STUDIES IN BLOOD COAGULATION AND HAEMOPHILIA.

I. Blood Coagulation in Haemorrhagic Diseases.

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It has long been known that whereas in many diseases the coagulation time of the blood may be a true index of the haemorrhagic tendency, in others it is not. Thus in most cases of Jaundice and Purpura where there is a proven coagulativeness deficiency, the coagulation time is normal. In the following observations the coagulation times have been taken not only of the first drop of blood but also of successive drops from a single puncture, in accordance with the method described by Gibbs, and plotted in a curve. (Fig. I.) (Details of the procedure and the coagulometer used may be found in his original article.)

Thus not only do we find the spontaneous coagulability of the blood, but also an index of the reaction of the blood and tissues against haemorrhage. It is not the first drop of blood from a wound which stops haemorrhage, it is the blood which has been rendered more coagulable by the many influences brought into play when the wound was made.

From what is known at the present time, we would divide this reaction into two main groups, the tissue reaction, and the platelet reaction.

1). Tissue Reaction. The bruising of any cellular tissue (Thromboplastin Substance) liberates a substance, Thrombokinase, necessary to the clotting of blood and acting quantitatively, i.e. the greater the amount present the more rapidly will clotting take place. (Howell, Addis, Mollanby, etc.)
2) **Platelet Reaction.** There is a popular belief that the role of the Blood Platelets in coagulation has been discredited, but a survey of the literature on the subject will show that there is no foundation for this. Even Pickering, who with his colleagues claims to have shown that Thrombocytes are not essential to coagulation, admits that "they must play an important part in arresting haemorrhage."

It has been definitely shown (Duke, Hayem, and others) that when blood is shed the platelets agglutinate into masses or become adherent to the injured intima of the blood vessels. The action of this mechanism can be demonstrated long before the laying down of fibrin has commenced. It has also been shown (Morawitz, Bayne-Jones, Howell, Quintin, & Pepper,) that on disintegration these platelets liberate free Prothrombin in large quantities, and a smaller amount of Thrombokinase. Thus the platelets will tend to stop haemorrhage, first by their mechanical agglutinating properties, and secondly by concentrating the supply of Prothrombin and Thrombokinase at the bleeding surface.

By the method of taking the coagulation time of successive drops of blood, we can trace this mechanism from start to finish, and can show any deficiency in the coagulability of the blood, the tissue reaction, or the platelet reaction. Thus the coagulation time of the blood is shown in the first drop; the next one or two drops are an index of the tissue reaction; and subsequent drops are an index of the platelet reaction. It is not suggested that the tissue reaction stops after the second or third drop, but merely that its action must become less and less marked, since the tissues...
were only once bruised, and that before the first drop was taken. The platelets on the other hand are becoming more and more concentrated round the bleeding surface, thus concentrating the supply of Prothrombin, and also liberating an increasing amount of Thrombokinase. Thus although the tissue reaction must diminish in intensity after a while, the coagulability of the blood coming from the wound does not decrease, since the platelet reaction is taking the place of the tissue reaction.

The normal initial coagulation time by this method varies from 1 min. 40 secs. to 2 min. 50 secs. with an average of 2 min. 8 secs. from 193 adult cases with no haemorrhagic history, taken at random in a surgical ward. (Personal communication,—Miss Croskery.) The average coagulation curve is shown in Fig.I, the shaded area denoting the normal limits of variation as found in 30 adult medical cases with no history of haemorrhage. The main cause for this comparatively slight variation is evidently the texture of the patient's skin, a fine skin giving slightly higher readings than a coarse one.

The time that elapses between the taking of the drops of blood undoubtedly affects the coagulation time of each individual drop. Since in the taking of these curves only one coagulometer was used, this interval varies considerably in each curve and in different parts of the same curve, but we have found that this does not affect the ultimate character of the curve taken as a whole.
Purpura.

It has been shown that in a large majority of cases of Purpura, the blood clotting elements are normal in quantity and quality, the coagulation time remaining unaffected and the only change in the blood being a very marked diminution of blood platelets, with an increased bleeding time. (Howell, Hess, Duke, Pratt, Bedson, etc.) Why this denudation of the blood of its platelets should not cause any decrease of prothrombin in the blood, has been explained by Hess, who has shown that the platelets are merely broken up, their constituent parts passing into solution in the blood. This explains why the actual coagulation time should remain normal. The platelet reaction, however, as defined above, must necessarily be deficient. The coagulation curve is typical. (Fig. II, III, IV, V & VI.)

In all but one of the cases described, the initial point was within normal limits, denoting a normal coagulation time. And in all, the second point fell in the normal manner, thrombokinase having been added from the tissues. Subsequent points however remained well above the normal limits, there being practically no platelet reaction, and consequently no concentration of prothrombin or platelet thrombokinase.

It must be noted that in Purpura as in Jaundice, the coagulation curves of many patients in whom the symptoms were not very pronounced, were within normal limits, although definitely above the average.

Case V (Fig. VI.) is of special interest in that, when first observed, his clinical picture was highly suggestive of a simple
purpura haemorrhagica, and his coagulation curve was almost exactly the same as that found in Fig. IV. Coincident with the aplastic change in his blood picture, however, there was a progressive uniform elevation of his coagulation curve, till just before death we have the curve shown in Fig. VI. As we would expect, the aplastic change must have been accompanied by a decreased formation of platelets. This would produce a quantitative deficiency of prothrombin, which, superimposed on the lysis of what platelets were formed, would account for the very abnormal nature of the curve. Unfortunately no estimation of the prothrombin content was possible, but the coagulation curve definitely indicates that there was some deficiency in the coagulative elements.

It has been observed by others that an increase of the coagulation time in Purpura is a bad prognostic sign, and I would suggest that it always denotes this aplastic change wherein there is a deficiency of platelet formation superimposed on the platelet destruction found in simple Purpura.

**Jaundice.**

The cause of the haemorrhagic tendency in Jaundice has not yet been satisfactorily explained, although many theories have been advanced. (Morawitz, Whipple, etc.) It has been definitely established that in certain cases of extreme liver inefficiency the amount of fibrinogen in the blood is diminished, (Whipple) but this by no means applies to all cases of Jaundice. Many of those cases of obstructive or simple catarrhal Jaundice, which are such a menace to the surgeon, have been shown to have a normal coagulation time. (Duke) The coagulation curves, however, as shown in 2 cases examined of deep jaundice, (Fig. VII, & VIII.) show a definite
abnormality, almost identical with that found in Purpura, suggesting that the haemorrhagic tendency is due to a deficient agglutination of the platelets. It seems rational that this should be so, since as far as we know the agglutination is purely a physical process, which might readily be prevented by the lowering of surface tension produced by the presence of bile salts in the blood. The absence of any purpuric symptom would then be explained by the experiments of Bedson, who showed that two factors are essential in the production of purpura—diminution of platelets and the presence of a toxin which will damage the capillary endothelium. I would suggest that in Jaundice this toxic factor is absent except in those isolated cases where definite purpuric symptoms do appear. (Duke.)

In case VII (Fig. VIII) the increased initial coagulation time and associated heightening of the whole curve will be due to the lack of fibrinogen shown by Whipple to occur in cases of marked liver inefficiency. It is interesting to note that this curve corresponds very closely to Case V (Fig. VI), where there was also a quantitative coagulative deficiency in the blood, superimposed on a deficient platelet reaction.

Haemophilia.

All observers agree that a marked prolongation in the coagulation time of the blood is a constant feature in this disease, and practically all the clotting elements have been blamed in turn for this defect. Thus a deficiency of Thrombokase (Sahli, Morawitz & Lossen, Wolf & Harry), Calcium (Wright), and an excess of Anti-thrombin (Weil, Feissly) have been said to be the causal feature, but more recent investigators have been unanimous in putting it down to some deficiency in prothrombin, whether it be quantitative (Howell, Hurwitz & Lucas, Klinger), or Qualitative (Addis,
Sajous, Monot & Lee, Wohlsch, Christie Davies & Stewart). Pickering & Gladstone have recently advanced the theory that it is due to an excess of protective colloid in the prothrombin-fibrinogen complex. In an article now in the press, Christie, Davies, & Stewart have to a certain extent confirmed the work of Howell, Monot & Lee, and Pickering & Gladstone, and have gone on to show that the abnormality in Haemophilia is due to an undue functional stability of the formed elements of the blood, revealing itself, from a coagulative point of view, in the slow liberation of prothrombin from the blood platelets, and also the slow formation of thrombin from prothrombin.

Now what effect will this have on the coagulation curve? As was found by Gibbs in his single case of Haemophilia, I found a high initial coagulation time, followed by the usual fall, and then a rise to well above the original level, this being constant in numerous observations in 6 of the 8 cases of true haemophilia examined (Figs. IX, X, XI, XII). Here the initial coagulation time will be lengthened by the slow liberation of prothrombin. The thrombokinase, of which it has been proved there is no deficiency, will then cause the curve to fall by the law of mass action. The subsequent rise seems to indicate some definite deficiency in the platelet reaction superimposed on the slow liberation of prothrombin, but we have not been able to demonstrate experimentally any defect in the agglutinating properties of the platelets.

In the two other cases of haemophilia examined, the prolongation of the coagulation time was so great as to make the end point on Gibbs's coagulometer impossible to determine. This was evidently due to the fact that even after coagulation had commenced, it proceeded so slowly as to allow of the formation of small particles of thrombin, which ultimately formed a suspension in the revolving drop of serum.
**Pseudo Haemophilia.**

Lastly we come to those cases where, with no haemorrhagic family history, there is a definite tendency to haemorrhage after injury. In 8 cases examined with histories of excessive haemorrhage the coagulation curve was abnormal in 3 (Cases XII, XIII, XIV), and normal in 5. Of the latter 5, 3 had minor operations performed upon them subsequent to the taking of the coagulation curves, with no excessive haemorrhage.

Unfortunately neither time nor circumstance permitted of a thorough blood examination in those cases which showed an abnormal curve, so we can only submit the curves as a possible method of differentiating these dangerous cases from the much larger class where there is no contraindication to operation.

It is interesting to note that in one of the cases (Case XIV) the curve (Fig. XV) has a slight but definite haemophilic tendency, showing a rise after the initial fall. In this case, although there was a definite haemorrhagic history, the coagulation time was normal, but the coagulation curve was very definitely abnormal.

**Conclusions.**

1) By taking the coagulation time of successive drops of blood from a single stab wound, we get not only the coagulation time of the blood, but also an index of the tissue reaction and platelet reaction, and thus a true index of the haemorrhagic tendency.

2) In Haemophilia we get a characteristic curve, indicating a deficiency in the coagulative elements of the blood, and also a deficiency of the platelet reaction.

3) In Purpura and Jaundice we get a characteristic curve, indicating a deficiency of the platelet reaction.
4) Three cases are described where although there is a definite haemorrhagic history, the coagulation time is normal. The coagulation curve in these cases is definitely pathological.

Cases.

I. M.J., Female, Aet 33. History of intermittent febrile attacks, accompanied by vomiting, colicky abdominal pain, and slight pain and swelling in ankle, rapidly followed by the appearance of a dense purpuric rash on trunk and limbs with occasional epistaxis. Examined during severe attack with extensive purpura and epistaxis. Platelets in film very scanty.

Case II. E.Y., Female, Aet 22. History of "Influenza" followed by appearance of purpura rash on legs, and painful joints. Rash soon spread to trunk, and to lesser extent to arms. Slight epistaxis.


Case V. J.T., Male, Aet 13. Admitted with history of febrile attack followed by edema of face, hemorrhage from gums and nose, and the appearance of a purpuric rash on limbs. Blood picture: Hemoglobin 12% with color index of 1.1. 40% lymphocytes with a few myeloblasts. Platelets 60,000. After blood transfusion slowly improved but Hb never rose above 20%. Readmitted 6 months after first attack with temperature, bleeding from nose and gums, and purpuric rash all over body. Blood picture typical of Aplastic Anemia. Went rapidly downhill, and died 3 weeks after admission. Post mortem: typical of Aplastic Anemia.


Case VIII. J.C., Male, Aet 25. Family history: Definite of Haemophilia for 1 generation (previous family history not known); 4 definite cases in family & 4 indefinite (died in infancy). Symptoms: Bruises easily; typical hemorrhage into joints, gums, and nose. Very severe hemorrhage from trauma on 4 occasions, once almost fatal.

Case IX. G.M., Male, Aet 7. Family history: Definite of Haemophilia for 2 generations, 5 definite cases in family. Symptoms: Bruises easily; typical hemorrhages into joints and gums; almost fatal hemorrhage from scalp injury.

Case X. J.M. Yale, Aet 40. Family History: Definite of Haemophilia for 5 generations, 12 cases on record. Symptoms: Bruises easily; typical hemorrhages into joints, kidneys, and gums; numerous severe hemorrhages after trauma.
Case XI. J.C., Male, Aet 70. Family history: Definite of Haemophilia for one generation only, 3 haemophilic brothers, one haemophilic cousin. Symptoms: Bruises easily; frequent epistaxis and bleeding from gums, sometimes leading to severe anaemia; very severe haemorrhage from trauma on 3 occasions, once almost fatal. No haemorrhage into joints. A comparatively mild haemophilia.

Case XII. A.B., Male, Aet 30. No family history of bleeding. Tooth pulled 9 months ago, haemorrhage excessive, and socket still bleeds when teeth are brushed. Tonsilectomy 3 weeks ago, haemorrhage very excessive, and still occasional bleeding. Hernia operated on one week ago, haemorrhage not excessive, but haematoccele rapidly developed afterwards.

Case XIII. W.Y. Male, Aet 35. No family history of bleeding. Always bleeds freely from shaving cuts. Can only remember having had one accident, when he cut his hand; the wound bled for over a day, a considerable amount of blood being lost. Had had teeth pulled twice; on both occasions great difficulty was experienced in controlling haemorrhage, the gums bleeding on one occasion for 4 days, and on the other for 7 days.

Case XIV. W.D. Male, Aet 40. No family history of bleeding. Has always bruised very easily and sometimes extensively, and has been led to believe on this account that he has a "thin skin". Gums frequently bleed (no pyorrhoea). Has only had one accident that he can remember, when his front teeth were loosened. At this time his gums bled for 7 days, leaving him very weak and anaemic. Inguinal Hernia operated on 7 days ago, haemorrhage excessive, and there is now a large haematoma at the site of operation.
In conclusion I must thank Prof. G. Lowell Gulland for the trouble he has taken in collecting suitable cases, and for his permission to publish them. Without his co-operation it could have been impossible to investigate such a comprehensive series of cases.
References.

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Blood Coagulation in Haemorrhagic Diseases

Coagulation Curves.
Fig 1

Successive Drops.

Normal coagulation curve with limits of variation in 30 normal cases.
Fig III.
Case II

Successive Drops.
Coagulation Curve in Purpura
Successive Drops

Coagulation Curve in Purpura
Successive Drops
Coagulation Curve in Aplastic Anemia
with Purpura.

Fig VI
Case V
Fig VII

Case VI

Successive Drops

Coagulation Curve in Tannolice
Fig IX
Case VIII

Coagulation Curve in Haemophilia
Fig X.
Case IX

Successive Drops
Coagulation Curve in Hemophilia
Fig XI.

Case X

Successive Drops

Coagulation Curve in Hemorrhagia.
Fig. XII

Case XI

Successive Drops

Coagulation Curve in Haemophilia
Successive Drops
Coagulation Curve in Pseudo-Haemophilia

Fig XIII
Case XII
Fig. xiv

Case xiii

Successive Drops

Coagulation Curve in Pseudohaemophilia
Successive Drops

Coagulation Curve in Pseudo-Hemophilia

Fig XIV
Case XIV
Studies in Blood Coagulation and Haemophilia

II. Observations on Haemic Functions in Haemophilia

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At one time or another every factor known or believed to be concerned in the coagulation of blood has been declared to be deficient or inoperative in haemophilia. From this mass of contradictory statements it is impossible to extract more than a very few which may be regarded as well-established, and still fewer which are without doubt. Much of the obscurity, doubtless, is due to the unsatisfactory nature of our knowledge of the mechanism of blood coagulation, concerning which two main and several subsidiary hypotheses appear to be warring for supremacy, while confusion has been worse confounded by the wholesale introduction of synonyms - in many cases descriptive of merely hypothetical substances.

It becomes necessary, therefore, to preface any discussion of the causation of haemophilia by a glossary of the terms employed and by a brief account of the particular hypothesis of blood coagulation to which the authors adhere. Further, if the discussion is to be of any value, it is imperative to limit the terms employed to the minimum, and, as far as is possible, to found all deductions on those parts only of the mechanism of coagulation concerning which there is reasonable unanimity of opinion.

The nomenclature of the hypothesis of coagulation adopted in this paper are essentially those of Morawitz. (1)

Fibrinogen/

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Fibrinogen, present in unshed blood, is converted to fibrin by
the action of thrombin. Thrombin is **already in fibrin** not
present in unshed blood, but is formed from pre-existing **pre-thrombin**
by the combined action of **calcium salts** and **thromboplastic substance**.
We prefer to use this last term in preference to that employed by
Morawitz himself - **thrombokinase** - since doubt has been cast on the
idea that the substance functions as an enzyme. Indeed, the term
we have adopted, though less committal than that of Morawitz is
itself unsatisfactory for the factor it connotes may be a condition
rather than a substance. However, we have employed it in preference
to introducing a new term.

It is thoroughly established that fibrinogen is a globin
occurring in the blood plasma; the distribution of prothrombin,
however, is not so clear. Mellanby describes it as being in
close association with fibrinogen, and his evidence is accepted
apparently without further examination - by Pickering. On the other
hand, a number of workers claim that only very small amounts of pro-
thrombin are present in the circulating plasma, and that the great
part of that required in coagulation is liberated, when the blood
is shed, from the platelets, and, to a lesser extent, from the
leucocytes. 

**Thromboplastia** substance must be absent from the circulating
plasma, as, were it present in appreciable amount, it would bring
about the formation of thrombin, and so cause intra-vascular
clotting or negative phase blood, unless of course one postulates
an anti-thrombin as does Howell. Many workers have claimed that
plasma, completely denuded of all formed elements, is still capable
of coagulation, though they admit that in these circumstances
clotting is extremely slow. Their results, however, do not
necessarily indicate the presence of thromboplastic substance in
circulating plasma. In the first place, a certain amount may have
entered the plasma during its manipulation, and secondly, a catalyst
is classically defined as a substance capable of accelerating a
chemical reaction though not of initiating it. Of these objections
the first holds whatever view be taken of the action of thrombo-
plastic substance, the latter only if one agrees with Morawitz that
it is enzymic. In any case, thromboplastic substance whatever its
mode of action appears to be necessary for the rapid clotting of
blood. It is usually described as present in the cellular tissues
including the formed elements of blood, and to be found especially
in such tissues as brain and testis which are rich in phosphatides.
Howell, indeed, who introduced the name we employ, thromboplastic
substance, identifies it with kephalin, and believes its action to
consist in the neutralisation of an anti prothrombin which he terms
heparin and considers to be also a phosphatide.

It appears, then, that whatever the mechanism by which thrombin
is formed, the velocity of the reaction will be increased by any
means which will accelerate the pouring of prothrombin and thrombo-
plastic substance into the plasma. It is well known, for example,
that blood clots more rapidly if it is allowed to flow over a
bruised tissue surface - an effect especially noticeable in avian
blood, whereby it receives additional thromboplastic substance.
Similarly, acceleration of clotting has frequently been observed
when lysis or switching has destroyed the cellular elements.

In this connection, some experiments of our own yielded interest-
ing results. Firstly, we studied the relation between the
coagulation time and the amount of lysis. For this purpose
haemophilic blood was used since the greatly increased coagulation
time facilitated manipulation, and allowed a number of obser-
vations to be made on each sample. Blood was drawn by vein
puncture using a short wide bone paraffined needle. 2 c.c. were
added to each of a series of test tubes placed in a water-bath
at 38° C., and containing quantities of distilled water varying
from 0.0 to 3.0 c.c. The contents of each test tube were rapidly
mixed, and the time of coagulation taken, the blood being con-
sidered to have clotted when the test tube could be inverted
safely/
safely. Table I shows the results of typical experiments. In every case the time of coagulation decreased with the addition of water, reaching a minimum when complete lysis was attained. Further addition of water lengthened the coagulation time, an effect which was probably one of mere dilution. At the optimum, a reduction of 60% in the coagulation time was attained.

Next, again using haemophilic blood, we investigated the effect of switching with a bundle of fine wires. In this series our first aim was to ascertain the amount of switching required to produce the optimum effect, for with the quantity of blood usually available it was not possible to carry out a variety of experiments on each sample. The time of coagulation decreased as the time of switching increased up to about two minutes, remained roughly constant up to 4 minutes; thereafter defibrination became appreciable and the coagulation time again increased. A number of experiments on different samples then showed that two minutes' switching of the blood was capable of reducing the coagulation time by about the same amount as was complete lysis, i.e. 60% (Table II)

Turning then to normal blood, we investigated the effect on the coagulation time of two minutes switching, and of lysis by the same amount of distilled water as was employed for haemophilic blood. (Since the fragility of haemophilic corpuscles determined by the method described by Meaumont and Dodds had been found to be normal, it was assumed that the optima for the two series would coincide). To our surprise we found that whereas switching produced an acceleration of clotting comparable with that obtained in haemophilic blood, lysis lowered the coagulation time very slightly (Table III).

The bearing of these experiments on the causation of haemophilia will be discussed later; at present it is intended to consider only their implications with respect to the process of coagulation. Lysis, under the experimental conditions, destroyed, at any rate, almost/ all the formed elements of the blood, for Minot and Lee have found that the platelets are largely destroyed when the tonicity is reduced to such an extent as to cause almost complete lysis of
the erythrocytes. Hence in our experiments there must have been liberated a large amount of the prothrombin, and also any thromboplastic substance existing in the cells. In switching, cell destruction did not take place to anything like the same extent, yet a much greater acceleration of clotting was produced. Whilst it is obvious that much further work is necessary before an adequate explanation of this phenomenon can be given, we would suggest tentatively that it affords an indication of the non-existence of pre-formed thromboplastic substance. It may be that it is formed in the breakdown of cells in contact with air or certain surfaces, but that when the cells are suddenly disintegrated as in lysis only some of the precursor substances are able to react in the normal way. On this view, switching, by increasing the breakdown (in the normal way) of the cells, would increase the supply of thromboplastic substance and so accelerate coagulation; lysis, by preventing the formation of much of the thromboplastic substance would have little or no accelerating action.

As regards the mode of action of the various participants, we are not prepared to be so definite as was Porawitz. Thus he named thrombokinase with the idea that it is an enzyme, a question on which, in view of later work dealing especially with the quantitative nature of its action, we prefer to keep an open mind. A knowledge of its exact mode of action does not as yet appear to be essential to our work. The work of Haxthausen appears to indicate a definite quantitative relationship between the amount of thrombin and the amount of fibrin produced. Taken in conjunction with the observation that an amount of thrombin below a certain limit can never produce complete coagulation, this would indicate that thrombin, at any rate, does not function as an enzyme, but forms a compound or complex with the fibrinogen.
Turning now to the more particular question of the abnormalities discoverable in haemophilia, we may first review briefly some of the suggestions which have been advanced by previous workers.

In the first place, it must be emphasised that the evidence points strongly to the conclusion that the slowing of coagulation in haemophilia occurs prior to the formation of thrombin. Wohlich (15) has shown that the fibrinogen is normal both in behaviour and amount. Addis(47) and others (8, 15, 18), too, have found that haemophilic blood is clotted as readily by thrombin as is normal blood, and that the thrombin of haemophilic blood and that of normal blood possess equal coagulating power. Hence the fibrinogen and thrombin of haemophilic blood are deficient neither in quantity nor in quality.

There remain, then, three possibilities. The defect may be due to a deficiency (qualitative or quantitative) in the calcium, the thromboplastic substance, or the pro-thrombin.

Wright (19) has claimed that a deficiency of calcium is a cause of haemophilia. It has, however, frequently been found, as by the present authors (Table ) that the calcium content of haemophilic serum is within the normal range, and that addition of calcium salts does not cause any acceleration of clotting. Further in such diseases as tetania parathyreopriva, where the blood calcium is much lowered, there is no corresponding increase in the coagulation time.

A shortage of thromboplastic substance has been suggested by Sahli(20) as the cause of the delayed clotting in haemophilia, and this suggestion has been supported by Morawitz and Lossen (24) while Nolf and Harry (22) consider the thromboplastic substance to be altered in quality rather than in quantity. Addis, (16) however, has concluded that there is deficiency of thromboplastic substance neither in the formed elements of the blood nor in the body tissues.

It would seem, then, that the defect lies in the pro-thrombin and/
and various investigators (23, 24, 25) have stated that in haemophilic blood this substance is present in smaller amounts than in normal blood. The consensus of opinion, however, appears to be (26) that the defect is qualitative rather than quantitative. It does not, however, follow that the constitution of the prothrombin is abnormal in haemophilia, though that would readily account for its slow conversion to thrombin. Indeed, the production of a normal thrombin which has been shown to take place, would strongly indicate a normally constituted prothrombin. It seems more probable that the defect consists in an abnormally slow availability of prothrombin as has been suggested by Macleod (26), and by Howell (27). Evidence in favour of this view is adduced from the experiments described below.

Still other hypotheses regarding the cause of haemophilia have been advanced. Macleod and Fieslet (28) have suggested an increased activity of the plasma, and Fieslet (28) has claimed that haemophilic plasma contains a substance which prevents the formation of prothrombin. Pickering and Gladstone (29) explain the disease on the basis of the theory of blood clotting advanced by Pickering as due to the presence of a relative excess of a stable protective colloid.

14x15
Localisation of Coagulation Defect.

Since all modern investigators are agreed that the deficiency in haemophilia does not lie in either the thrombokinase or fibrinogen we have not thought it worth while to repeat their experiments. We have confined ourselves to a study of the circulating prothrombin and calcium, the former being generally accepted as defective, and the latter being claimed by some to be occasionally deficient.

That the thrombin activity of haemophilic serum is as great as that of normal serum has been shown by several investigators (Addis, Minot and Lee, Wohlsch, Mills). The importance of this observation has led us to repeat the experiments at body temperature. Table I definitely supports the view that the thrombin content in haemophilic serum is as great as that in normal serum, since each accelerates the clotting of normal blood to the same degree. Since the amount of fibrin is known to be normal and the amount of thrombin has been shown to be normal in clotted haemophilic blood, the actual quantity of prothrombin present in the unclotted blood must be normal, since prothrombin acts qualitatively.

Having decided that any prothrombin defect was not quantitative, we proceeded to define further and isolate this defect by liberating the prothrombin from the platelets. To do this we repeated and amplified the admittedly inconclusive experiments of Minot and Lee (1908). First we determined the fragility of haemophilic corpuscles by observations on their resistance to hypotonic saline solutions (Baumont and Dodds) and found this to be perfectly normal, thus confirming the observations of Minot & Lee. We then proceeded to determine the action of lysis. Table II shows the effect of varying degrees of lysis on the coagulability of haemophilic blood. As has been explained, the greatest reduction in the coagulation time was at that point where lysis was completed, with a minimal amount of dilution. Tables III VI show the effects of this lysis at the optimum dilution in normal as compared with haemophilic blood. The difference is very striking in that whereas/
whereas lysis of haemophilic blood produces a 60% decrease in the coagulation time, lysis of normal blood produces only a 7% decrease. Two factors however must be excluded before any conclusion can be reached. First, we have broken up not only the platelets but also the corpuscles. To exclude the latter as being responsible for the decrease, a platelet suspension in plasma was prepared according to the method described by Bayne-Jones (14), the presence of platelets being demonstrated microscopically. Tables V, VI, VII show almost the same degree of difference between the decrease of coagulation time in lysed normal and lysed haemophilic platelet suspension as was found with whole blood.

The other factor which must be excluded is that not only the prothrombin but also a small amount of thromboplastic substance is liberated by the lysis of platelet (Quintin & Peffer (15), Bayne-Jones (14), Morowitz (16), Howell (17)). To exclude the possibility of this thromboplastic substance being responsible for the decrease we compared the effect of switching normal and haemophilic bloods (Tables IV, V). Here, since we are bruising the cellular elements of the blood thromboplastic substance will be liberated (13, 12, 30). The experiments showed that haemophilic and normal blood respond almost the same degree, indicating that it is not the liberation of thromboplastic substance which is responsible for the abnormally large decrease in the coagulation time when haemophilic blood is lysed.

One may conclude that in haemophilia lysis of the platelets brings about a marked decrease in the coagulation time by liberating prothrombin. In normal blood, with a normal liberation of prothrombin lysis brings about little if any acceleration of clotting.

This would indicate that the deficiency in haemophilia consists in part in a slow liberation of prothrombin from the platelets. That there is probably some further defect is clearly shown by the fact that lysis of the platelets alone does not bring the clotting time of haemophilic blood to within normal limits.
The Acid Base Balance of the Blood.

Investigation of the acid-base balance of the blood was made in each case and was as complete as the material and facilities at our disposal permitted. Direct determinations of hydrogen ion concentration were unfortunately not possible.

The first observation made was a single point on the carbon dioxide dissociation curve of case III. This fell within normal limits, and the $p_H$, calculated by means of the Henderson Hasselbalch equation was normal. This confirms the findings of Hurwitz and Lucas, and does not support the suggestion of Mellanby and other authors that undue alkalinity of the blood may be a causative factor in haemophllia. Subsequent observations in this and other cases confirmed this initial finding, but in addition revealed a hitherto unsuspected abnormality. When the carbon dioxide dissociation curve of the blood of any individual is determined by the method described by Meakin and Davies, it has been found that the carbon dioxide combining power (percentage increase or decrease of carbon dioxide content as compared with that of the blood of Haldane at the same carbon dioxide pressure) remains approximately the same for any carbon dioxide pressure within or even beyond the physiological range. In these haemophilic cases, however, with increasing pressures of carbon dioxide, the carbon dioxide combining power of the blood (expressed as above) diminished. In other words there was an abnormal flattening of the carbon dioxide dissociation curve. Hence for any increase in carbon dioxide pressure the amount of bicarbonate in the blood did not increase to a normal extent, and assuming the Henderson Hasselbalch equation to be valid in these cases, the $p_H$ change for any given change of carbon dioxide pressure was greater than normal. That is to say that the buffering mechanism of the blood in these cases was deficient.

This is shown in Fig. I and Table VIII. The estimations of carbon dioxide content were made upon duplicate samples of 2 c.c. each by means of the Haldane blood-gas apparatus, and in the majority of cases duplicates agreed to within 0.5 vol. per cent. On each occasion/
occasion the estimations were made on successive portions of a single sample of oxalated blood preserved by the addition of a trace of sodium fluoride (Lovatt Evans (32) ) and in a vessel surrounded by crushed ice. It can be seen that in the cases of the two normals (H.W.D. and R.V.C.) the carbon dioxide combining power of the blood increased with increasing carbon dioxide pressure. In other words the carbon dioxide dissociation curves of these two individuals were, at the physiological range, slightly steeper than that of Haldane. The flattening shown by the patients is not always marked, nor is it constant from day to day in a given case. Further work alone can show whether this variability is correlated with the variability in coagulation time which is known to occur from day to day in haemophilia. In case I the flattening was more marked on 13.4.26 than on 16.4.26 and 22.4.26. Similar flattening was observed in case II on 5.6.26 (Table) but the curve for this individual was not included in Fig. I in order to avoid undue confusion. In case IV flattening was marked on 17.5.26 but almost absent on 21.9.26 (Figs. I and III). In no case, however, was a curve steeper than that for Haldane's blood observed.

From the above findings it can be seen that the buffering mechanism of the blood in this group of cases was less efficient than in the cases of Haldane, H.W.D. and R.V.C. although it is difficult to state definitely whether this diminution of buffering power was greater than may possibly occur in a normal individual. It is significant, however, that the curves of all the patients examined were flatter than the published curve of Haldane's blood (34) while those of the two normals (determined by precisely the same technique) were slightly steeper.

The normal buffering of the blood is dependent upon a number of factors. These have been discussed in a quantitative manner by Van Slyke (35), L.G. Henderson (36) and others. The principal factors, however, are as follows: (1) The liberation of base from loose chemical combination with haemoglobin and with the plasma proteins and (2) Combination of carbonic acid with portion of the base of di-sodium phosphate, and (3) The interchange of ions between plasma and corpuscles.

These/
These factors were severally investigated. Estimation of haemoglobin percentage and of oxygen combining power revealed no abnormality of the haemoglobin. Estimations of total and of non-protein nitrogen in the plasma revealed no quantitative abnormality. The phosphates were normal (see Table 1). It was therefore decided to investigate the ionic interchange between plasma and corpuscles. For this purpose methods essentially similar to those of Joffe and Poulton (37) were used.

Sufficient blood for complete investigation (200-250 c.c.) was drawn by venepuncture without stasis and with the forearm immersed in water at about 45°C. This was oxalated and in order to prevent glycolysis a trace of sodium fluoride was added. The blood was then placed under paraffin in a cylindrical jar standing in a larger beaker of crushed ice. The ice was replaced as it melted. In this way blood could be kept for 12-18 hours without loss of carbon dioxide combining power. The amount of fluoride added was not sufficient to interfere with the chloride estimations, and being added to the whole bulk of blood, was constant in each successive portion used.

For the last estimation approximately 100 c.c. of blood were placed in a 400 c.c. cylindrical saturating flask such as was used by Christiansen, Douglas and Haldane (37). The carbon dioxide was removed as far as possible by exposing the blood to a vacuum at 37°C. for about ten minutes. Room air was then re-admitted and the saturator placed in the thermostat bath for fifteen minutes, the pressure being released at the end of the first five minutes. At the end of fifteen minutes the saturating flask was removed and wrapped in warm cloths. Two 2 c.c. samples of blood were withdrawn for estimation of carbon dioxide percentage from which the pressure of carbon dioxide was calculated. The remainder of the blood was then run under paraffin into a centrifuge tube and rapidly centrifuged. After 20-25 minutes centrifugation samples of plasma were removed from beneath the paraffin layer for duplicate estimations of carbon dioxide and chloride. The remainder of the plasma was pipetted off into a vessel surrounded by ice and successive portions of it used for the determination of the carbon dioxide combining power of separated plasma as well as for total...
non-protein nitrogen.

The remaining determinations were made in similar manner, only 25 c.c. of blood being used for each point. This amount was sufficient to allow of duplicate 2 c.c. samples of whole blood being used for carbon dioxide content and duplicate determinations of chlorides and carbon dioxide on the plasma after centrifugation under paraffin. The chlorides were estimated by the method of Wetmore (32).

Results obtained in this manner on cases III and IV are shown in Figs. 2 and 3 (detailed figures being given in protocols 1 and 2). The investigation revealed the surprising finding that no alteration of plasma chloride occurred at different carbon dioxide pressures, except to a very slight extent at extreme ranges of pressure. From the data of L.J. Henderson (34) it can be seen that if in fully oxygenated blood the carbon dioxide pressure changes from 40 mm. to 50 mm. the plasma chloride concentration changes from 99.5 to 98.6 mm. per litre (from 577 to 572 mg. %). Similarly for large variations in carbon dioxide pressure Dautrebande and Davies (39) found more considerable alterations in plasma chloride concentration. Thus in the blood of H. W.D. when the carbon dioxide pressure was changed from 42.5 to 143.3 mm. the plasma chlorides changed from 662 to 587 mg. per cent. and in L.D. a pressure change from 6.8 to 195 mm. was associated with a chloride change from 563 to 600 mg. per cent. Since it is in the form of hydrochloric acid that chlorine passes into the corpuscles with increasing carbon dioxide pressures there remains in the plasma a larger amount of available base (mainly sodium) for combination with the increasing amount of carbon dioxide. The extent of this increased carbon dioxide carrying power of the plasma brought about by ionic interchange between cells and plasma, is shown by the difference in slope of the curves for true plasma and separated plasma in the results of Joffe and Poulton (37). Van Slyke and Cullen (40) found that the chlorine transfer from plasma to corpuscles was sufficient to account for 72 per cent. of the alkali increase in the plasma when the carbon dioxide pressure of the blood was raised from 29 to 53 mm. In our results (figs. 2 and 3) the increased amount of alkali in the true plasma with increasing carbon dioxide pressures was/
Effect of varying degrees of lysis on Hamostichi Blooms

Blood kept in water bath at 37°C.

<table>
<thead>
<tr>
<th>Amount of Ag. Dist. added</th>
<th>Percentage Ag. Dist. present</th>
<th>Case IV</th>
<th>Case V</th>
<th>Degree of lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 cc</td>
<td>0%</td>
<td>45 min</td>
<td>60 min</td>
<td>None</td>
</tr>
<tr>
<td>2.5 cc</td>
<td>11%</td>
<td>43 &quot;</td>
<td>44 &quot;</td>
<td>None</td>
</tr>
<tr>
<td>5 cc</td>
<td>20%</td>
<td>28 &quot;</td>
<td>27 &quot;</td>
<td>Trace</td>
</tr>
<tr>
<td>10 cc</td>
<td>33%</td>
<td>23 &quot;</td>
<td>20 &quot;</td>
<td>Trace</td>
</tr>
<tr>
<td>1.5 cc</td>
<td>43%</td>
<td>20 &quot;</td>
<td>19 &quot;</td>
<td>Complete</td>
</tr>
<tr>
<td>2 cc</td>
<td>50%</td>
<td>20 &quot;</td>
<td>19 &quot;</td>
<td>Complete</td>
</tr>
<tr>
<td>3 cc</td>
<td>60%</td>
<td>38 &quot;</td>
<td>33 &quot;</td>
<td>Complete</td>
</tr>
<tr>
<td>0 cc</td>
<td>0%</td>
<td>46 &quot;</td>
<td>59 &quot;</td>
<td>None</td>
</tr>
</tbody>
</table>
**Effect of Lysin vs. Swatching on Haemophilic Blood**

Lysin - 1.5 cc. Dext added to 2 cc. blood
Swatching - Swatched for 2 minutes with bundle of fine wires

<table>
<thead>
<tr>
<th>Case</th>
<th>Control</th>
<th>Lysin</th>
<th>Swatching</th>
<th>Temp</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>35 min</td>
<td>14</td>
<td>13 min</td>
<td>37°</td>
</tr>
<tr>
<td>I</td>
<td>46 &quot;</td>
<td>21</td>
<td>23 &quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>I</td>
<td>34 &quot;</td>
<td>15</td>
<td>24 &quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>I</td>
<td>60 &quot;</td>
<td>25</td>
<td>8 &quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>I</td>
<td>38 &quot;</td>
<td>14 min</td>
<td>14 &quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>IV</td>
<td>45-56 &quot;</td>
<td>20 min</td>
<td>18 &quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>V</td>
<td>60 &quot;</td>
<td>19 &quot;</td>
<td>23 &quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>V</td>
<td>50 &quot;</td>
<td>25</td>
<td>-</td>
<td>&quot;</td>
</tr>
<tr>
<td>V</td>
<td>102 &quot; (Plasma)</td>
<td>-</td>
<td>60 &quot; (Plasma)</td>
<td>17°-37°C</td>
</tr>
<tr>
<td>V</td>
<td>60-65 &quot;</td>
<td>27 &quot;</td>
<td>20 &quot;</td>
<td>37°C</td>
</tr>
</tbody>
</table>

**Average**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Lysin</td>
<td>Swatching</td>
</tr>
<tr>
<td>21.0&quot;</td>
<td>18.4&quot;</td>
<td></td>
</tr>
</tbody>
</table>

Average decrease in coagulation time after Lysin **59.7%**
Swatching **62.9%**
Effect of Lysis & Scratching on normal blood

1.5 cc Ag. Dust added to 2 cc blood

Scratching - scratched for 2 minutes with bundle of fine hairs

<table>
<thead>
<tr>
<th>Case</th>
<th>Control</th>
<th>Lysis</th>
<th>Scratching</th>
<th>Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16 min.</td>
<td>5 min.</td>
<td>4 min.</td>
<td>37°C</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>14</td>
<td>9</td>
<td>18°C</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>11</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>10</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>14</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>10.5</td>
<td>11</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>13</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>12</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>17</td>
<td>15</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>14</td>
<td>2 (defibrinated)</td>
<td>6-9</td>
</tr>
</tbody>
</table>

Average decrease in coagulation time after Lysis = 7.4 h

Scalp scratching = 46%
Table IV

Thrombin activity of Haemophili as compared with normal serum

Sera used in any one experiment of the same age or never more than 30 hours old.
Blood kept at 37°C. Normal & Haemophili serum added in equal amounts.

<table>
<thead>
<tr>
<th>Case</th>
<th>Control</th>
<th>Normal Serum</th>
<th>Haemophili Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>35 min</td>
<td>5 min</td>
<td>5 min</td>
</tr>
<tr>
<td>II</td>
<td>46½”</td>
<td>11”</td>
<td>11½”</td>
</tr>
<tr>
<td>III</td>
<td>35”</td>
<td>11½”</td>
<td>14”</td>
</tr>
<tr>
<td>VI</td>
<td>60”</td>
<td>14½”</td>
<td>14”</td>
</tr>
</tbody>
</table>

Effect of lysin on rabbit's normal plasma

Blood collected with 1 cc of 1% Sodium cacodylate in 9% Sodium Chloride to every 8 cc blood. Then centrifuged for 15 minutes at 1,000 rpm per min., 8 plasma mixed with 1 cc 5% Calcium Chloride in Ag. Dent., to every 2 cc plasma.

2 cc plasma used in each, 21.5 cc Ag. Dent added to produce lysis of platelets.

Plasma kept at room temperature.

<table>
<thead>
<tr>
<th>Case</th>
<th>Control</th>
<th>Lysin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20 min</td>
<td>17 min</td>
</tr>
<tr>
<td>2</td>
<td>11 &quot;</td>
<td>10 &quot;</td>
</tr>
<tr>
<td>3</td>
<td>26 &quot;</td>
<td>21 &quot;</td>
</tr>
<tr>
<td>4</td>
<td>12 &quot;</td>
<td>10½ &quot;</td>
</tr>
<tr>
<td>5</td>
<td>13 &quot;</td>
<td>12½ &quot;</td>
</tr>
<tr>
<td>6</td>
<td>17 &quot;</td>
<td>15 &quot;</td>
</tr>
<tr>
<td>Average</td>
<td>16.5 &quot;</td>
<td>14 &quot;</td>
</tr>
</tbody>
</table>

Average decrease in coagulation time after lysis 15%.

Note: The shortening of the coagulation time in both normal and lysed blood is due to the fact that Calcium was added in excess of the optimum concentration (Howell).
Effect of lysin on recalcified Hamophelic plasma

Technique as with recalcified normal plasma.

Blood kept at room temperature

<table>
<thead>
<tr>
<th>Case</th>
<th>Control</th>
<th>Lysin</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>186 min</td>
<td>104 min</td>
</tr>
<tr>
<td>V</td>
<td>154 &quot;</td>
<td>91 &quot;</td>
</tr>
<tr>
<td>V</td>
<td>251 &quot;</td>
<td>115 &quot;</td>
</tr>
<tr>
<td>V</td>
<td>148 &quot;</td>
<td>107 &quot;</td>
</tr>
<tr>
<td>V</td>
<td>220 &quot;</td>
<td>140 &quot;</td>
</tr>
<tr>
<td>Average</td>
<td>190 &quot;</td>
<td>119 &quot;</td>
</tr>
</tbody>
</table>

Average decrease in coagulation time after lysin 38%
Comparison of Tables

<table>
<thead>
<tr>
<th></th>
<th>Normal decrease</th>
<th>Hemophili Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis of whole blood</td>
<td>7 %</td>
<td>59 %</td>
</tr>
<tr>
<td>Swelling of whole blood</td>
<td>46 %</td>
<td>62 %</td>
</tr>
<tr>
<td>Lysis of necrophors' plasma</td>
<td>15 %</td>
<td>38 %</td>
</tr>
<tr>
<td>Date</td>
<td>CO₂ content of initial venous blood, vol. %</td>
<td>CO₂ combining power, %</td>
</tr>
<tr>
<td>------------</td>
<td>--------------------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>12.6.26</td>
<td>30.4</td>
<td>43.8</td>
</tr>
<tr>
<td>17.6.26</td>
<td>27.7</td>
<td>43.5</td>
</tr>
<tr>
<td>9.4.26</td>
<td>38.7</td>
<td>49.3</td>
</tr>
<tr>
<td>30.4.26</td>
<td>25.5</td>
<td>42.6</td>
</tr>
<tr>
<td>15.4.26</td>
<td>33.7</td>
<td>49.1</td>
</tr>
<tr>
<td>16.5.26</td>
<td>32.6</td>
<td>48.7</td>
</tr>
<tr>
<td>5.6.26</td>
<td>32.2</td>
<td>49.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Further figures for cases III & IV are given in the protocols.
Venous blood drawn without stasis (forearm immersed in hot water).
Oxalate and fluoride added. Blood kept in vessel under paraffin and surrounded by ice.

at 93.2 mm. carbon dioxide pressure.

CO₂ content of whole blood 64.2 vol.%
" true plasma 76.5 "

Chlorides (as NaCl) in true plasma 569 mg.%

CO₂ content of whole blood 53.2 vol.%
" true plasma 64.8 "

Chlorides (as NaCl) in true plasma 575 mg.%

CO₂ content of whole blood 52.1 vol.%
" true plasma 78.8 "

Chlorides (as NaCl) in true plasma 575 mg.%

CO₂ content of whole blood 46.8 vol.%
" true plasma 62.8 "

Chlorides (as NaCl) in true plasma 575 mg.%

CO₂ content of whole blood 46.7 vol.%
" true plasma 55.6 "

Chlorides (as NaCl) in true plasma 575 mg.%

CO₂ content of whole blood 44.3 vol.%
" true plasma 52.7 "

Chlorides (as NaCl) in true plasma 575 mg.%

CO₂ content of whole blood 48.5 vol.%
" true plasma 55.3 "

Chlorides (as NaCl) in true plasma 575 mg.%

CO₂ content of whole blood 20.4 vol.%
" true plasma 24.7 "

Chlorides (as NaCl) in true plasma 575 mg.%

The plasma remaining from this determination was used as "separated plasma."
(Protocol I contd.)

**Separated plasma**

Non-protein nitrogen 32 mg.

Total nitrogen 232 mg.

At 33.9 mm. CO₂ pressure separated plasma took up 34.7 vol.% of CO₂ 49.9

Total nitrogen 256 mg.

**Protocol II. Case IV. 21.9.26** (See Fig. 3)

Venous blood drawn without stasis (forearm immersed in hot water)
Oxalate and fluoride added. Blood kept in vessel under paraffin and surrounded by ice.

Clotting time in test tube at 37°C, was 55 minutes.

At 92.1 mm. carbon dioxide pressure

CO₂ content of whole blood 62.1 vol.%

" true plasma 75.8 "

Chlorides (as NaCl) " 538 mg. %

At 56.4 mm. carbon dioxide pressure

CO₂ content of whole blood 55.6 vol.%

" true plasma 64.6 "

Chlorides (as NaCl) in " 538 mg. %

At 43.6 mm. carbon dioxide pressure

CO₂ content of whole blood 50.4 vol.%

" true plasma 59.9 "

Chlorides (as NaCl) in " 538 mg. %

At 32.4 mm. carbon dioxide pressure

CO₂ content of whole blood 45.3 vol. %

" true plasma 53.3 "

Chlorides (as NaCl) in " 538 mg. %

At 11.4 mm. carbon dioxide pressure

CO₂ content of whole blood 29.8 vol. %

" in true plasma 36.1 "

Chlorides (as NaCl) in " 544 mg. %
The plasma remaining from this determination was used as "separated plasma".

**Separated plasma**

Non-protein nitrogen 34 mg. %

Total nitrogen 256 mg. %

At 2.05 mm CO₂ pressure separated plasma took up 24.8 vol.% of CO₂:

<table>
<thead>
<tr>
<th></th>
<th>23.5</th>
<th>40.6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40.9</td>
<td>45.3</td>
</tr>
<tr>
<td></td>
<td>113.2</td>
<td>56.4</td>
</tr>
</tbody>
</table>

Case III. J.W.S. Act. 41. Male

**Family history** - Definite of haemophilia for 3 generations, 12 cases on record.

**Symptoms.** Bruises easily. Typical haemorrhage into joints, kidneys, bowel, gums and nose. Many trivial accidents followed by severe haemorrhage.


**Family history** - First cousin of Case I.

**Symptoms.** Bruises easily. Typical haemorrhage into joints, gums, and nose. Very severe haemorrhage from trauma on 4 occasions, once almost fatal.

Case V. P.J.D. Act. 34. Male.

**Family history** - Definite of haemophilia for 3 generations, 6 cases in family.

**Symptoms.** Bruises easily. Typical haemorrhage into joints, kidneys, lungs, gums and nose. One almost fatal haemorrhage after accident.

Case VI/
Case I. W.E. aet. 25. Male.

Family history - Definite of haemophilia for one generation (previous family history unknown) 4 definite, 4 indefinite (died in infancy) cases in family.


Case II. J.C. aet. 20. Male.

Family history - Definite of haemophilia for one generation only. 3 haemophilic brothers. One haemophilic cousin.

Symptoms. Bruises easily. Frequent epistaxis and bleeding from gums sometimes leading to severe anaemia. Very severe haemorrhage from trauma on 3 occasions, once almost fatal. No haemorrhage into joints.

Case III. J.W.S. Aet. 41. Male

Family history - Definite of haemophilia for 5 generations. 12 cases on record.


Case IV. J.C. aet. 25. Male.

Family history - First cousin of Case I.

Symptoms. Bruises easily. Typical haemorrhage into joints, gums, and nose. Very severe haemorrhage from trauma on 4 occasions, once almost fatal.

Case V. F.J.D. Aet. 34. Male.

Family history - Definite of haemophilia for 3 generations. 6 cases in family.


Case VI/

Family history - First cousin of Case III.


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FIG. 1

Case I
Case II
Case III
(Average)

T. W. C. (normal)
A. V. C.
III. The Treatment of Haemophilia.

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THE TREATMENT OF HAEMOPHILIA.

With our scanty knowledge of the true nature of blood coagulation it is not surprising that our knowledge as to the deficiency in Haemophilia should be even more fragmentary. Practically all the known coagulative elements of the blood have in turn been blamed and subsequently exonerated.

The older school believed that there was a deficiency in Thrombokinase, while more recently Weil and Feissly have claimed to have found an excess of anti-thrombin. Practically all modern authorities, however, agree that the deficiency lies in the prothrombin. Rational treatment must therefore be either directed towards replenishing the supply of available prothrombin or stimulating its formation.

Blood Transfusion by Direct Method. This is probably the earliest method described, by which haemorrhage in haemophilia can be successfully controlled. Here we have introduced into the blood stream a certain quantity of normal prothrombin which will act quantitatively in reducing the coagulation time. Fig. 1 shows the effect of transfusing a severe haemophiliac (Case I) with 725 c.c. of normal blood. As will be seen there was a very marked decrease in the coagulation time and lowering of the whole curve immediately after the transfusion, but this was followed by a steady rise till be the end of 7 days his blood had resumed its former degree of prothrombin deficiency.
Since it is typical of the disease that the coagulability of the blood and the severity of the symptoms vary from day to day, it is essential that clinical observations should be made over prolonged periods. So many extraneous factors influence the appearance of symptoms, that these can only be taken as an approximate index of progress, unless some artificial form of trauma be applied, such as the extraction of teeth. Observations on the coagulability of the blood remain as the most sound method of judging progress. As one of us has shown in another article, the most accurate method of gauging this coagulability is by means of the coagulation curve. Here the coagulability of successive drops of blood from the same puncture is taken according to the method described by Gibbs, the results being plotted in a curve. Thus an index is obtained, not only of the coagulability of the blood, but also of the tissue reaction and platelet reaction, the first, and last being deficient in Haemophilia (Macleod and Christie Davies & Stewart).

I. Introduction of normal Prothrombin.

a) Blood Transfusion by Direct Method.

This is probably the earliest method described, by which haemorrhage in haemophilia can be successfully controlled. Here we have introduced into the blood stream a certain quantity of normal prothrombin which will act quantitatively in reducing the coagulation time. Fig. I shows the effect of transfusing a severe haemophilic (Case I) with 725 c.c. of normal blood. As will be seen there was a very marked decrease in the coagulation time and lowering of the whole curve immediately after the transfusion, but this was followed by a steady rise till be the end of 7 days his blood had resumed its former degree of prothrombin deficiency.
b) Blood Transfusion by Citration Method.

Here again prothrombin is being introduced into the bloodstream in conjunction with a certain quantity of sodium citrate. That the amount of sodium citrate used (1.8 grammes) does not detract from the benefit of the transfusion is clearly shown by comparing Figs.II,III, & IV with Fig.I. This is only to be expected, since it has been proved beyond doubt that the calcium content in haemophilic blood is normal, and it is an accepted fact that a citrated transfusion does not decrease the coagulability of a normal recipient's blood. The possibility of there being any therapeutic value in the sodium citrate will be discussed later.

It will be seen by studying the coagulation curves (Figs.II, III,IV) of the 4 cases in which a transfusion of citrated blood was carried out, that, as with whole blood transfusion, the improvement was transient, lasting from 5 to 7 days. We got no subsequent increase of the coagulation time, as was suggested by Addis.

Case II is of special interest, in that the haemorrhagic tendency closely followed the changes in the coagulation curve after transfusion, extraneous influences having been reduced to a minimum. In this case a molar tooth was extracted after prolonged serum treatment. Steady haemorrhage continued from the tooth socket for 11 days after extraction, the haemoglobin dropping from 105% to 40%. The patient was then transfused (Fig.III). All haemorrhage had stopped ten minutes after its completion and did not recur.

It will be seen from the results of these transfusions that the beneficial effect lasts from 5 to 7 days. That the prothrombin introduced remains active for this period of time although the blood platelets have been shown to survive only 3 to 4 days (Duke), is
readily explained by the work of Morawitz, Bayne-Jones, Quintin & Pepper, Hess, and others, who have shown that on disintegration the prothrombin from the platelets passes into solution in the blood, and is still available for coagulative purposes.

c) Transfusion of Defibrinated Blood.

Here we are introducing a suspension of the cellular elements of the blood in serum, the active ingredient of which will be almost pure thrombin. (Mellanby). We have no practical experience of this line of treatment, and can only quote the work of Minot & Lee (Sajous), who have shown that there is a very definite but transient improvement in the coagulability of the blood after a transfusion of 600 c.c. of defibrinated blood. This improvement had almost disappeared by the end of 5 days, which is very similar to what we have found in citrated and whole blood transfusion. Since there must have been a very much smaller quantity of prothrombin (in the form of thrombin) present in the defibrinated blood used by Minot & Lee than in the whole blood and citrated blood used by us, we would suggest that it is the degree of improvement and not the duration of improvement that depends on the quantity of prothrombin introduced. Thus although in Fig I 30% more blood was used than in Fig II, the duration of improvement was similar, as in Fig V, there was no cumulative action with regard to duration after 2 transfusions.

d) Intravenous Fresh Human Serum.

The serum used was obtained by centrifuging normal blood which had been collected by venepuncture and allowed to clot. As with defibrinated blood, the serum would be poor in prothrombin content but rich in thrombin (Mellanby). In no case was the serum used more than 6 hours after preparation. This is well within the limits of safety, since it has been shown that serum retains its thrombin activity for 3 to 4 days (Weymouth & others).
Fig. V shows the effect of 12 intravenous injections of fresh human serum (average quantity 6.2 c.c.) at intervals of 48 hours, in Case II. As can be seen, the improvement was only slight, and reached its maximum after the 3rd injection, thereafter remaining stationary. After the last injection a carious molar tooth was extracted. That the improvement must have been only slight was shown by the haemorrhage that took place from the socket, this only being controlled by the blood transfusion (Fig. III) already described.

These results, although roughly agreeing with what was found by Howell & Addis, by no means support the optimistic views of Faile-Weil and Sajous.

We have not attempted to reinject the patient's own serum after clotting has taken place, but can see no reason why this should not be just as efficacious as serum from normal blood, since it has been conclusively shown that the thrombin content is the same in quantity and quality. (Addis, Minot & Lee, Sajous; Chiti, Davis & Stewart)

e) Introduction of Sheep Serum.

On only one case (Case I) did we try the effect of this form of treatment. Unfortunately no coagulation curve could be obtained, but the coagulation time by Dale & Laidlaw's method, and by the simple method of stirring the blood with a wire until a thread of fibrin became attached, was observed from time to time. In all, the patient had 15 subcutaneous injections of sheep serum, the total amount being 56 c.c. The coagulation times showed no improvement whatever, and if anything the symptoms were worse after treatment. Unfortunately the serum used was by no means fresh, its age varying from 1 to 4 weeks in the different injections.
f) Introduction of Horse Serum.

This form of treatment was only given a brief trial on one case, and as with sheep serum no coagulation curves were obtained. 10 c.c. of horse serum was administered intravenously, and although previous intradermal tests had been negative, pronounced anaphylactic phenomena were observed. This was followed by no clinical improvement, but by a slight improvement in the coagulation time.

II. Introduction of other Coagulative Elements.

a) Introduction of Calcium.

The administration of Calcium by the mouth, which has been abandoned by practically all modern investigators, probably owes its popularity to the fact that administration is easy, and something is better than nothing. The only published case that we can find where definite improvement is claimed is that of Max Kahn (Sajous), in which there was admittedly no haemophilic family history, and the blood calcium was definitely below normal, a thing unique in true haemophilia. We have tried prolonged administration of Calcium Lactate in 4 cases, (Cases I, II, V, VI), with completely negative results.

Intravenous injection of Calcium Chloride in animals has been shown by Gratia to produce disintegration of the platelets without thrombus formation. According to modern conception of haemophilia, this form of treatment might be of benefit, and to our knowledge has never been tried.

b) Administration of Hemostatic Serum.

This is another popular form of treatment, the value of which has been, in our opinion, very much overrated. The pamphlet issued by the manufacturers states that the active ingredients are: -
1) Prothrombin.
2) Thrombokinase.
3) Anti-antithrombin, which in modern terminology is identical with Thrombokinase.

It is a recognised fact that should thrombokinase be injected intravenously, as is advised with this serum, either you get intravenous clotting with instantaneous death if injected rapidly, or, if injected slowly, the production of "negative phase" blood with decreased coagulability (Mellanby, Mills). Thus the introduction of Thrombokinase can only do harm.

We have analysed a sample of the serum and found a considerable percentage of Calcium to be present (% ), which would ensure the transformation of the Prothrombin and Thrombokinase into Thrombin. We believe this latter to be the active ingredient of Hemoplastin, and since we have found experimentally that anything less than 10% of Hemoplastin in haemophilic blood in vitro does not hasten coagulation to any appreciable degree (Table I), it seems improbable that so small an amount as 2-4 c.c. should affect the 4-6 litres of circulating blood where the concentration would be '03 to '1%.

<table>
<thead>
<tr>
<th>Case</th>
<th>Amount of Blood</th>
<th>Amount of Hemostatic Serum</th>
<th>Dilution</th>
<th>Temperature</th>
<th>Clotted</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>1 c.c.</td>
<td>-</td>
<td>-</td>
<td>17</td>
<td>6 hrs 30 min.</td>
</tr>
<tr>
<td></td>
<td>1 c.c.</td>
<td>.001 c.c.</td>
<td>.001</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1 c.c.</td>
<td>.002 c.c.</td>
<td>.002</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>1 c.c.</td>
<td>.01 c.c.</td>
<td>.01</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1 c.c.</td>
<td>.02 c.c.</td>
<td>.02</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1 c.c.</td>
<td>.1 c.c.</td>
<td>.1</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1 c.c.</td>
<td>.2 c.c.</td>
<td>.2</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>Normal</td>
<td>1 c.c.</td>
<td>-</td>
<td>-</td>
<td>17</td>
<td>9</td>
</tr>
</tbody>
</table>

We have tried the effect of Hemoplastin on 3 cases:

1) Case I.
   4 intravenous injections of 2 c.c. Hemoplastin at 4-6 day/ inter-
intervals. No definite improvement in coagulation time, but followed by freedom from symptoms for 3 weeks. After last injection marked anaphylactic phenomena.

2) Case II.

3 injections of 4 c.c. hemoplastin intramuscularly at intervals of 34 hours. No improvement in coagulation curve. Marked pain and tenderness in buttock after last injection, probably due to haemorrhage. 1 injection of 4 c.c. hemoplastin intravenously followed by marked anaphylactic phenomena. No improvement in coagulation curve.

3) Case VI.

Has had 3 c.c. hemoplastin intramuscularly at intervals of 3 months, for 3 years. No clinical improvement.

Thus it would appear that Hemoplastin is of little or no therapeutic value in Haemophilia. Given intravenously it endangers life and given intramuscularly we and others have found that there is a definite danger of formation of a haematoma at the site of injection.

c) Administration of "Tissue Fibrinogen" ("Fibrogen- Morrell").

This is another preparation which has a considerable vogue in the treatment of haemophilia, although we have not been able to find any authenticated case where definite improvement followed its use. Its active principle is admittedly thrombokinase, so whether administered orally or subcutaneously it must necessarily pass through the bloodstream before distributing itself in the tissues, and must therefore expose itself to the factors described in the previous paragraph, thus rendering it either useless or harmful.

In Case II we gave the recommended subcutaneous dose at 12-hourly intervals, till 3 injections had been given. No improvement was found in the coagulation curve. In Case III we gave the recommended subcu-
taneous dose \((\frac{1}{2}-1\text{ c.c.})\). A marked local reaction resulted, with slight involvement of the glands in the axilla. No improvement was found in the coagulation curve.

**III. Stimulation of Coagulation.**

a) **Introduction of Sodium Citrate.**

The evident success which has attended the use of this drug in other haemorrhagic diseases has led us to give it a fairly extensive trial in Haemophilia. That the intravenous injection of small quantities of Sodium Citrate is definitely followed by a hyper coagulability of the blood in a normal subject, has been shown by Weil, Pickering & Hewitt, and others, although the actual mechanism of this improvement has never been demonstrated. It is not the actual presence of the citrate that is responsible, since this is very rapidly oxidised and removed from the tissues (Sabbatini, Salant & Wise), but is probably something of the nature of a recalcification in vivo (Pickering & Hunter).

In the cases described each injection has been of 6 G. Sodium Citrate in distilled water, given intravenously.

Case V. 9 injections in all, at intervals of 3 weeks. No improvement in blood coagulation, but has been quite free from symptoms, and has firm belief in the benefit which has accrued from his injection.

Case III. 7 injections at 4-day intervals, followed by 8 injections at weekly intervals. No improvement in coagulation, but symptoms relieved apart from bruising. On returning home from his last injection, he fractured his patella. This was followed by extensive haemorrhage into the joint and surrounding tissues, the leg being swollen and discoloured from hip to toe. With each of the first three injections the coagulation time was taken immediately before and 15 minutes after the injection, by means of placing venous blood in a test tube at 37 C.
The following figures show a definite increase in the coagulability.

<table>
<thead>
<tr>
<th>Coagulation time before injection</th>
<th>After injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) 35 minutes</td>
<td>16 minutes</td>
</tr>
<tr>
<td>2) 46 &quot;</td>
<td>34 &quot;</td>
</tr>
<tr>
<td>3) 47 &quot;</td>
<td>35 &quot;</td>
</tr>
</tbody>
</table>

Coagulation curves however taken 6 hours later showed no improvement.

Case IV. 16 injections at 3-4 day intervals, followed by 4 at 7 day intervals. No improvement in coagulation curves. Patient states however that he never remembers being so free from symptoms as he has been since commencing injection. Has only had occasional slight bleedings into right elbow.

Thus it would seem that intravenous sodium citrate produces a very transient increase in the coagulability of the blood. In the 3 cases described this was followed by a permanent clinical improvement, but the degree of this improvement does not warrant the assumption that it was due to the injection of Sodium Citrate.

b) Administration of Peptone.

This form of treatment has not yet been thoroughly investigated, but what has been done gives little hope of success (Sagous). We gave Case I a series of intravenous injections of Armour's No 2 peptone 5% starting at 0.1 c.c. and finishing at 1.5 c.c., 11 injections, and 11.2 c.c. in all, at intervals of 3-4 days. The patient is convinced that there was no clinical improvement.

c) Protein Shock.

In the two cases described we have been unable to confirm the work of Vines and Mills, who claim a definite improvement after anaphylactic shock or protein sensitisation.

Case I, (a)10 c.c. horse serum intravenously. Slight anaphylactic phenomenon, followed by appearance of pronounced serum rash. No
improvement in symptoms. Slight improvement in coagulation time.

(b) 4 c.c. hemostatic serum intravenously. Very marked anaphylactic phenomena. No improvement in symptoms nor coagulation time.

Case II. 4 c.c. hemostatic serum intravenously. Very marked anaphylactic phenomena. No improvement in symptoms nor coagulation curve.

d) Administration of Thymus extract.

This form of treatment is advised in many text-books, but no definite evidence has been produced to prove its utility. Case I took it by the mouth for some months with no beneficial results. The injection of Thymus Nucleic Acid will be dealt with later.

Miscellaneous. Amongst the many other substances that have been tried with no proven success are Gelatin, raw meat juices, milk, thyroid extract, ergot, adrenalin, turpentine, perchloride of iron, and practically every known stiptic. Locally we have found that fresh human blood applied in cotton wool after all useless clots have been removed is the most efficacious coagulant.

Prophylaxis.

It is obvious that trauma of any sort must be strictly avoided, but apart from this we have little evidence as to what causes the exacerbation which are so typical of the disease. Case I, a well educated and very intelligent patient, is quite certain that the use of any strong purge is very often instrumental in starting a sequence of haemorrhages. Cases III, IV, and V are equally convinced that while leading an open-air life the haemorrhagic tendency is diminished.

Suggestions as to further methods of Treatment.

a) Disintegration of Platelets, thus liberating prothrombin for coagulative purposes. Both Antiplatelet serum (Bedson) and intravenous
Calcium Chloride (Gratia) have been shown to disintegrate the platelets in vivo in animals, and to our knowledge neither has been tried in haemophilia. The dangers of intravalvular clotting and the production of purpura would have to be carefully excluded.

b) A defibrinated transfusion of the patient's own blood should be effective in cases of emergency, as has already been suggested.

c) Thymus Nucleic Acid in small quantities has been shown to accelerate clotting (Pickering & Taylor) and to our knowledge has never been tried in Haemophilia.

Conclusions.

1) The only means by which the coagulability of Haemophilic blood can be increased to any appreciable extent, and the symptoms definitely controlled, is by blood transfusion, whether it be whole blood, citrated blood, or defibrinated blood. Of these we believe the citration method to be the best.

2) This improvement lasts from 5 to 7 days, it being the degree and not the duration of improvement which depends on the amount of blood given. We found no negative phase as was suggested by Addis.

3) A slight but transient improvement was obtained after intravenous injections of fresh human serum. Subsequent injections produced no cumulative action.

4) Hæmostatic serum, Sheep serum (not fresh), Horse serum (not fresh), "Fibrogen Norrell", Peptone, Calcium, Thymus Extract, and Protein shock have been tried with negative results.

5) Intravenous sodium citrate has been given an extensive trial, and appears to be of some slight therapeutic value.

6) Locally we have found the most effective coagulant to be fresh human blood soaked in cotton wool and applied after removal of all
useless clots.

7) Anti-platelet serum, Intravenous Calcium Chloride, and Thymus Nucleic Acid are suggested as being worthy of a trial in the treatment of Haemophilia.

Case I. F.J.D., Male, Aet. 34.

History: Definite of Haemophilia for 3 generations. 6 definite cases in family.

Symptoms: Bruises easily. Typical haemorrhage into joints, kidneys, lungs, gums, and nose. One almost fatal haemorrhage after an accident.

Case II. J.C., Male, Aet. 30.

History: Definite of Haemophilia for one generation only. 3 haemophilic brothers, one haemophilic cousin.

Symptoms: Bruises easily. Frequent epistaxis and bleeding from gums, sometimes leading to severe anaemia. Very severe haemorrhage from trauma on 3 occasions, once almost fatal. No haemorrhage into joints. A comparatively mild haemophilic.

Case III. W.E., Male, Aet. 25.

History: Definite of Haemophilia for one generation. (Previous family history not known.) 4 definite cases in family, 4 indefinite, died in infancy.


Case IV. J.C., Male, Aet. 25.

History: First cousin of Case III.

Symptoms: Bruises easily. Typical haemorrhages into joints, gums, and nose. Very severe haemorrhage from trauma on 4 occasions, once almost fatal.
Case V. J.W.S., Male, Aet. 41.

History: Definite of Haemophilia for 5 generations. 13 cases on record.


Case VI. G.M., Male, Aet. 7.

History: Definite of haemophilia for 2 generations. 5 definite cases in family.


References.

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Treatment of Haemophilia

Coagulation Curves
Blood Transfusion - Whole Blood

Successive Drops

Before Transfusion & 7 days after
6 days after Transfusion

Fig 1

Case I

1 2 3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6
Fig III
Case III

Successive Drops

Blood Transfusion - Citrated Blood.

Before Transfusion
5 Days after Transfusion
3 " " 
1 " " 
4 Hours " " 

1st 2nd 3rd 4th 5th 6th 7th 8th 9th
Fig IV

Case 11

7 Days after 2nd Transfusion
Before Transfusion

3 Days after 2nd Transfusion
5 " 2nd "

2 " 2nd "
1 " 1st "
1 " 2nd "

Successive Drops

Blood Transfusion — Citrated Blood

(2nd Transfusion 70 hours after 1st)
Fig V
Case II

Before Treatment

After 3 Injections

After 6 Injections

After 12 Injections

After 8 Injections

Successive Drops
intravenous human serum