA?**

The defect is believed to be due to a deficiency in Haemophilic blood of Anti Haemophilic Globulin (A.H.G. or Factor VIII) which is present in normal plasma.

When the presence of this factor was postulated there was no means by which its function in the normal coagulation process could be demonstrated. A curious method was devised for the diagnosis of Haemophilia:-

A sample of blood taken from an individual suspected of suffering from Haemophilia was added to a sample of blood of a known Haemophiliac. If the coagulation time of the known Haemophilic blood could not be shortened by the addition of the suspected blood then the suspected case was labelled a Haemophiliac.

The pathologist relied on an accurate Clinical Diagnosis being made in the first place!

In 1953 Biggs and Macfarlane found seven cases of *Supposed* Haemophilia in which there was *no* deficiency of AHG. but a deficiency of another factor previously unrecognised, and named it Christmas Factor (Factor IX). To add to the confusion this factor is also called Plasma Thromboplastin Component (P.T.C.).

Many "known" Haemophilic families including those of the Tenna Valley in Switzerland are now identified as suffering from deficiency of Christmas Factor. Also in 1953 Rosenthal observed in six supposed cases of haemophilia that there was neither deficiency of A.H.G. nor of Christmas Factor but deficiency of a factor labelled Plasma Thromboplastin Antecedent (P.T.A. or Factor XI).

#### **WHAT WERE THESE NEW DEFICIENCY DISORDERS TO BE CALLED?**

The classical view of Haemophilia could now be regarded as covering THREE disease entities all having the criteria, necessary to be fitted into Bullock and Fildes' interpretation of Haemophilia. Many new classifications were suggested to cover this new state of affairs. That they are three separate entities can be shown by the fact that A.H.G. added to a sample of blood lacking Christmas factor will not lower the clotting time of that sample, a sample of Haemophilic blood added to that of Christmas Disease reduces the clotting time. Biggs and Macfarlane use the terms Christmas Disease for the cases deficient in Factor IX and Rosenthal Syndrome for those deficient in P.T.A., reserving Haemophilia for those patients whose blood shows a deficiency of A.H.G. (1)

A further anomaly was added by the observation that some families existed suffering from a relatively mild form of Haemophilia in which the clotting time might be within normal limits but with grossly abnormal levels of A.H.G. (Merskey et al. 1949). Thus whilst a long clotting time probably indicates a severe defect, a short clotting time does not necessarily mean that the patient is mildly affected and has little diagnostic or prognostic significance.

#### **AETIOLOGY**

##### **WHAT IS THE AETIOLOGY OF HAEMOPHILIA?**

There seems little doubt now that although there may be other factors deficient, the *essential cause* of the Haemophilic clotting defect is A.H.G. deficiency, either apparent or real, since the clotting time of Haemophilic blood can be restored to normal by the addition of A.H.G. The observation by Tocantins et al in 1951 (4) that the coagulation of Haemophilic plasma can be made to approach that of normal plasma by nothing more complicated than appropriate and optimum dilution is intriguing.

On reflection, although the apparent anomaly of Haemophilic blood having a normal Bleeding Time can be explained on the basis of the vascular factor necessary for Haemostasis functioning normally, why does a Haemophilic patient develop Haemarthroses so consistently?

A Haemophiliac, if he can survive the rigours of teeth extraction and other traumatic experiences in childhood that could cause him to bleed to death, nevertheless may spend a considerable amount of his time in Hospital receiving medical and/or orthopaedic treatment for Haemarthroses and their consequences.

The tendency to such serious effusions and haemorrhages into joints, muscles and various organs in Haemophiliacs (in Great Britain there is a plea from several quarters to replace the three wheeler invalid cars supplied to Haemophiliacs with well sprung modern saloons in order to minimise minor traumata and so minimise the incidence of joint effusions and haemorrhages) all point to the existence of a vascular abnormality which is not fully explained by the defect in coagulation. Pavlovsky (1958) suggested that an undue fragility of the blood vessels was responsible.

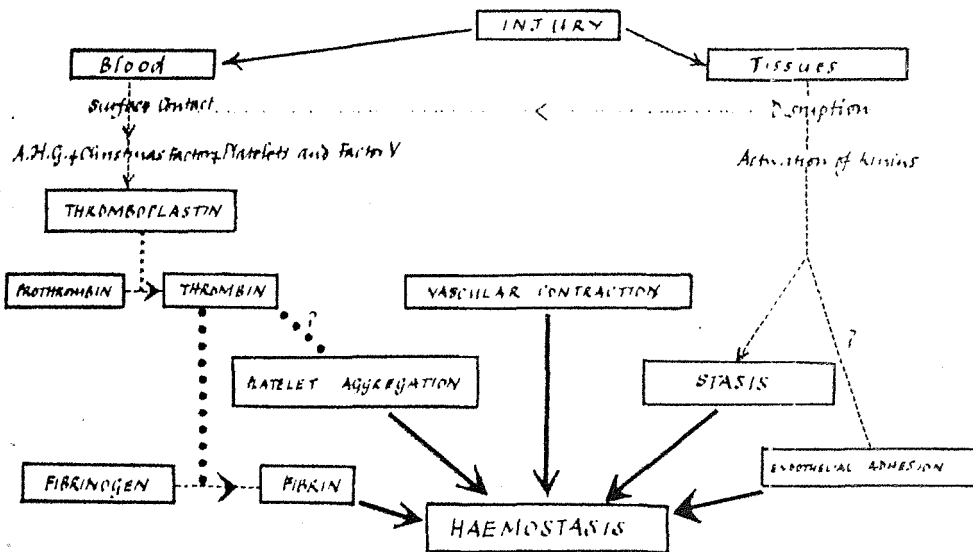
### WHAT OF THE THIRD FACTOR NECESSARY FOR HAEMOSTASIS — THE PLATELETS?

Here again there had been an anomaly. When a sample of normal blood is withdrawn from a vein the platelets quickly disappear before clotting occurs. But in Haemophilic blood, under the same conditions, the platelets may be found in the still fluid blood hours afterwards. The phenomenon of viscid metamorphosis (first described by Wright and Minnon in 1917) or Platelet Aggregation had not occurred.

In 1956 Bergsagel showed that following contact with a foreign surface A.H.G., Christmas Factor and  $Ca^{++}$  react to form an "Intermediate Product" which then causes the aggregation of the platelets.

As a result of these changes a granular material is discharged from the platelets which, in the presence of Factor V is extremely active in converting Prothrombin. A deficiency of A.H.G. will prolong the time which is required for the initial changes to take place — thus prolonging clotting time. What may be more important is that it will reduce the amount of Thromboplastin formed and the amount of Prothrombin which is converted during the process of coagulation. The end result of the coagulation process had been accepted to be Fibrin formation. (see Fig. 1)

FIGURE 1 : COAGULATION



**WHY THEN IS AFIBRINOGENAEMIA LESS OF A DISABILITY THAN HAEMOPHILIA?**

Afibrinogenaemia is a rare condition in which there is either Hereditary absence or an acquired absence of Fibrinogen. It presents a clinical picture resembling Haemophilia except that Haemarthrosis is very rare. Thrombin formation is normal and Platelet Aggregation occurs.

Thus it seems that Thrombin formation is more important to the Haemostatic mechanism as a whole than is Fibrin formation.

**HOW CAN A DISEASE WHICH SYSTEMATICALLY ERADICATES ITS SUFFERERS BE CONSIDERED TO BE AN INHERITED DISEASE?**

Haemophilia has been considered as a classical example of a sex-linked inherited disease. Perpetuation of the disease being considered to arise from the fact that a female may have one of her X chromosomes affected for Haemophilia but still be to all intents and purposes normal in phenotype. The mating of a Heterozygous (for Haemophilia) female with a nor-

mal Hemizygous (only one X) male producing sons in the ratio of one haemophilic to one normal and daughters in the ratio of one heterozygous to one normal. (see Fig. 2). In this way the apparently normal Heterozygous female can, it seems, transmit the disease in perpetuity, her one normal X chromosome being sufficient to prevent manifestation of her abnormal X. But selection against such an event is strong. Every time the gene is passed on to a male the chances of it surviving are considerably reduced.

When taking a Family History of Haemophiliacs it is necessary to enter into considerable detail.

Despite this, about a third of all cases in Britain will not show a Family History of the Disease apparently arising spontaneously. It was thought that these cases arose as a result of a mutation, the mutation occurring more frequently in males than in females (Haldane 1946). It was suggested that the genetic abnormality arose in the germ cell of the patient's maternal grandfather.

The pedigree of Haemophilia of the Royal Families of Europe strongly suggests that Queen Victoria was Heterozygous for Haemophilia. Since her father was normal, and there is nothing to suggest that her mother was a carrier, it seems likely that a mutation had occurred, most probably in her father's gametes. It is well known that the Romanoff family gene came to an abrupt end in 1817 and that the Spanish Royal Family branch of the Hapsburg family gene was extinguished as a result of car accidents. (see Haemophilia 3, Rizzo Naudi)

It has been calculated that the average life of a gene determining Haemophilia is little more than three generations (5). It is, of course, possible that the gene may sometime have been handed down through several generations of carrier females all of whom by a succession of fortuitous chances producing *only* normal sons, normal daughters and carrier daughters. This is highly unlikely. (see Haemophilia 2, Olivieri Munroe)

One could consider Haemophilia occurring in succeeding generations in the manner shown in Figure 3.

Thus it might be expressed that Haemophilia is a disease which occurs as a result of a mutation, may be transmitted to succeeding generations, and has a tendency to disappear from the line.

FIGURE 2

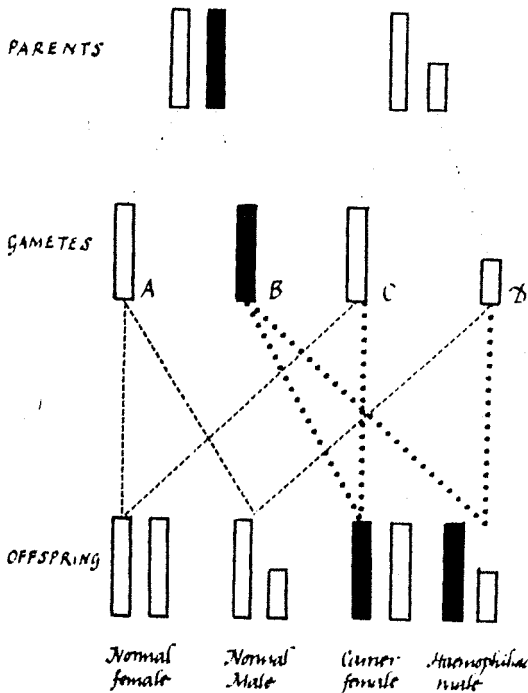
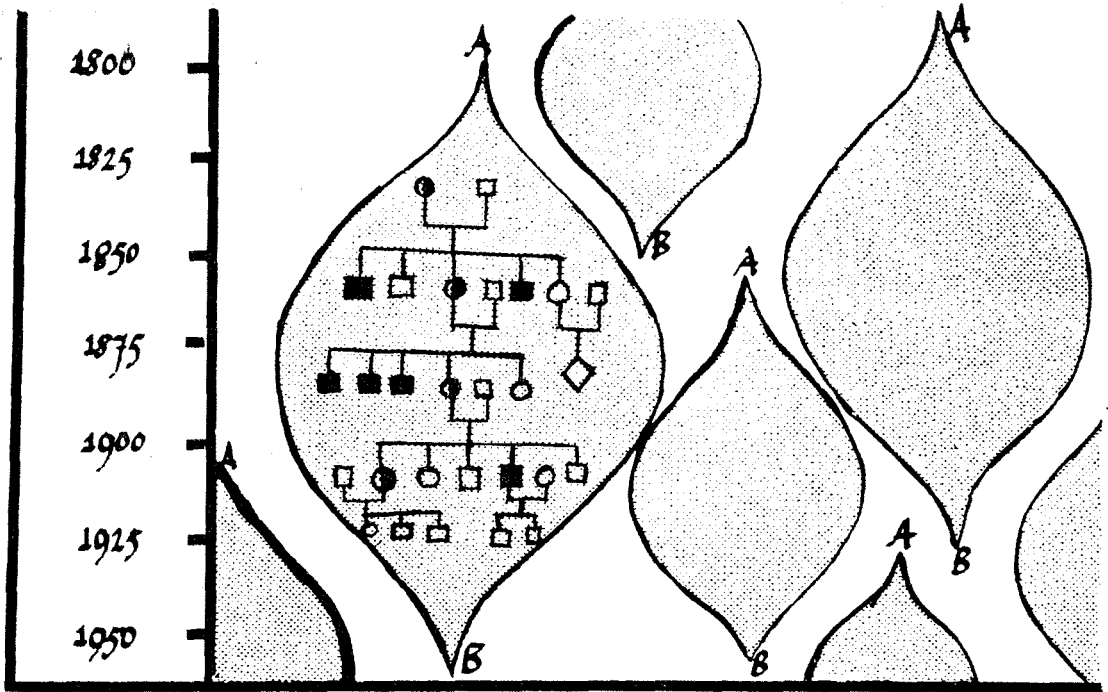


FIGURE 3

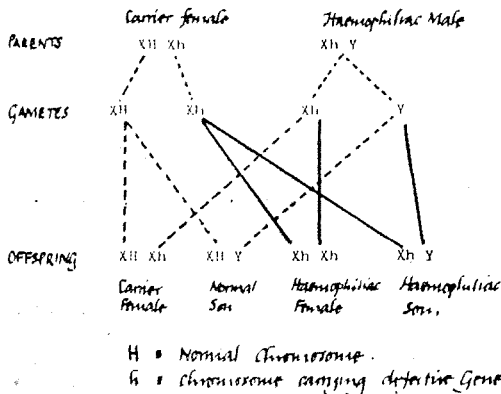


A - Mutation occurs initiating line  
 B - Natural or otherwise end of line

CAN ONLY MALES SUFFER FROM HAEMOPHILIA?

The union of a Heterozygous Female with a Male sufferer could theoretically arise:-

FIGURE 4



Graham and Brinkhouse succeeded in breeding Haemophilic dogs in this manner and producing the expected ratio of Haemophilic females. From figure 2 it may be postulated that a Haemophilic female could arise if gamete C had undergone a mutation. It was thought that females Homozygous for Haemophilia did not exist either because the combination  $Xh Xh$  ( $h$  being gene for Haemophilia) was a lethal combination to the zygote, or that they did exist (despite the chances against the above union being very high) but occurred so very rarely that they were unrecognised for what they were.

Cases of Haemophilia occurring in females have now been well documented, (1) which are almost certainly examples of such a union. It is calculated that only one female in 100,000,000 females can be expected to suffer from Haemophilia (5).

**WHY IS IT THAT THESE FEMALE HAEMOPHILIACS BLEED NO MORE SEVERELY THAN MALE HAEMOPHILIACS?**

Mary Lyon suggests that only one X Chromosome remains functional per cell of a diploid individual and that every female is a mosaic of two kinds of cells — those with the paternal X chromosome still acting and those with only the maternal X still acting.

In females one X only is acting the other X condenses to form the Barr body, in general present only in nuclei of female cells. This hypothesis could possibly explain the fact that a female haemophiliac (XhXh) will not suffer more severely than a male haemophiliac (Xh).

Might it be that, in a female heterozygous for haemophilia there could be a mechanism which relegates only the affected X chromosome to become the Barr body? This could account for the apparently normal phenotype of such a female. Or could it be that the mosaic effect is retained so that only half (say) of the Xh chromosomes are acting at any one time which would render a carrier female to show half the grade of severity of her hemizygous male counterpart? Macfarlane et al have shown that carrier females were more liable to excessive haemorrhage after tooth extraction than were the normal controls (6).

**AETIOLOGY: A HYPOTHESIS:—**

**CAN HAEMOPHILIA BE ACQUIRED?**

Another perplexing consideration can now be introduced. Various observations show that a disorder clinically indistinguishable from classical Haemophilia may be acquired during an individual's lifetime (7,8,9,10,16). Several cases have been well documented of such a condition arising following therapy with Penicillin. Other cases have been described following Horse Serum Therapy and after treatment with Sulphonamides. Specific Anticoagulants directed against A.H.G. were discovered to be present in those patients who were described as having a Haemophilia-like disease.

A well defined specific anticoagulant directed against the conversion of Prothrombin to Thrombin has been found with some frequency (10%) in patients with Lupus Erythematosus (16). Circulating anticoagulants directed against A.H.G. have been reported to occur spontaneously in otherwise normal individuals, and in as high as 21% with congenital Haemophilia (7).

Women in 3rd or 4th decades may suffer an onset of severe haemorrhage several weeks to years after parturition. From these observations Haemophilia could be represented as the main Theme and all these other conditions variously labelled as Pseudo-Haemophilia, Haemophilia-like diseases or Para Haemophilia as variations.

Haemophilia-like diseases or Para Haemophilia as variations.

If one considers individuals who manifest a tendency to excessive haemorrhage and in whose blood can be demonstrated lack of A.H.G. (whether this is due to an absolute deficiency or not) then such cases may be classified as follows:-

**HAEMOPHILIA or the HAEMOPHILOID States:**

*I Congenital.*

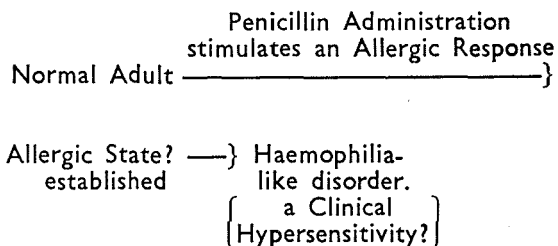
- a) With family history of disease.
- b) No family history. Possibly result of mutation.

*II Acquired*

- 1) As a result of a Drug Reaction (Penicillin, Sulphonamides, Horse Serum).
- 2) After Pregnancy — Iso-immunisation?
- 3) After administration of Porcine A.H.G.
- 4) Associated with Collagen Disease: Systemic Lupus Erythematosus, Rheumatoid Arthritis, Temporal Arteritis, Regional Ileitis.

**CONSIDERATIONS:**

1. A Haemophilia-like state developing in a patient after Penicillin therapy might indicate an unfavourable reaction occurring twixt the Penicillin and the individual. In a recent article Green (8) showed that neither Penicillin nor its analogues are satisfactory antigens for A.H.G. inhibitors. Neither did the Penicillin form a coat on the A.H.G. molecule. A possible explanation could be that the disorder is the result of the altered allergic state that results from the Penicillin.



Hypersensitivity could be said to be Auto Immune — but affected by an exterior source (18).

2. The appearance of circulating anticoagulants in cases of Systemic Lupus Erythematosus, Rheumatoid Arthritis, Temporal Arteritis and other so-called Collagen Diseases, might indi-

cate that the appearance of Pseudo-Haemophilia in these patients could fit in with Damashek's concept of the Hidden Iceberg (13). (see Fig. 5).

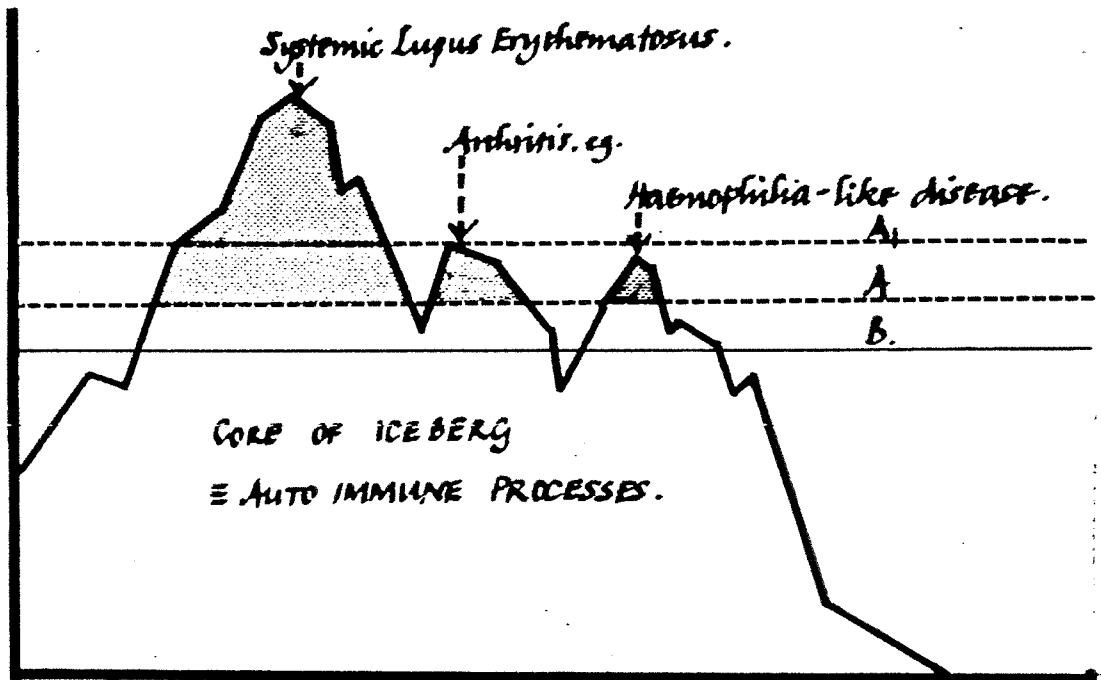
3. In those patients who develop a Haemophilia-like disease after treatment with Penicillin, therapy with *Prednisone* brought about a remission (7). Abilgaard et al in 1965 noted the cessation of Haematuria in 3 Haemophiliacs after 6 to 48 hours therapy with Intravenous Hydrocortisone (50 mg every 8 hours) or oral Prednisone (20-25mg every 6 hours for 1 to 4 days). (11)

4. Kasper et al in 1964 observed that in 5 pregnant known carriers of Haemophilia with low A.H.G. levels *there was a significant rise in A.H.G.* which averaged twice the non pregnant levels. This increase was not reflected in cord blood. One of the pregnancies resulted in a severely haemophilic newborn — both cord and baby's blood were practically devoid of A.H.G. (12)

5. Most of the anticoagulants developing in patients with classical Haemophilia have been shown to inhibit Factor VIII specifically in a reaction dependent both on time and tempera-

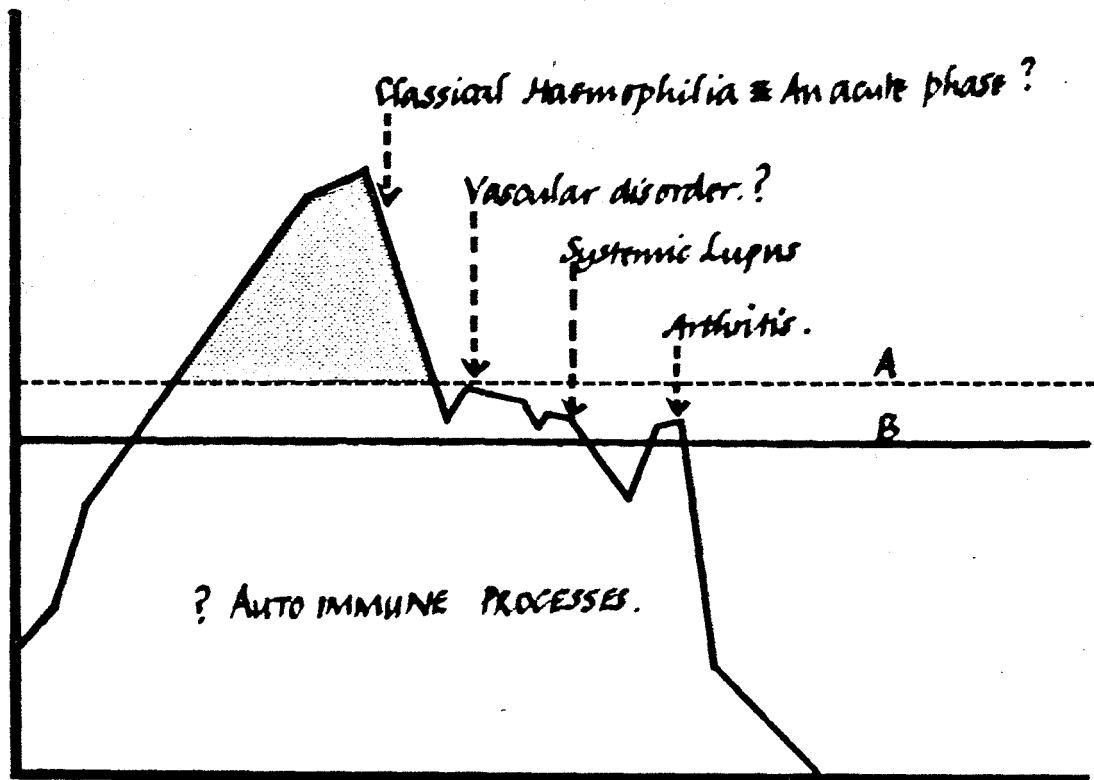
*Often, more than one type of Auto Immune lesion may occur in the same individual.*

FIGURE 5



*A<sub>1</sub> - Level at which Clinical recognition was possible in 1940.  
 A - Level at which Clinical recognition is now possible.  
 B - Normality Level.*

FIGURE 6 .



ture suggesting to some workers that the inhibitor was an enzyme. Other workers believed these circulating anticoagulants to be antibodies since they could be recovered from the gamma globulin fraction after electrophoresis of serum or plasma. Green showed that the A.G.H. inhibitors of both Spontaneous and Haemophilic origin were IgG globulins. (9)

In view of the above points it is tempting to think of adding "Haemophilia" to the growing list of Diseases which are labelled Auto-Immune. (see Fig. 6).

Could it be that the mutant Gene or one of its intermediate or end products is not recognised as being "Self"? William Damashek in his Introduction to a Symposium (published 'Blood' in 1954) wrote:-

"Until recently Haemophilia was easily defined and readily recognised. However, in the last few years various active workers in the field have unearthed new facts, developed new hypotheses and have had the temerity to claim that Haemophilia may not be actually what it seems".

#### CONCLUSION

The Question — "Why is there an apparently higher Incidence of Haemophilia in Malta?" — has now developed a greater significance. In genetic terms Malta appears to be an ideal population isolate. The Hardy-Weinberg equation can not be applied to the case of Haemophilia since the possession of the affected gene reduces fertility and will progressively be eliminated from the population (excluding mutational possibilities). Also there are unlikely to be random matings — since haemophilic males tend not to marry (as the result of genetic counselling), and the female sibs of known Haemophiliacs are warned that they ought not to have children.

If one considers any single individual Maltese now living — say Mr. X, then Mr. X would have 2 parents, 4 grandparents, and 8 great grandparents etc. Assuming that in any one century there occur four generations and if there was no consanguinity amongst Mr. X's ancestors, then the number of ancestors would be 2 to the power of n where n represents the number of ancestral steps removed from the



individual. (17) Over a period of 400 years then  $2^n$  would be 2 to the power of 16 = 65,136. Thus the population of Malta in 1560 should have been at least 65,000 for there not to have been consanguineous marriages. In 1530 a preliminary report made for the Knights of Malta assessed the population of the Island at 12,000 inhabitants. (14) (According to this calculation the population of the World should have been one million million ancestors for there to have been no consanguinity. The estimated population of the world 300 years ago was only 500 million at the most. The Brotherhood of Mankind is a genetic reality!). Although consanguinity will affect the recessive alleles and tend to expose recessive traits, where a lethal gene is concerned a high rate of consanguinity will tend to remove it at a faster rate than in a population isolate where the rate of consanguinity is less.

Can the higher incidence of Haemophilia in Malta be linked with the high incidence of Diabetes Mellitus? (15) What is the relative incidence of other known Auto-Immune diseases in Malta, and could there be present some factor to a greater degree in Malta than elsewhere?

A higher mutation rate is unlikely — despite Malta's incomparable climate — since Haemophilia appears to be rarer in surrounding countries.

Nossal has described Medicine as having entered The Golden Age of Immunology. Im-

munologists made Cardiac Transplantation a reality — the "Iceberg" may soon have its depths revealed.

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