

T H E S I S

on

HAEMORRHAGE IN JAUNDICE.

Presented for the Degree of

Ch. M.

by

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VOLUME 1.

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HAEMORRHAGE IN JAUNDICE.

It is established that in toxic-infective and obstructive forms of jaundice there commonly develops a state characterised by an undue liability to haemorrhage.

The bleeding may occur from a mucous or serous surface or into the skin. It commonly gives rise to epistaxis, haematemesis, melaena, purpura. The danger of haemorrhage is greatest in the period immediately after operation, and herein lies its special importance from the surgical standpoint. The usual experience is that no excessive bleeding is noticed during the operation, and haemostasis is generally secured without difficulty; the bleeding takes place a few days later, and the period of greatest /

greatest danger lies between the third and the sixth day. Such post-operative bleeding may occur at the sites already mentioned, but more commonly it takes place from the raw surfaces in the operative field. It is very apt to take the form of a slow ooze into the depths of the wound, forming a haematoma there or perhaps leaking to the surface at the incision.

Haemorrhage in jaundice is thus a condition of no little surgical importance. In the investigation reported here I have aimed at the solution of three problems related to the subject, the cause of the bleeding tendency, its recognition and its prevention or treatment.

The work is based upon a study of 50 cases of jaundice under treatment in the Edinburgh Western Hospital and the Edinburgh Royal Infirmary, including 12 cases in which spontaneous or post-operative haemorrhages developed. It comprises clinical and laboratory investigations, with special reference to the coagulability of the blood and other factors concerned in the arrest of haemorrhage. Special attention has been directed to the prothrombin /

prothrombin content of the blood, and evidence is presented in support of the view that a prothrombin deficiency is an important factor predisposing to haemorrhage in jaundice. The results of prothrombin estimations on 34 jaundiced cases are given, and the value of this test as a method of gauging the risk of haemorrhage is discussed. The cause of the prothrombin deficiency is considered, and the evidence attributing it to faulty absorption or faulty utilisation of a vitamin is studied. Finally, observations are recorded on the effect of administering preparations containing the vitamin to jaundiced patients.

I. HISTORICAL NOTES.

That acute observer, Matthew Baillie⁽²⁾, appears to have been the first to draw attention to the bleeding tendency in jaundice. His brief reference to the subject is contained in his book on "The Morbid Anatomy of Some of the Most Important Parts of the Human Body", which was published in 1793. Under the heading "Common Tubercle of the Liver", which appears to denote coarse cirrhosis, he states that in prolonged jaundice "the blood is found not to be coagulated at all after death, or to be coagulated very loosely". The cause he attributed to "the chemical influence of a mixture of a certain proportion of bile with the blood".

Between 1793 and 1878, brief references to the risk of bleeding in jaundice are contained in the works of Budd⁽⁹⁾, Murchison⁽⁴⁸⁾, Frerichs⁽²³⁾ and Leyden⁽³⁸⁾, but except in relation to one disease the literature of this period appears to contain no more exact account of the condition.

The one exception is the occurrence of haemorrhage, particularly umbilical haemorrhage, as

a complication of jaundice in young infants. The first record of this subject appears to have been made by Alexander Campbell⁽¹²⁾, of Edinburgh, in the first volume (1844) of the Northern Journal of Medicine.

Campbell described four cases— one seen in his own practice, three contributed by colleagues in the city - in which haemorrhage occurred as a complication of icterus neonatorum due to congenital obliteration of the bile ducts. The bleeding took place at the umbilicus of three of the infants, originated at about the 7th day and caused death by the 10th day after birth. The remaining infant passed safely through this danger period of umbilical sloughing and survived to the age of six months, when a massive haematemesis precipitated the fatal issue.

Subsequent to 1844 the subject of umbilical haemorrhage in jaundiced infants attracted considerable attention, and Grandidier⁽²⁵⁾, writing in 1859, collected records of 81 cases, in 75 of which the issue proved fatal. Grandidier noted that quite commonly (35 cases) in addition to haemorrhage from the umbilicus there were other indications of a bleeding /

bleeding tendency, for example, petechiae and more extensive ecchymoses. He also distinguished clearly between the common mild type of icterus neonatorum, physiological rather than pathological, which causes no bleeding tendency, and the dangerous type due to congenital maldevelopments, inflammations and cirrhoses of the liver.

With the advent of surgical intervention for diseases of the biliary tract, the subject of haemorrhage in jaundice assumed a new importance, for operative trauma often precipitates haemorrhage and calls attention to the bleeding tendency. Consequently, it is not surprising that since Bobbs' first uncertain cholecystotomy in 1867, there have been increasingly frequent records of this post-operative complication.

Marion Sims⁽⁵⁸⁾, the famous American surgeon and obstetrician, who did so much to establish biliary surgery on a sound footing, appears to have been the first, in 1878, to report an instance of haemorrhage after operation in a jaundiced subject, and it is of interest to note that this also was one of the first cases of obstructive /

obstructive jaundice ever submitted to operation.

Sims' patient was a woman of 45 years. She had suffered for four months from jaundice which was insidious in onset, painless and progressive. From the description of the operation and autopsy findings, the cause would appear to have been a stone in the common bile duct.

Sims operated and drained the gall-bladder, which contained a number of stones. The operation was carried out with antiseptic precautions and under the carbolic spray. For the first few days the patient progressed well, but on the fifth day there was some bleeding from the gums. It continued on the sixth day, and there was a slight ooze of blood from the wound. The bleeding from both sources became more copious on the seventh day. On the eighth day there was a haematemesis, and shortly after that she died. Post-mortem examination revealed also a collection of blood in the peritoneal cavity.

II. FREQUENCY OF HAEMORRHAGE IN JAUNDICE.

Sims' case illustrates well an all too common experience in the surgery of jaundice. The frequency of haemorrhage as a post-operative complication has been estimated variously by different authorities. Thus, Walters⁽⁶⁶⁾ has stated that fully 50 per cent. of deaths after operation in jaundiced subjects are due to haemorrhage, but most surgeons would probably assess the frequency at a very much lower figure than this.

To give a proper indication of the frequency, Table 1 has been compiled from the figures given in four papers by American and continental writers, relating to nearly 4,000 operations on jaundiced subjects. In this series there were 442 post-operative deaths, and 61 of these, or 13.8 per cent., were attributed to haemorrhage. This figure tallies very closely with the 12 per cent. given by Sir John Fraser⁽²²⁾ in his recent paper based on experience in this country.

The problem of haemorrhage in jaundice is thus one of considerable magnitude especially when /

when it is noted that the figures quoted above refer only to fatal cases. In non-fatal cases the liability to haemorrhage also has an importance in influencing morbidity, and especially as a cause of delay in wound healing through the formation of a haematoma.

In my own series of 50 cases there were 12 patients who developed haemorrhage. In seven of these the haemorrhage followed operation (in four of them it proved fatal). In the remaining five cases the haemorrhage developed apart from operation, and in four of these it proved fatal.

The incidence of haemorrhage in my series is thus considerably in excess of the general experience, but this can readily be understood when the type of case under treatment is considered. Most of the 50 cases examined, and all except two of the cases with haemorrhage, were under treatment in the Edinburgh Municipal Hospitals, whither it is still the custom to send patients who are too ill for treatment in other institutions. Thus many of the cases were admitted in the late stages /

stages of malignant jaundice, at a time when the bleeding tendency was very apt to develop. The severity of the disease in the cases under treatment may indeed be judged from the fact that of the 12 patients with haemorrhage, only seven were sufficiently fit for operative treatment to be undertaken.

III. THE SITES OF HAEMORRHAGE IN JAUNDICE.

Attention has already been directed to two important sites of haemorrhage - the umbilicus (in young infants) and the wound (after operation). It is clear that in these two examples the site of haemorrhage is determined by the existence of a raw surface from which oozing may readily occur. In post-operative cases for a similar reason any anastomosis, e.g., a cholecyst-gastrostomy, is an important site for haemorrhage. Where no such predisposing factor obtains, the haemorrhage may occur from any mucous or serous membrane or into the skin or interstitial tissues.

The sites of haemorrhage in my series of 12 cases are indicated in Table 2. It will be seen that six of the seven operated cases had bleeding from the wound, and two had bleeding from the stoma of a cholecyst-gastrostomy. Post-operative haematemesis was noted once, uterine haemorrhage once, epistaxis twice, and melaena three times.

In the five unoperated cases,
epistaxis /

epistaxis was noted once, haematemesis twice, melaena twice, and haemorrhage into the integuments three times.

In one patient (Case 15) after the gallbladder had been drained externally, there was bleeding from the drainage tube. The blood was intimately mixed with the bile, and it appeared likely that the haemorrhage had originated in the liver or bile ducts, possibly as a result of biliary decompression and thus comparable to renal bleeding after cystostomy.

IV. THE INCIDENCE OF HAEMORRHAGE IN JAUNDICE.

It is commonly stated that the liability to haemorrhage becomes manifest most often in deep jaundice resulting from malignant obstruction of the ducts. To determine the accuracy of this statement, I have collected the relevant figures from the literature and the records of my own cases, and set them down in the following paragraphs.

IV.A. Relation of the Bleeding Tendency to the Duration of Jaundice.

Table 3 shows the figures given in Petré'n's paper⁽⁵¹⁾, which is based on 58 cases of post-operative haemorrhage in jaundiced patients treated in various Swedish hospitals during the period 1892 to 1915. This is the only reference I can find in the literature to the duration of the jaundice in relation to the risk of haemorrhage, and its value is diminished by the fact that there is no indication of the total number of cases in each of the duration groups. Petré'n's figures are, however, important in one respect, namely that they show clearly that post-operative haemorrhage is by no /

no means limited to cases of long standing; in no fewer than 19 of Petré'n's cases the jaundice had been present for less than one month.

Table 4 shows the duration of the jaundice in the 50 cases of the present series, including the 12 cases with haemorrhage. It will be seen that in the group of 22 cases with jaundice of less than one month's duration there were only 3 cases with haemorrhage; the frequency of this complication appeared to increase as the jaundice was prolonged, and in 5 cases with jaundice of over 4 months, there were no fewer than 4 with haemorrhage.

Before accepting these figures as proof that the risk of haemorrhage increases as the jaundice is prolonged, it is necessary to consider two possible fallacies.

The first arises from the fact that the haemorrhage is often a post-operative event, and operation is rarely undertaken in the early stages of jaundice. To throw light upon this point Table 5 has been constructed, similar to Table 4, but referring only to unoperated cases. It will be seen /

seen that here again the relation between the duration of the jaundice and the risk of haemorrhage seems evident.

The second possible fallacy arises from the fact that many of the cases with jaundice of short duration were cases of mild toxic jaundice, whilst those of long duration were almost all severe cases of obstructive jaundice.

It has commonly been observed that in acute yellow atrophy and other severe forms of toxic jaundice, haemorrhage commonly occurs at an early stage in the disease, and it seems quite possible that the apparent increased risk of haemorrhage in the later stages in the cases in my series may be due not so much to the duration of the jaundice as to the fact that as a result of prolonged biliary obstruction certain secondary toxic changes develop. Chief among these is the secondary hepatic impairment so constantly noted as a late effect of biliary obstruction, and it seems that the diverse observations recorded above may well be correlated on the basis that the haemorrhagic tendency is related to the degree of liver damage, /

damage, for it is well established that such damage is greatest in the early stages of toxic jaundice, but in the later stages of obstructive jaundice.

IV.B. Relation of the Bleeding Tendency to the Depth of Jaundice.

Table 6 shows the figures given by W. Walters⁽⁶⁶⁾, to indicate the relation of the bleeding tendency to the depth of jaundice. They refer to a series of 29 post-operative deaths in jaundiced patients, 15 of which were attributed mainly or wholly to haemorrhage. It will be seen that in the first group of 5 cases with slight jaundice only one death was due to haemorrhage; with increasing depth of jaundice the frequency of haemorrhage increased, so that in the last group of 9 cases with deep jaundice no fewer than 7 deaths were due to this cause.

Table 7 shows the relation between the duration of jaundice and the bleeding tendency in my own series of 50 cases, including the 12 cases with haemorrhage. The cases have been classified into four groups according to the Icteric Index as estimated by Meulengracht's method. It will be seen that /

It will be seen that bleeding occurred in only one of the 11 cases with Icteric Index below 50, and that with increasing depth of jaundice there was an increased incidence of haemorrhage. That this is not an adventitious result depending upon the operation rate for different grades of jaundice is seen from Table 8, which refers only to unoperated cases.

IV.C. Relation of the Bleeding Tendency to the Lesion Causing Jaundice.

It is generally agreed that haemorrhage is practically unknown as a complication of catarrhal jaundice, but it is commonly seen in other forms of toxi-infective jaundice and in obstructive jaundice. Thus, it is well known as a complication of jaundice in such toxic conditions as acute yellow atrophy, spirochaetal jaundice, chloroform poisoning, phosphorus poisoning, and various acute infections. Similarly, haemorrhage has been observed in obstructive jaundice due to congenital atresia of the ducts, traumatic stricture of the common duct, stone in the common duct, carcinoma of the pancreas and other diseases.

The frequency with which these causative lesions are encountered naturally varies in /

in different institutions; in surgical wards, calculous and malignant obstructions preponderate. The lesions responsible in two series of cases published respectively by Petré⁽⁵¹⁾ (Sweden) and Boland⁽³⁾ (Mayo Clinic) are indicated in Table 9, whilst Table 10 gives the lesions responsible in the 12 cases with haemorrhage in my own series.

V. EARLIER VIEWS ON THE CAUSE
OF THE BLEEDING TENDENCY.

Many varied theories have been put forward in the past to account for the undue liability to haemorrhage in jaundice. Among the more important have been the theories ascribing it to the retention of bile in the blood and to a deficiency of the calcium or fibrinogen content of the blood. It will be advisable to consider briefly the observations relating to these theories before proceeding to the views held at the present time.

V. A. Retention of Bile as the
Cause of the Bleeding Tendency.

Matthew Baillie⁽²⁾ was the originator of the earliest theory when he stated that "most probably the faulty coagulation depends on the chemical influence of a mixture of a certain proportion of bile with the blood". This theory obtained some support from the observation that the addition of a sufficient quantity of bile to blood in vitro did, in fact, render it incoagulable. However, more recent observations have shown that the /

the amount of bile required to bring about this result represents a concentration many times greater than is ever known to be present in vivo, and thus it would appear that further proof is required before this theory can be accepted.

There are three principal constituents of the bile which may be retained in the blood stream, namely, cholesterol, the bile pigments, and the bile salts. It is accepted that cholesterol is quite innocuous, and in any case, the cholesterol content of the blood in jaundice rarely exceeds that found in normal pregnancy. The bile pigments were formerly thought to have a harmful effect upon the blood, and the experiments of King and Stewart⁽³⁴⁾ appeared to give confirmation to this view; but more recently the observations of Horrall and Carlson⁽²⁸⁾ make it clear that they are quite innocuous. The bile salts are well known to have a harmful effect, mainly in virtue of their property of reducing surface tension. However, it has been shown (Morawitz and Bierich⁽⁴⁶⁾; Brakefield and Schmidt⁽⁵⁾; Rowntree, Greene and Aldrich⁽⁵⁶⁾;) that the bile salts in the blood after increasing in /

in amount for two or three weeks, later fall to the normal level, whereas it is known that the bleeding tendency only becomes manifest at a considerably later period. This would seem to indicate that the retention of bile salts is not responsible for the bleeding tendency.

Perhaps the most definite evidence on this score is that given by Wildegans⁽⁶⁹⁾. This worker anastomosed the common bile duct to the inferior vena cava in dogs, so that the whole bile secretion entered the blood stream. All the dogs died within a few days of operation, but none showed delayed coagulability or any tendency towards haemorrhage.

V. B. Calcium Deficiency as the Cause
 of the Bleeding Tendency.

As long ago as 1894, Mayo Robson⁽⁴⁴⁾ suggested that a calcium deficiency might be responsible for the bleeding tendency, and this view has been widely held almost up to the present time. Originally suggested on empiric grounds, the theory of calcium deficiency as a cause of haemorrhage in jaundice /

jaundice has been supported both clinically and experimentally by a large number of observers, but denied by an even larger number. The consensus of opinion at the present day is against the theory.

Difficulty arises in the first place from the fact that our knowledge of the part played by calcium in the normal process of coagulation is far from exact. Formerly, it was presumed that ionised calcium alone took a part in the process, and many observations were made to determine whether any change could be demonstrated in the ionised calcium of the blood in jaundiced subjects. The work of Vines⁽⁶⁵⁾, and of Stewart and Percival⁽⁶²⁾ however, indicates that the ionised calcium is of little importance in this connection, and that the essential factor is a complex non-dialysable compound of calcium with the blood proteins, the amount of which cannot be estimated with any degree of accuracy.

A considerable literature has accumulated on these and other aspects of the relation of calcium to the bleeding tendency in jaundice. To avoid needless repetition and for the sake of lucidity /

lucidity it will be sufficient to summarise the more important work in six sections as follows:-

1. The Serum Calcium in Jaundice.

Earlier workers claimed that a diminution in the total calcium content of the blood is a constant feature in jaundiced patients and animals (King, Bigelow and Pearce⁽³³⁾; Buchbinder and Kern⁽⁸⁾). It has also been claimed that the reduction affects particularly the ionised calcium of the blood (Kirk and King⁽³⁵⁾). More recent observations by competent observers have, however, made it appear clear that these claims were unfounded, and there is now authentic evidence that in jaundiced patients and adult animals (though perhaps not in immature animals) the total blood calcium and the ionised blood calcium are both unchanged in amount (Snell and Greene⁽⁶¹⁾; Gunther and Greenberg⁽²⁶⁾; Zimmerman⁽⁷⁰⁾).

The serum calcium has been estimated in 24 cases of my series including eight of those in which haemorrhages developed. The figures obtained /

obtained in these cases, and in 30 normal controls are given in Chart 1. It will be seen that although the figures obtained in the cases showing haemorrhages were, on the whole, somewhat lower than in the control series, they were, with one exception, within the normal range.

2. Skeletal Decalcification in Jaundice.

Although direct evidence of a disturbance of calcium metabolism has thus been unobtainable, a certain amount of indirect evidence does exist in the observation that in prolonged jaundice some decalcification of the skeleton takes place.

Experimentally, such skeletal decalcification has been observed in puppies with obstructive jaundice, though not in adult dogs (Buchbinder and Kern⁽⁷⁾). A similar decalcification occurs also in cases of biliary fistula, a condition which, like jaundice, is associated with an abnormal liability to haemorrhage.

It must be remarked, however, that the mere demonstration of skeletal decalcification cannot be accepted as evidence that calcium deficiency /

deficiency is responsible for the bleeding tendency. Skeletal decalcification does not imply a deficiency of any part of the calcium content of the blood — indeed in parathyroid osteo-dystrophy it is associated with a raised blood-calcium — nor is skeletal decalcification in other affections (old age, decubitus, carcinomatosis, osteomalacia, etc.) associated with a tendency towards haemorrhage.

3. Effect of Calcium In Vitro.

It has been claimed by Lee and Vincent⁽³⁷⁾ that the addition of calcium to jaundiced blood in vitro accelerates coagulation. They have made this the basis of a clinical test, which consists in adding 3 drops of a 1 per cent. solution of calcium chloride to 1 cc. of blood, and estimating the coagulation time of the mixture.

If it could be shown that in vitro calcium accelerates the clotting of jaundiced blood to a greater extent than normal blood, the observation could be regarded as evidence ^{that} ~~of~~ calcium deficiency ^{is} ~~as~~ a cause of the faulty clotting in jaundice. However, although their test has been widely /

widely adopted for clinical use, the observations on which it is based lack confirmation. There is, indeed, evidence to indicate that calcium in vitro often fails to accelerate clotting in jaundice. Linton⁽³⁹⁾, for example, has been unable to show any constant acceleration of clotting by the addition of calcium in vitro, and in 17 of a series of 23 jaundiced cases, the calcium appeared actually to delay coagulation.

5. Effect of Parathormone.

It was claimed by Cantarow⁽¹³⁾ and others that the coagulation time in jaundice — and consequently the risk of haemorrhage — could be reduced by the administration of parathyroid extract. This was regarded as additional proof that the bleeding tendency in jaundice is attributable to a calcium deficiency. However, no confirmation has been forthcoming of Cantarow's work. Indeed, Zimmerman⁽⁷⁰⁾ found no constant effect on the coagulation time in either normal or icteric patients or in animals by the injection of parathyroid hormone, even though sufficient were injected to raise the serum calcium to double its normal value. Moreover, it has been shown /

shown by Simpson and Rasmusson⁽⁵⁷⁾ and by Ravdin and his colleagues⁽⁵⁴⁾ that when the serum calcium is reduced — even to the point of tetany — by parathyroidectomy, there is no disturbance of the blood coagulation.

6. Effect of Calcium Therapy.

Since Mayo Robson first advised the administration of calcium, many surgeons have testified to its value in the prevention of post-operative haemorrhage. Formerly it was the practice to administer calcium lactate or calcium chloride by the mouth. Later, Walters⁽⁶⁶⁾ in several papers based on his experience at the Mayo Clinic, advocated the intravenous administration of calcium chloride. In recent years, calcium gluconate has been preferred for intravenous use.

Despite the widely held clinical belief, there is little real evidence of the value of calcium administration in jaundice, and there appears to be no record of a long series of cases with adequate control observations. Moreover, there is no doubt that post-operative haemorrhage may /

may occur despite the use of calcium; for example, Judd⁽³⁰⁾ reported 4 deaths from haemorrhage in a series of 142 operations for stone in the common bile duct where this treatment was carried out. Further evidence of the ineffectiveness of calcium is provided by the observations of Linton⁽³⁹⁾ who found that in 7 out of 9 cases the intravenous administration of calcium chloride actually delayed coagulation.

In my own series of cases calcium chloride or calcium gluconate has been given intravenously in 5 cases and in each a careful study of the coagulation time has been made during the subsequent period. In no case did the administration of calcium have any significant effect on the coagulation time. Two of the patients thus treated subsequently died of haemorrhage.

The following two cases illustrate my experience of the effect of calcium administration in jaundice:

Case 7. This was a woman aged 60, suffering from jaundice due to carcinoma of the pancreas. She also was regarded as a case of scurvy and reacted well to treatment by vitamin C (see p. 35).

Chart 2 illustrates the coagulation time /

time as measured by the capillary tube method during a period of 5 days. It will be seen that throughout the period of observation the coagulation time varied between wide limits. After the intravenous injection of 5 ccs. of 10 per cent. calcium chloride solution the coagulation^{time} appeared to be reduced within a few hours from 16 minutes to $11\frac{1}{2}$ minutes, but the reduction was a transient one, and within 11 hours of the injection the coagulation time was raised to 21 minutes.

Case 15. This was a woman aged 70, (Chart 3) also suffering from jaundice due to carcinoma of the pancreas. Operation was carried out on the fourth day after her admission to hospital and the gallbladder was drained externally.

Calcium gluconate (15 ccs. of 10 per cent. solution) was administered intravenously as a pre-operative measure, and appeared to have the effect of reducing the coagulation time (estimated 10 hours later) from 8 minutes to 6 minutes.

Subsequently the coagulation time rose, and on the tenth day after admission it stood at 15 minutes. A similar injection of calcium gluconate at this point, however, did not effect any reduction, and on the contrary, the coagulation time (estimated 10 hours later) rose to 18 minutes.

V. C. Fibrinogen Deficiency as the Cause of the Bleeding Tendency.

In 1905, Doyon⁽²⁰⁾, in the course of an investigation into the effects of various poisons on the liver, noted that liver damage produced in dogs by /

by the action of chloroform gave rise to a state of impaired blood coagulability associated with a deficiency of fibrinogen. As a result of this observation he concluded that fibrinogen is normally produced in the liver. Subsequent workers have suggested that defective production of fibrinogen as a result of liver damage might be responsible for the bleeding tendency in jaundice.

Doyon's experiments have been repeated on many occasions and there is a considerable amount of evidence to confirm his view that fibrinogen is normally produced by the liver (Foster and Whipple⁽²¹⁾, Mann and Bollman⁽⁴³⁾). Moreover it has been amply confirmed that there is a diminished fibrinogen production in severe chloroform poisoning - though in mild chloroform damage the fibrinogen may on the contrary be raised (Whipple and Hurwitz⁽⁶⁸⁾). The application of these observations to the problem of bleeding in jaundice, is not, however, permissible, for the work of Gram⁽²⁴⁾, Moss⁽⁴⁷⁾ and many others make it clear that the fibrinogen content of the blood in jaundice is not reduced, indeed it is sometimes /

sometimes present in excess.

This is seen in my own series, in which the fibrinogen content of the blood has been estimated in 22 cases, including 8 of the cases in which a liability to haemorrhage became manifest. Reference to Chart 4 will show that of the 22 cases only 3 presented a low fibrinogen content; all but one of the cases with bleeding proved to have an adequate amount of fibrinogen present, whilst in one of these cases the fibrinogen content was actually in excess of the normal (0.81 gms. per cent.).

VI. RECENT OBSERVATIONS ON THE CAUSE
OF THE BLEEDING TENDENCY.

In seeking the cause of haemorrhage in jaundice we must bear in mind all the factors concerned in the normal arrest of haemorrhage — the state of the capillary vessel walls and the character of the blood clot, as well as the time required for clotting to take place.

My observations lead me to believe that three distinct, though possibly related, abnormalities may sometimes be found in jaundice, which may be responsible either singly or together for the bleeding tendency; (1) Fragility of the capillary blood vessels; (2) Softness and friability of the blood clot; (3) Delayed coagulability of the blood;

In the following pages, the significance of these three factors is discussed. It will be shown that capillary fragility is a somewhat infrequent feature in jaundice, and that its relation to the bleeding tendency is far from clear. The second factor, friability of the blood clot, is commonly observed, and its importance in regard to haemorrhage /

haemorrhage cannot be doubted. Most important of all, however, is delayed coagulability of the blood. Its frequency is noted in the following pages, and subsequently the cause of the delayed coagulability is discussed.

VI. A. The Factor of Capillary Fragility.

Fragility of the capillary blood vessels is well recognised as the major factor concerned in the bleeding tendency in scurvy and certain forms of purpura, but hitherto it has attracted little attention as a factor in jaundice, and I can find no reference to it in the literature in this connection. In my own series a capillary fragility has been observed in a small number of cases, and a special study has been made to determine its significance in relation to the bleeding tendency.

The method used was to bring about a state of venous congestion in the upper limb by means of an inflatable tourniquet, and subsequently to count the number of petechial haemorrhages thus caused. As a routine the cuff was inflated to 70 mms./

70 mms. Hg. for a period of 10 minutes. For the petechial count a circle 5 cms. in diameter was marked out on the anterior aspect of the forearm, its centre 5 cms. below the flexure of the elbow. A hand lens was used in making the count. In normal subjects under such conditions a count of from 0 to 5 may be expected.

The test was applied to 20 cases in my series (Table 11). In 6 of these the first examination showed a considerable capillary fragility, the petechial count ranging from 20 to over 70. In 3 of these cases, the fragility diminished rapidly, reaching normal within a few days after the patient's admission to hospital; in the other 3, it persisted for a longer period.

Four patients who subsequently developed haemorrhages were included in the 20 submitted to the tourniquet test. One of them showed persistent capillary fragility, one temporary fragility, and the remainder showed no abnormality in this respect.

A special study was made of the 3 cases /

cases with persistent capillary fragility to determine if possible the cause of the condition, with special reference to the question of a vitamin deficiency, and to determine its significance in relation to the bleeding tendency.

Case 7. This patient (see Chart 5) was a woman, aged 60, admitted to the Edinburgh Western Hospital, deeply jaundiced, emaciated and cachectic, suffering from carcinoma of the pancreas. She died eight days after admission. Autopsy showed that the immediate cause of death was a large haemorrhage into the small intestines.

On admission she was seen to have numerous blotchy, cutaneous and subcutaneous haemorrhages. Coagulation time and bleeding time were both greatly lengthened (15 minutes and 16 minutes respectively). The tourniquet test gave a strongly positive result. The erythrocyte sedimentation rate was greatly increased.

The woman was known to have been living alone in a basement flat, subsisting on a small allowance. In view of this, and despite the absence of bleeding from the gums (she was edentulous) the possibility was considered of scurvy superimposed upon the jaundice.

Accordingly ascorbic acid (60 mgm.) was injected intravenously. This promptly arrested the bleeding tendency, and although the coagulation time and bleeding time remained raised, the tourniquet test became negative and no further spontaneous haemorrhages developed until the seventh day after admission. At this point, further subcutaneous /

subcutaneous haemorrhages were noted. Again the tourniquet test became strongly positive and again a further injection of vitamin C. proved beneficial.

In this case the conclusion reached was that the haemorrhagic tendency was scorbutic in origin, and not clearly related to the jaundice. However, it was regarded as a possibility that in jaundice the liver damage might so impair the storage of vitamin C as to predispose to scurvy. Thus the capillary fragility in jaundice might prove to be due to a deficiency of vitamin C despite a diet adequate in this respect. To put this theory to the test, the observations recorded below were therefore carried out.

Case 15. This was a woman aged 70, who was admitted deeply jaundiced as a result of carcinoma of the pancreas. After a short period of observation, operation was performed, and in view of her debilitated condition the operation was limited to external drainage of the gallbladder. Subsequently there was bleeding from the wound, from the gallbladder and from the nose.

The pre-operative coagulation time was $4\frac{1}{2}$ minutes. A tourniquet test (see Chart 6) gave a reading of 36 petechiae. In view of the experience of the earlier case, a possible deficiency of vitamin C was considered, although the diet had been more than /

than adequate in this respect. Accordingly, vitamin C. was given intravenously, in the form of 500 mgm. Redoxon. Reference to the Chart will show that this had no significant effect on the capillary fragility.

To obtain more definite evidence of the ascorbic acid content in jaundice, a vitamin C. tolerance test was carried out in this case. For this purpose, the ascorbic acid content of the urine (24-hour samples) was estimated before and after the administration of Redoxon. The estimation, which was performed by Dr. H. Scarborough in the Clinical Laboratory of the Royal Infirmary, gave the results shown in Table 12.

This investigation indicates that in this case the output of ascorbic acid was normal; it gives no evidence of a deficiency of ascorbic acid.

The further progress of this case provided interesting evidence of the relation of capillary fragility to the bleeding tendency in jaundice (see Chart 6). After four days' observation, operation was performed and the gallbladder drained. In order to achieve gradual decompression of the liver, the drainage tube was carried upwards to a bottle situated 30 cm. above the level of the wound, and maintained in this position for 48 hours.

The capillary fragility which had persisted during the four days' pre-operative observation, disappeared immediately after operation and /

and the tourniquet test then remained negative for 10 weeks. The coagulation time, on the other hand, showed a tendency to rise after operation, and on the third, fourth and fifth post-operative days the coagulation time reached 15 minutes, 17 minutes and 18 minutes respectively.

The bleeding was first noted on the third post-operative day. It thus occurred at a time when no capillary fragility was evident.

These observations appeared to indicate that the capillary fragility sometimes found in jaundice is not directly related to the bleeding tendency. It must, however, be borne in mind that, as many workers have pointed out in relation to scurvy and purpura, the clinical tests at present available for estimating capillary fragility give very inconstant results which do not tally with the variations in the bleeding tendency in those diseases. Thus, in scurvy the capillary fragility, as estimated by the tourniquet test, may vary from day /

day to day, and may disappear for prolonged periods even though the diet remains deficient in vitamin C. In scurvy also and in purpura haemorrhages may develop at times when the tourniquet test is negative.

The relation between capillary fragility and the prothrombin content of the blood could not be estimated pre-operatively in this case, as the technique of prothrombin estimations had not then been developed. An opportunity occurred, however, 10 weeks later (see Chart 7) when again a transitory phase of capillary fragility was noted. It will be seen that during this phase the prothrombin index was low (approximately 40 per cent.) and that the eventual disappearance of the capillary fragility coincided with the rise of the prothrombin content to normal. This case thus afforded some evidence of correlation between capillary fragility and prothrombin deficiency.

Case 16. This was a man aged 66, who was admitted to hospital with jaundice of five weeks' duration, insidious in onset and painless. /

painless. On admission, he was deeply jaundiced, the icteric index being 115. He was kept under observation for 18 days, and the provisional diagnosis of carcinoma was reached. At that point he developed dysentery of Sonne type, and shortly succumbed. No autopsy could be obtained.

On admission to hospital he presented marked capillary fragility, the tourniquet test giving a count of 21 petechiae. The fragility persisted more or less continuously until the 14th day (Chart 8).

Observations were carried out in this case to determine the relation of the capillary fragility to a deficiency of either vitamin P. or vitamin C. First, a proportion of vitamin P. (Hesperidin) was administered in doses of 4 tablets thrice daily for 4 days. Reference to the chart will show that this had no significant effect on the capillary fragility.

On the 7th day after admission, vitamin C. (500 mgm. Redoxon) was administered intravenously. The petechial count, which previous to the injection was 30, was thereupon reduced to 11, but the reduction was not maintained and within 48 hours had risen to over 50.

Thus the observations in this case gave no evidence of a deficiency of vitamins P. or C. as the cause of the capillary fragility in jaundice.

From the observations recorded above, certain provisional conclusions may be reached:-

- (1) Capillary fragility is evident in a small proportion of cases of jaundice.
- (2) It /

- (2) It does not appear to bear a direct relationship to the bleeding tendency.
- (3) It does not appear to be attributable to a deficiency of vitamin P. or vitamin C.
- (4) There is some evidence to suggest that it is related to a deficiency of prothrombin.

VI. B. The Factor of Clot Friability.

It has frequently been observed that the blood clot in jaundice is bulky, soft and friable; and it has been suggested that this feature may be one of the factors concerned in the bleeding tendency, on the supposition that the soft clot fails to retract and does not adhere to the wall of the wounded vessel and so is unable to staunch the flow of blood.

Alternatively, it has been suggested (McNealy, Shapiro and Melnick⁽⁴¹⁾) that the abnormally fragile clot adheres at first, but is readily dislodged by post-operative vomiting or restlessness.

The soft, bulky character of the clot in jaundice is perceptible on naked eye examination.

When /

When removed from a test tube on to filter paper, the clot wets a large surface area; and it promptly falls to pieces when handled. The bulkiness of the clot has been confirmed by measurement by Boyce and McFetridge⁽⁴⁾, who have employed a simple test in which the volume of the clot is compared with the volume of serum separated after standing the blood for four hours at room temperature. Under these conditions the clot formed in normal blood should approximately equal the serum in volume; the clot formed in jaundiced blood considerably exceeds the serum.

I have carried out this test in 13 cases of jaundice, including 4 cases in which haemorrhage occurred. The method adopted has been to allow from 5 to 10 ccm. of venous blood to clot in a graduated conical tube. After four hours at room temperature the clot is gently separated from the wall of the tube and lifted out by a platinum hook. The volume of serum remaining is then read, and compared with the original blood volume.

Normally, the volume of serum is approximately 50 per cent. of the total blood volume. Reference to Table 13 will show that of the 13 cases examined, /

examined, only two gave this normal figure. In all the remainder, the serum accounted for considerably less than half the total blood volume - in other words, the clot was unduly bulky.

The faulty clot formation was formerly attributed to a deficiency of fibrinogen, but since it is now known that fibrinogen is not deficient in jaundice, this theory cannot be maintained. In 1934 Carr and Foote⁽¹⁴⁾ suggested that the clot defect is due to the presence of cystein and other sulphur-containing amino-acids in the blood. Normally such products of protein metabolism are converted in the liver into sulphates, and it was presumed that in jaundice the damaged liver was unable to perform this function and passed the products unchanged into the general circulation.

The observations of Carr and Foote were backed by much evidence derived mainly from animal experimentation, but so far there has been no confirmation of their findings. Since estimation of the cystein content of the blood is impracticable, it has not been possible to obtain direct /

direct evidence on this point. Some information, however, can be obtained by estimating the glutathione content of the blood and this has been done for me in the Department of Medical Chemistry of the University in 11 of my series, including 2 which subsequently showed a bleeding tendency. In all the cases the glutathione was within the normal limits. Thus this brief investigation gives no support to the view put forward by Carr and Foote.

I have found a certain amount of evidence to suggest that the defective clot formation may, like the delay in clotting, be related to a prothrombin deficiency. The evidence is based on observations I have made in the course of my investigation of the prothrombin content of the blood in jaundice.

The method of estimating the prothrombin content of the blood consists essentially in determining the coagulation time of the blood under certain controlled conditions (see Appendix). In carrying out the test, it is an invariable finding that whereas in normal blood the clotting time is short /

short and the actual gel formation is completed within a very short time (a second or less), in blood deficient in prothrombin not only is clotting delayed, but the process of gel formation occupies a longer period and the end-point is much less exact. The clot thus formed is notably bulky and soft. Moreover, the extent of the deviation from the normal varies with the degree of prothrombin deficiency; in severe deficiency the process of gel formation occupies 15 to 30 seconds, and the clot forms a bulky, soft, "thin" gel which floats in the plasma and does not adhere to the tube.

Further evidence suggesting that the defective clot in jaundice is due to a prothrombin deficiency is provided by the observation that a similar clot may be produced in blood by the addition of anti-prothrombin (Heparin). The heparin may be added to freshly drawn blood in vitro, or may be injected intravenously before the blood is withdrawn. In either case the effect is the same, the blood coagulating slowly as in jaundice and giving rise to a bulky, friable, non-adherent clot. /

clot.

VI. C. The Factor of Delayed Coagulability.

It is established that in jaundice delayed coagulability of the blood is frequently evident, and there can be little doubt that this is one of the major factors responsible for the bleeding tendency.

I have made 194 estimations of the blood coagulation time in 30 cases of jaundice, including 8 cases in which haemorrhages developed. The results are shown, along with 100 control blood coagulation tests, in Chart 9.

It will be seen that in all the control cases the coagulation time was less than 9 minutes. Of the 121 estimations carried out on jaundiced cases without haemorrhage, 41 gave a reading of over 9 minutes, whilst of the 73 estimations carried out on jaundiced cases with haemorrhage, no fewer than 54 gave a reading of over 9 minutes.

Thus there is ample evidence that the blood coagulation is generally increased in jaundice, and especially so in the presence of a bleeding tendency. /

tendency.

It will be noted that there are exceptions to this general rule. In particular, 19 estimations carried out on jaundiced patients with haemorrhage gave a normal reading (9 minutes or less). Attention will be directed to this observation on a later page, in connection with the diagnosis of the bleeding tendency. At present it will be sufficient to indicate that in many of these cases, the normal coagulation time was a pre-operative finding, whereas in the dangerous phase after operation the coagulation time was more often raised. Only rarely is the coagulation time within the normal limits at a time when the bleeding tendency is manifest.

VII. PROTHROMBIN DEFICIENCY AS THE CAUSE
OF THE BLEEDING TENDENCY.

Recently Quick and his Colleagues⁽⁵²⁾ at Milwaukee have devised a comparatively simple method of estimating the prothrombin content of the blood. On the basis of observations made with this test, they have shown that a prothrombin deficiency is commonly present in jaundice, and they have claimed that this is the factor responsible for the tendency to bleed. They and subsequent workers (Snell⁽⁶⁰⁾; Brinkhous, Smith and Warner⁽⁶⁾; Magath⁽⁴²⁾) have claimed moreover that the prothrombin test may be used as a criterion of the risk of haemorrhage, and, finally, have shown that measures designed to remedy the prothrombin deficiency are effective in preventing haemorrhage.

The technique of Quick's test is described in the Appendix, where also its significance and reliability are assessed. It will suffice here to say that the method is a form of blood coagulation test, so modified by the addition of optimal /

optimal amounts of the other clotting elements that the only variable is prothrombin. The amount of prothrombin present may then be gauged by the clotting time of the mixture (prothrombin time) and by comparison with normal controls the prothrombin index may be calculated, the normal index being rated at 100 per cent.

I have carried out approximately 220 prothrombin estimations on jaundiced patients, with a similar number of observations on normal control cases. The results of my observations are given in the following pages.

VII. A. Prothrombin Estimations
in 34 Cases of Jaundice.

The 34 cases in which prothrombin estimations have been carried out fall into two categories, a group of 14 cases of toxi-infective jaundice and a group of 20 cases of obstructive jaundice.

Most of the former group were cases of catarrhal /

catarrhal jaundice of mild character, and in them only one or two prothrombin estimations were done. In the more severe cases of toxic jaundice and the majority of the cases of obstructive jaundice a more thorough study was made, estimations being carried out twice weekly during the patients' stay in hospital. In this way the effect produced on the prothrombin content by various diets, drugs and operative procedures was studied.

The prothrombin index, as estimated at the initial examination of the 14 cases of toxic jaundice, is given in Table 14. The cases have been arranged according to the level of the prothrombin index (column 3). If the normal index is taken as 100 ± 10 , it will be seen that the first five cases in the list fall within this range. Of the remainder, the majority show a moderate deficiency of prothrombin; in only one, the last case in the list, is the prothrombin deficiency severe.

Table 15 shows the prothrombin index as estimated at the initial examination of the 20 cases of obstructive jaundice. Here again the cases have been arranged according to the level of the /

the prothrombin index (column 3).

It will be seen that only in three cases of this series does the prothrombin index fall within the normal range, whilst in no fewer than nine cases the index is below 50 per cent., an indication of a severe degree of prothrombin deficiency.

VII. B. Relation of Prothrombin Deficiency to Depth and Duration of Jaundice.

To determine the relationship between the degree of prothrombin deficiency and the depth and duration of the jaundice, the 50 cases of my series have been classified as shown in Tables 16 and 17.

In Table 16 the cases have been arranged in three groups, according to the Icteric Index as estimated by Meulengracht's method. It will be seen that in the group of 9 cases with slight jaundice (as judged by an icteric index of 50 or less) 6 cases had a normal prothrombin index and in 2 of the remaining 3 cases the prothrombin deficiency was of moderate degree. In the groups of /



of cases with deeper jaundice, on the other hand, only two had a normal prothrombin index, and of the remainder a large proportion showed a severe prothrombin deficiency.

In Table 17 the cases have been arranged according to the duration of the jaundice. It will be seen that in the 19 cases with jaundice of less than one month's duration, 7 had a normal prothrombin index, and that the prothrombin deficiency was of moderate degree in 8 of the remainder; whereas, in the cases with jaundice of longer duration only 1 had a normal prothrombin index, and the prothrombin deficiency was of severe degree in a considerable proportion of the remainder.

Thus there appears to be a certain amount of evidence to suggest that the prothrombin deficiency is related to the depth and duration of the jaundice. That this relationship is not a close one, however, is indicated by individual cases which form exceptions to the general rules.

Thus in Case 46, a man suffering from multiple carcinomatous metastases, the prothrombin deficiency was severe although the jaundice (icteric index 55) was of comparatively slight degree; and in /

in Case 29, a woman with puerperal toxic jaundice, the deficiency was again severe although the jaundice had been present for only one week.

A similar lack of correlation is also seen in the contrast presented by Cases 27 and 24. The former, a man aged 55, suffering from carcinoma of the pancreas, with jaundice of 4 weeks' duration and moderate depth, presented the low prothrombin index of 18 per cent.; whereas the latter, a man of 59 suffering from the same disease, with jaundice of similar duration but slightly deeper, presented a normal prothrombin index.

From the evidence available, therefore, it must be concluded that although a general tendency is discernible towards correlation between the prothrombin deficiency and the depth and duration of jaundice, yet this relationship is by no means close. It is, indeed, clear that the prothrombin deficiency is influenced by factors other than these. One such other factor may be the degree of liver damage.

VII. c. Relation /

VII. C. Relation of the Prothrombin Deficiency to Liver Damage.

There is some evidence to suggest that in jaundice the prothrombin deficiency is related to liver damage.

Experimentally, Smith, Warner and Brinkhous⁽⁵⁹⁾ have shown that in dogs the impairment of liver function brought about by the administration of chloroform is associated with a deficiency of prothrombin. On the clinical side, evidence may be gained from a study of individual cases or from consideration of the whole group of cases examined.

Case 19 has an importance in this connection. This was a woman aged 45, who gave a history that six weeks before admission to hospital she was taken ill with malaise, abdominal pain, nausea, vomiting and jaundice. After three weeks the jaundice faded, but 14 days before admission a rigor developed and subsequently the jaundice recurred with increased intensity. On admission she was deeply jaundiced, highly toxic and very ill. The provisional diagnosis was made of a gallstone in the common duct with cholangitis. At operation, however, /

however, a condition of subacute yellow atrophy was found, and confirmed by microscopic examination of a portion of liver. After operation she progressed well; the jaundice faded in the course of three to four weeks and the toxic manifestations gradually lessened.

At the time of her admission to hospital there was a prothrombin deficiency of moderate extent, the prothrombin index being 63% (Chart 10). The deficiency was maintained throughout the 6 weeks' stay in hospital, and did not lessen as the jaundice faded. In view of the gross liver damage found at operation, it was evident that considerable impairment of hepatic function must persist for a considerable time, and it seemed probable that this functional impairment was responsible for the continued prothrombin deficiency.

Further evidence may be obtained from a study of the 14 cases of toxic jaundice given in Table 14. It will be seen that 9 of these cases were diagnosed as catarrhal jaundice. All these had jaundice of mild type with little impairment of health, and no evidence, apart from the icterus, of liver damage. It is significant that in all these /

these cases the prothrombin index was either normal or but little diminished. In contrast with these, the other 5 cases presented severe constitutional effects and clinically they appeared to have a considerable impairment of hepatic function; thus, one case was regarded as suffering from subacute yellow atrophy, whilst another suffered from a fulminating and rapidly fatal puerperal toxæmia. Conforming to the more severe liver damage the prothrombin deficiency in three of these cases was considerably more marked, and in the most severe of all (the fatal puerperal case) the prothrombin index was reduced to the low figure of 24 per cent.

It is not possible to adduce such strong evidence from the Table in Fig. 15 relating to obstructive jaundice, for this Table does not afford any clear criterion of the relative amount of liver damage present. Clinical observations made upon these cases do, however, support the view that the prothrombin deficiency runs parallel with the liver damage. Thus, it was noted that Case 24, which heads the list, was a man who showed little impairment of health apart from the icterus; whereas, /

whereas, Cases 39, 26, 41, which are close to the foot of the list, were all patients who presented clear evidence of marked deterioration of general health - indeed they were almost moribund when admitted to hospital.

Thus it may be claimed that there is a certain amount of evidence to link the extent of the prothrombin deficiency with the degree of liver damage. This relationship is considered further on a later page, in connection with the relation of vitamin K. to prothrombin.

VIII. THE PROTHROMBIN INDEX AS A GAUGE
OF THE BLEEDING TENDENCY.

It is accepted that hitherto there has been no accurate criterion of the risk of haemorrhage in jaundice.

All who have had experience of the blood coagulation tests in clinical use are familiar with the inconsistency and unreliability of the results obtained. Not only does the coagulation time as judged by such tests vary between wide limits in normal subjects, and even in the same subject at successive estimations, but furthermore it depends greatly upon such variable factors as the rate of flow of blood from the finger prick or venepuncture, the amount of venous congestion induced in the limb and the amount of pressure or squeezing applied to the part.

Moreover, apart from these technical inconsistencies, it is accepted that in jaundice the coagulation time is not an accurate index of the risk of haemorrhage. It is true that delayed coagulability is a common feature in jaundice (see p. 46), and in general is most marked in jaundiced patients /

patients who show the bleeding tendency; nevertheless it is a not infrequent experience that haemorrhage may occur after operation in patients whose pre-operative coagulation time has been normal, whilst in some cases the coagulation time has been found to be within the normal range after operation and within a few hours of the onset of haemorrhage.

Nygaard⁽⁴⁹⁾ has sought to overcome the technical defects of coagulation tests by his method of estimating the clotting time of recalcified plasma. In this method, oxalated venous blood is transferred to the laboratory, the cells are separated by spinning, and the plasma is then recalcified by the addition of a known quantity of calcium chloride. The coagulation time of the recalcified plasma is then estimated under known conditions of temperature and humidity. Nygaard's method represents a great advance in comparison with the clinical methods, but it still leaves much to be desired, for the end-point of clotting in the recalcified mixture is far from exact, and the clotting time in successive estimations from the same /

same patient varies over a considerable range.

Ivy⁽²⁹⁾ has attacked the problem from a different standpoint, and has tried to find an improved method of estimating the bleeding time. The customary test for the bleeding time is highly unreliable, for it varies according to the depth and size of the puncture and the amount of retraction of the ruptured capillary walls. Ivy used a standard size of stylet which was introduced to a uniform depth, and to overcome the factor of capillary retraction he introduced the method of inducing passive venous congestion in the limb by the use of a tourniquet. His method has, however, one major disadvantage, namely that it takes no account of the varying thickness of the skin in different patients. In many subjects with obstructive jaundice, owing to loss of weight the skin is inelastic and thick. I have repeatedly found that under these conditions the stylet introduced into the forearm as described by Ivy causes very little bleeding or may completely fail to draw blood, whereas in thin-skinned subjects it produces a free flow lasting several minutes.

In /

In contrast with these estimations, the prothrombin test is highly satisfactory to work with, for its end-point is exact and the results obtained show a remarkable degree of consistency. (See appendix).

The value of the prothrombin test as a method of assessing the risk of haemorrhage can only be judged satisfactorily on an experience of a large number of cases thus complicated. My observations do, however, give some indication that the test will prove useful in this respect.

In my series of 34 jaundiced patients whose prothrombin index was estimated, there were six who developed haemorrhages. In three of these cases the bleeding arose after operation, in two it was discovered post-mortem in patients who had not been subjected to operation, whilst in the sixth it developed whilst the patient was under observation preparatory to operation.

Case 25.

This was a woman aged 50, suffering from jaundice of 5 weeks' duration. She had previously had one breast removed for carcinoma, and /

and the jaundice proved to be due to the pressure of a solitary metastasis on the common bile duct.

At the time of her admission the prothrombin index was 58 per cent. (see Chart 11). As a result of pre-operative measures it rose to 95 per cent and immediately before the operation it stood at 90 per cent.

At the operation the malignant mass was detected and, in view of the patient's grave condition, a simple cholecystostomy was performed. After operation there was free drainage of bile (approximately 10 ounces daily) and the degree of icterus diminished, but the patient's general condition deteriorated and there was clinical evidence of a progressive impairment of hepatic function.

The prothrombin index fell rapidly after operation and on the seventh day it reached the low figure of 28 per cent. Bleeding from the wound was noted on the third day after operation. There was a free ooze of blood which was controlled temporarily by the application of a pack, but it recurred later and persisted irregularly until death.

Case 26.

This was a man aged 70, who was admitted to hospital suffering from jaundice of 11 weeks' duration, insidious in onset and painless.

On admission he was found to be gravely ill and almost moribund. Operation was considered inadvisable, and he gradually sank and died twelve days later. He proved to have a stricture of the lower end of the common bile duct with cholangitis.

At the time of his admission the prothrombin index was 23 per cent. (see Chart 12). It rose to 44 per cent. on the fifth day, but subsequently fell and reached 18 per cent shortly before death. Autopsy showed the immediate cause of death to be a large haemorrhage into the stomach.

Case /

Case 27.

This was a man aged 55, who suffered from carcinoma of the pancreas with jaundice of 4 weeks' duration. After a period of observation in hospital operation was carried out and a cholecyst-gastrostomy was performed. For a short time the anastomosis was effective, but then, as a result of encroachment of the growth up the common duct to its junction with the cystic duct, the flow of bile through the anastomosis was interrupted. Accordingly, a further operation was performed, and the dilated upper part of the common duct was anastomosed to the stomach.

The prothrombin index at the time of admission was 18 per cent. - the lowest figure in the whole series, (Chart 13). After pre-operative therapy it rose to 50 per cent. at the time of the operation. Two days after the operation the prothrombin index had fallen to 32 per cent., and at this stage there was a haemorrhage from the wound, consisting of a free ooze amounting to several ounces of blood. The bleeding was controlled by a pack. Subsequently the prothrombin index rose, and no further bleeding took place.

A similar haemorrhage occurred after the second operation. Again there was a free ooze of blood from the wound, which, however, proved transient. At this time also there was a marked deficiency of prothrombin, the index being 34 per cent.

Case 41.

This was a man aged 71 who had suffered for 12 months from jaundice due to carcinoma of the pancreas. He was admitted to hospital in a moribund condition and died three days later. During his stay in hospital he vomited small quantities of blood on several occasions, and autopsy revealed an extensive haemorrhage into the stomach.

It was only possible to carry out one prothrombin estimation on this patient, on the day of his admission to hospital. The prothrombin index at that time was 21 per cent.

Case /

Case 44.

This was a woman aged 44, who for three months had suffered from anorexia, loss of weight and jaundice. She proved to have multiple metastatic growths in the liver, secondary, it was thought, to a primary carcinoma of the common bile duct.

Whilst under observation and awaiting operation, she developed a severe epistaxis - an occurrence to which she was not usually subject - and two days later she was noted to have sustained a considerable haemorrhage into the subcutaneous tissue of the thigh.

No prothrombin estimation was performed at the time of the haemorrhages, but a week later (2 days after exploratory laparotomy) the index was found to be 44 per cent. It is a curious fact that in this case operation caused no further haemorrhage.

Case 47.

This was a woman aged 48, suffering from jaundice of 4 weeks' duration, the result of impaction of a stone in the lower end of the common duct. Operation was performed, and drainage of the duct established. Three days after operation there was a considerable haemorrhage from the uterus, and this was repeated at intervals during the subsequent five days. No special treatment for the bleeding was carried out.

Prothrombin estimations had not been done before operation, and the initial estimation was carried out on the 4th post-operative day (whilst the bleeding was in progress). At this time the prothrombin index was 23 per cent. Three days later it had risen to 37 per cent. After a further period of four days, when the bleeding had ceased, the index had risen to 71 per cent. Subsequently it attained the normal level and no further haemorrhage took place.

Thus /

Thus in all six cases there was a considerable deficiency of prothrombin. The prothrombin index at the initial examination, and the index at the time of haemorrhage, is shown in Table 18.

The value of the prothrombin index as a guide to the bleeding tendency is illustrated further in Table 19, which refers to the whole series of 20 cases of obstructive jaundice in which prothrombin estimations have been carried out. The cases in this table have been arranged in order according to their initial prothrombin content (middle column). It will be seen that no case with a prothrombin index over 60 per cent. sustained a haemorrhage, whereas of the 13 cases with prothrombin index below 60 per cent., no fewer than five developed this complication. Moreover, of the remaining eight cases with prothrombin index below 60 per cent., six were not submitted to operation and thus escaped that most rigorous test of the bleeding tendency.

IX. THE CAUSE OF THE PROTHROMBIN DEFICIENCY;
A LACK OF VITAMIN K.

In seeking the cause of the prothrombin deficiency in jaundice we must bear in mind the fact that a similar prothrombin deficiency has also been shown to be responsible for the bleeding tendency observed in certain cases of external biliary fistula in which the whole biliary secretion is lost for a prolonged period. This observation was first made by Hawkins and Brinkhous⁽²⁷⁾ and I have been able to confirm it as shown in the following two case records.

Case 15.

This was a woman aged 70, who gave a history of jaundice of 5 weeks' duration, insidious in origin, painless and progressive (see Chart 14). She proved to have a carcinoma of the pancreas, and in view of her critical condition I performed a cholecystostomy, draining the bile externally. The jaundice gradually faded and 15 weeks later a cholecyst-gastrostomy was performed in order to re-direct the bile back into the alimentary tract. Prothrombin estimations were begun 10 weeks after the cholecystostomy and subsequently were carried out at weekly intervals. During the continuance of the biliary fistula the prothrombin index remained at about 40 per cent., except at one estimation when it reached 60 per cent. When the bile was redirected to the alimentary tract the prothrombin index rose rapidly and within a week it had regained the normal level.

Case /

Case 22.

This was a man aged 61, who gave a history of painless, insidious jaundice of 7 weeks' duration. He also proved to have a carcinoma of the pancreas (see Chart 15). After a fortnight's observation he was submitted to operation and again a cholecystostomy was performed. His whole biliary secretion was then discharged externally for a period of 67 days, at the end of which time the bile was redirected to the alimentary tract by the performance of a cholecyst-gastrostomy.

His prothrombin index on admission was 52 per cent. After establishing the biliary fistula and despite a wide variety of therapeutic measures the index never rose above 72 per cent. Once the biliary flow was directed into the alimentary tract the prothrombin index rapidly rose, attaining the normal range within 14 days.

Thus a prothrombin deficiency is found in two distinct conditions - jaundice and biliary fistula.

Now it is clear that one important feature which these two conditions have in common is that no bile reaches the alimentary tract - in one case it is dammed back in the bile passages, in the other it escapes externally - and thus its digestive value is lost. This suggests that the prothrombin deficiency may be due to faulty absorption of some substance from the intestinal tract owing to the lack of bile.

Now /

Now the earlier work of Dam⁽¹⁶⁾ in 1934 had drawn attention to yet another condition associated with a prothrombin deficiency. This is a disease of young chickens characterised by the development of subcutaneous and intramuscular haemorrhages. Dam and his colleagues have shown beyond all possible doubt that this disease is the result of a dietary deficiency. It can be produced by feeding the chicks on a diet lacking in certain substances and can be cured by supplying these substances. Accordingly, they attributed it to deficiency of a "Koagulations Vitamin" - Vitamin K. From this work it is an easy step to assume that a similar vitamin is lacking in jaundice, not in this case by deprivation from the diet, but as a result of faulty absorption in the absence of bile.

On the basis of this assumption, in 1937 Butt, Snell and Osterberg⁽¹⁰⁾ at the Mayo Clinic suggested that vitamin K. should be administered in jaundice, and since then preparations reputed to contain this substance have been put into wide clinical use in America. Favourable reports on the value of this form of therapy have been published by Brinkhous, Smith and Warner⁽⁶⁾ in America, and Dam and Glavind⁽¹⁸⁾ in Denmark, and recently the Mayo Clinic workers⁽¹¹⁾ have published a review of their experiences.

X. THE THERAPEUTIC EFFECT OF VITAMIN K. IN JAUNDICE.

I have made observations on the effect of administering a preparation reputed to contain vitamin K. in four of the cases of my series - three cases of jaundice and one of biliary fistula. The preparation used has been Cerophyl, supplied by the American Dairy Company. This is a preparation obtained from alfalfa grass, and in addition to vitamin K. it is reputed to contain many other substances including vitamins A. and C. Due care must therefore be observed in interpreting the results obtained as being attributable to any one constituent.

Case 25.

This was a woman aged 50, who gave a history of painless, insidious jaundice of five weeks' duration. She had previously had one breast removed for carcinoma, and the jaundice proved to be due to the pressure of a solitary metastasis on the common bile duct. On admission to hospital she had a prothrombin index of 58 per cent. (Chart 11). She was kept under observation for ten days and during the latter half of this period bile was administered, (5 ounces daily) by stomach tube, without influencing the prothrombin deficiency. Cerophyl was then administered, the dose being 2 drachms thrice daily, and the administration was maintained for sixteen days. During this period a considerable rise in the /

the prothrombin content took place, the index reaching the normal level and then fluctuating between 80 and 90 per cent.

Twenty-seven days after admission laparotomy was performed; the malignant mass was detected and in view of the patient's grave condition the gallbladder was drained externally. The administration of Cerophyl was stopped at this point.

After operation the patient did not improve and there was clinical evidence of a progressive impairment of hepatic function. Death took place eight days later.

The prothrombin index fell rapidly after operation and on the seventh day it was reduced to 28 per cent. A transfusion of citrated blood had only a very small effect on the prothrombin index.

In this case the administration of Cerophyl thus appeared to raise the prothrombin index before operation but did not maintain this effect after operation when the administration had been stopped. This experience appeared to suggest - (1) that the storage of prothrombin was highly deficient, and (2) that the administration of the vitamin should be continued during the post-operative phase.

Case 27.

This was a man aged 55, who was admitted to hospital with a history of jaundice of four weeks' duration. The jaundice proved to be due to the presence of a carcinoma of the pancreas. On admission to hospital he had a prothrombin index of 18 per cent. (Chart 12) the lowest figure in the series. After four days' observation Cerophyl was administered (2 drams thrice daily). Seven days later the dose was doubled and a bile salt preparation (10 grains Bilron thrice daily) was given with the object of facilitating absorption. During this period /

period the prothrombin index rose from 18 per cent. to 50 per cent. At this stage, cholecyst-gastrostomy was performed. There was a small post-operative fall in the prothrombin index to 32 per cent., after which it rose rapidly, attaining the normal range within six days of operation.

A fortnight later, however, it was noted that the prothrombin index had again fallen to 44 per cent. - and at this stage it was also clear that the jaundice had not been relieved by the anastomosis. It subsequently was found that the cholecyst-gastrostomy had been ineffective in relieving the jaundice owing to encroachment of the growth up the common duct and occlusion of the termination of the cystic duct.

After a further period of observation for ten days, when the prothrombin index proved to have fallen still further to 28 per cent., the Cerophyl was again administered, with bile salts. Following the administration, in seven days the prothrombin index rose to 90 per cent. After a further period of a week a second operation was carried out, and the dilated common duct above the growth was anastomosed to the stomach. Following this operation which proved effective in overcoming the jaundice there was again a transient fall in the prothrombin index to 34 per cent. after which it again rapidly attained the normal figure.

In this case the administration of Cerophyl on two occasions was followed by an immediate rise in the prothrombin index.

Case 28.

This was a man aged 60, who had suffered from jaundice, painless in origin and progressive, of seven weeks' duration. He was believed to be suffering from carcinoma of the head of the pancreas, but the diagnosis could not be confirmed by operation or autopsy.

On his admission to hospital the
prothrombin /

prothrombin index stood at 46 per cent. (Chart 16). Cerophyl was administered (2 drams thrice daily) and the administration was maintained for 40 days. After the first 21 days, bile salts were also given. During this period the prothrombin index rose progressively attaining the normal figure on the 36th day after admission. On the 44th day after admission the Cerophyl was discontinued, the administration of Bilron being maintained. From this point onwards a progressive fall in the prothrombin index was noted and on the 57th day, when the patient was discharged from hospital, the prothrombin index was 41 per cent.

Thus, in this case administration of Cerophyl was followed by a progressive elevation of the prothrombin index to normal, whilst cessation of the administration was followed by a progressive fall to the original level.

Case 22.

This was a man aged 61, who also complained of jaundice which proved to be due to carcinoma of the pancreas. On his admission he was deeply jaundiced and very debilitated, and accordingly the operation was limited to external drainage of the gallbladder.

After a period of 67 days his condition was sufficiently improved to justify a further operation in which cholecyst-gastrostomy was performed, the bile being thus redirected to the alimentary tract. Thus, for a period of 67 days there was a complete external biliary fistula.

On his admission to hospital the prothrombin index was 52 per cent. (Chart 15). After nine days' observation Cerophyl and the bile salts preparation were administered and the administration was maintained for 7 days. During this period the prothrombin index at first fell to 34 per cent. and then rose to 67 per cent. Subsequently, whilst the biliary fistula was present, Cerophyl was again administered for two periods of 7 and 11 days respectively. In the latter period 5 ounces of bile were /

were administered daily by stomach tube with the object of promoting absorption of the vitamin. On both occasions, however, the treatment appeared to have little significant effect upon the prothrombin index. Yet, after performance of the second operation, when the bile had been redirected to the alimentary tract, the prothrombin index rose spontaneously and within fourteen days attained the normal range.

Thus, in this case of biliary fistula the Cerophyl appeared to be without effect.

XI. THE NATURE AND MODE OF ACTION OF VITAMIN K.

During the past few years much work has been carried out by Dam and Glavind^(17,19) and by Almquist,^(1, 36) Doisy⁽⁶⁴⁾ and their respective colleagues to determine the distribution and character of the coagulation vitamin.

In such experiments the presence of the vitamin has been judged by its effect on young chicks. In the "therapeutic assay" the chicks are put on a vitamin-free diet until the bleeding tendency develops, and the substance to be tested is then added to the diet, its effect on the bleeding tendency being noted. In the "prophylactic assay" the substance to be tested is added from the first, the results being compared with those in a control series of chicks fed on a vitamin-free diet alone.

Dam and Glavind have shown that vitamin K. is widely distributed, especially in grasses, green vegetables and the leaves of several varieties of tree. One of the most plentiful sources of the vitamin is alfalfa grass and this has been the source from which it has been obtained for clinical use. /

use. The vitamin has also been found in moderate amount in cabbage, spinach and tomatoes. Recently Dem and Glavind⁽¹⁹⁾ have claimed that it may be obtained most abundantly from horse-chestnut leaves.

Osterberg⁽⁵⁰⁾ at the Mayo Clinic has obtained the vitamin from putrefied fish meal and has used this substance for clinical use. He claims that putrefaction increases the amount of vitamin present, and on the basis of this observation has suggested that one source of the vitamin in man may be from intestinal putrefaction.

Recently attempts have been made to obtain the vitamin in pure form by extraction. Doisy and his colleagues⁽⁶⁴⁾ claim to have isolated it in a crystalline form from alfalfa by extraction with petroleum ether, absolute alcohol and acetone. The crystals thus obtained were small, well formed, transparent, colourless plates, melting at 69° C.

Almquist⁽¹⁾ has isolated the vitamin in a relatively pure form from alfalfa, using a process which takes advantage of the high solubility of vitamin K. in absolute methyl alcohol. By this method he claims to have obtained a crystalline substance /

substance of low melting point, which appears to contain one or more benzene rings.

The observations recorded on other pages appear to afford evidence in support of the claim that vitamin K. is concerned in the formation of prothrombin, but they give little information regarding the manner in which this effect is achieved. Since so little is known of the character and chemical constitution of either the vitamin or prothrombin, it is clear that their relationship must remain largely hypothetical. Thus, it is not known if the vitamin after absorption is converted into prothrombin or combines with some other substance to form prothrombin; or, on the other hand, if its action is an indirect one, stimulating the synthesis of prothrombin or mobilizing it into the blood from some place of storage.

In health, as my own observations show, the prothrombin content of the blood is remarkably constant; moreover it is quite unaffected by operation or by the loss of a considerable quantity of blood. Thus it is clear that normally an adequate reserve of prothrombin is available both to cover periods of reduced vitamin-intake and to compensate /

compensate for sudden loss in haemorrhage.

In jaundice, on the other hand, it is a striking feature that the prothrombin content of the blood undergoes considerable daily variation. This is particularly evident in the few days after operation (see Case 25, in which the prothrombin index fell from 90 per cent. to 28 per cent. in seven days) but it is also seen, to a lesser extent, in jaundiced persons receiving no special treatment. From this it would appear that in jaundice the storage of prothrombin - or perhaps the storage of its precursor, the vitamin - is defective.

Thus the benefit resulting from the administration of the vitamin in jaundice may be due to replenishment of depleted reserves. It does not necessarily indicate that the prothrombin deficiency in jaundice is due to faulty absorption of the vitamin in the absence of bile; it does not even indicate that man is normally dependent upon an exogenous supply of the vitamin. It merely demonstrates a method by which the prothrombin deficiency in jaundice may readily be overcome.

TABLE I.

Frequency of Haemorrhage in Jaundice.

	<u>Operations on jaundiced subjects.</u>	<u>Total Deaths.</u>	<u>Deaths from Haemorrhage.</u>
Petrén (51)	1891	231	30
Tennesen (63)	549	80	4
Judd and (31) Marshall	1181	108	19
Ravdin (53)	155	23	8
Total ...	<u>3776</u>	<u>442</u>	<u>61</u>

TABLE 2.

Sites of Haemorrhage in 12 Cases of Jaundice.

<u>Case No.</u>	<u>Disease.</u>	<u>Operation.</u>	<u>Site of Haemorrhage</u>
1*	Carcinoma Pancreas	Cholecyst-gastrostomy	Stoma Intestines Wound
4*	Carcinoma Bile Ducts	Exploration	Nose Stomach Intestines Wound
7*	Carcinoma Pancreas		Skin Intestines
8*	Stone in common duct; Cholangitis		Skin Intestines
10*	Carcinoma Pancreas	Cholecyst-gastrostomy	Stoma Intestines Wound
15	Carcinoma Pancreas	Cholecystostomy	Nose Gallbladder Wound
25*	Metastatic Carcinoma	Cholecystostomy	Wound
26*	Stricture common duct; Cholangitis		Stomach
27	Carcinoma Pancreas	(1) Cholecyst-gastrostomy (2) Choledoch-gastrostomy	Wound
41*	Carcinoma Pancreas		Stomach
44	Metastatic Carcinoma		Nose Subcutaneous tissue
47	Stone in Common Duct	Drainage of common duct	Uterus

* Death in hospital following haemorrhage.

TABLE 3.

Duration of Jaundice in 56 Cases with Postoperative Haemorrhage (Petren).

<u>Duration of Jaundice</u>	<u>No. of Cases.</u>
Less than 1 month	19
1 to 2 months	22
2 to 4 months	6
4 to 6 months	3
Over 6 months	8

TABLE 4.

Duration of Jaundice in 50 Cases (Present Series)
Including 12 Cases with Haemorrhage.

<u>Duration of Jaundice.</u>	<u>Total Cases.</u>	<u>Cases with Haemorrhage.</u>
Less than 1 month	22	3
1 to 2 months	14	3
2 to 4 months	9	2
Over 4 months	5	4

TABLE 5.

Duration of Jaundice in 31 Unoperated Cases (Present Series)
Including 5 Cases with Haemorrhage.

<u>Duration of Jaundice.</u>	<u>Total Cases.</u>	<u>Cases with Haemorrhage.</u>
Less than 1 month	18	1
1 to 2 months	3	0
2 to 4 months	7	2
Over 4 months	3	2

TABLE 6.

Relation of the Bleeding Tendency to the Depth of Jaundice (Walters)⁽⁶⁶⁾

<u>Grade of Jaundice.</u>	<u>Total Deaths.</u>	<u>Deaths from Haemorrhage.</u>
1 (Slight)	5	1
2	9	4
3	6	3
4 (Deep)	9	7

TABLE 7.

Relation of the Bleeding Tendency to the Depth of Jaundice (Present Series).

<u>Icteric Index.</u>	<u>Total Cases.</u>	<u>Cases with Haemorrhage.</u>
50 or under	11	1
51 to 100	21	5
101 to 150	13	5
Over 150	5	1

TABLE 8.

Relation of the Bleeding Tendency to the Depth of Jaundice
in 30 Unoperated Cases. (Present Series).

<u>Icteric Index.</u>	<u>Total Cases.</u>	<u>Cases with Haemorrhage.</u>
50 or under	6	0
51 to 100	14	1
101 to 150	6	3
Over 150	4	1

TABLE 9.

Cause of the Jaundice in Cases of Postoperative Haemorrhage (Petren; Boland).

	<u>Petren</u> ⁽⁵¹⁾	<u>Boland</u> ⁽³⁾
Stone in common duct	28	12
Carcinoma of Pancreas	11	14
Carcinoma of bile ducts	10	16
Carcinoma of gall-bladder	2	
Various	7	8
Total ...	<u>58</u>	<u>50</u>

TABLE 10.

Cause of the Jaundice in 12 Cases with Haemorrhage. (Present Series).

Stone in common duct	2
Carcinoma of pancreas	6
Carcinoma of bile ducts	1
Metastatic Carcinoma	2
Stricture of common duct with Cholangitis	1

TABLE 11.

Capillary Resistance Test in 20 Cases.

	<u>Total Cases.</u>	<u>Cases with Haemorrhage.</u>
No Capillary Fragility	14	2
Capillary Fragility	6	2
Temporary	3	1
Prolonged	3	1

TABLE 12.

Vitamin C. Tolerance Test (Case 15).

<u>Urine.</u>	<u>Ascorbic Acid Content.</u>
Preliminary sample	40 mgm.
After Redoxon	46 mgm.

TABLE 13.

Serum Volume in 13 Jaundiced Cases.

<u>Case No.</u>	<u>Volume of Serum from 10 ccm. blood.</u>
4 *	4.3
5	4.1
6	3.9
7 *	4.6
8	5.0
9	3.0
10 *	3.1
11	3.8
12	2.8
13	3.3
14	3.0
15	5.4
16 *	3.8

* Indicates Cases with Haemorrhage.

TABLE 14.

Prothrombin Index in 14 Cases of Toxic Jaundice.

<u>Case No.</u>	<u>Disease.</u>	<u>Prothrombin Index(%)</u>
18	Catarrhal Jaundice	107
21	" "	100
45	" "	100
17	" "	98
30	" "	91
36	" "	83
43	Toxic Jaundice (^{cause} unknown)	83
49	Chronic Hepatitis	76
34	Catarrhal Jaundice	76
20	" "	74
38	" "	70
19	Subacute Yellow Atrophy	63
23	Toxic Jaundice (^{cause} unknown)	55
29	Fatal Puerperal Toxaemia	24

TABLE 15.

Prothrombin Index in 20 Cases of Obstructive Jaundice.

<u>Case No.</u>	<u>Disease.</u>	<u>Prothrombin Index (%)</u>
24	Carcinoma of Pancreas	100
31	Stone in common duct	100
40	Stone in common duct and Carcinoma of Pancreas	100
35	Carcinoma of Pancreas	83
32	Metastatic Carcinoma	80
37	" "	71
42	Carcinoma Common Duct	71
25	Metastatic Carcinoma	58
23	Carcinoma of Pancreas	54
22	" "	52
46	Metastatic Carcinoma	51
48	Stone in common duct	49
28	Carcinoma of Pancreas	46
44	Metastatic Carcinoma	44
50	Carcinoma of Pancreas	42
39	" "	32
26	Stricture of Common Duct with Cholangitis	23
47	Stone in common duct	23
41	Carcinoma of Pancreas	21
27	" "	18

TABLE 16.

Relation of Prothrombin Deficiency to Depth of Jaundice.

<u>Icteric Index.</u>	<u>Total Cases.</u>	<u>Prothrombin.</u>		
		<u>Normal.</u>	<u>Moderate Deficiency.</u>	<u>Marked Deficiency.</u>
50 and under	9	6	2	1
51 to 100	14	0	7	7
Over 100	11	2	6	3

TABLE 17.

Relation of Prothrombin Deficiency to Duration of Jaundice.

<u>Duration.</u>	<u>Total Cases.</u>	<u>Prothrombin.</u>		
		<u>Normal.</u>	<u>Moderate Deficiency.</u>	<u>Marked Deficiency.</u>
Under 1 month	19	7	8	4
1 to 2 months	8	0	6	2
2 to 3 months	5	1	1	3
Over 3 months	2	0	1	1

TABLE 18.

Prothrombin Index in 6 Cases with Haemorrhage.

<u>Case No.</u>	<u>Initial Prothrombin Index.</u>	<u>Index at Time of Haemorrhage.</u>
	%	%
25	58	50 and under
26	23	18
27	18	32
41	21	21
44	44	-
47	23	23

TABLE 19.

Incidence of Haemorrhage in 20 Cases of Obstructive Jaundice.

<u>Case No.</u>	<u>Prothrombin Index.</u>	<u>Haemorrhage.</u>
24	100	0
31	100	0
40	100	0
35	100	0
32	80	0
37	71	0
42	71	0
25	58	+
33	54	0
22	52	0
46	51	0
48	49	0
28	46	0
44	44	+
50	42	0
39	32	0
26	23	+
47	23	+
41	21	0
27	18	+

A P P E N D I X.

TECHNIQUE AND INTERPRETATION
OF QUICK'S PROTHROMBIN TEST.

According to Morawitz's theory, (45)
the normal clotting of blood proceeds in two
phases:-

1. The prothrombin content in the plasma is activated by thrombokinase (derived from tissue juices, damaged cells or platelets) in the presence of calcium to form thrombin.
2. The thrombin thus formed converts the soluble fibrinogen into insoluble fibrin or clot.

The whole process occurs primarily in the plasma; the erythrocytes and white blood cells are merely entangled in the spreading network of fibrin.

It has been shown that the first phase of clotting is the critical one in nearly all haemorrhagic disorders. Variations in the fibrinogen content have little or no effect on the clotting provided a certain minimal amount is present - a state /

state of affairs regularly observed in jaundice.

The basis of Quick's prothrombin test is to add to the blood being tested an excess of those elements required for thrombin formation except prothrombin - i.e., an excess of thrombokinase and calcium. The rate of thrombin formation - and consequently the rate of clotting - will then vary according to the amount of prothrombin present.

The actual technique is as follows. Blood from a vein is oxalated to permit transfer to the laboratory. It is then recalcified by adding an excess of calcium chloride, whilst an excess of thrombokinase is supplied in the form of an extract of brain. We already know that fibrinogen is present in excess - and estimation of the fibrinogen should be carried out in every case - so the only variable is prothrombin. Under these conditions, normal blood clots very rapidly - in from 20 to 40 seconds, varying with the sample of brain - whereas blood deficient in prothrombin may take three or four times as long to clot. The prothrombin index is then estimated as a percentage of the normal. Thus, if normal blood takes 30 seconds to clot, and jaundiced blood takes /

takes 60 seconds, the prothrombin index is 50 per cent.

The venous blood is obtained by syringe, and 4.5 ccm. are added immediately to 0.5 ccm. of a M/10 solution of sodium oxalate in water. The oxalated blood can be stored for two or three days (not longer) in a refrigerator.

The preparation of thrombokinase is made from brain tissue, obtained conveniently from the autopsy room. After removal of the meninges and vessels, the brain is thoroughly washed to remove all blood, and is pulped and ground into a uniform paste. It is then spread in a thin layer over sheets of glass, and dried by a hot electric blower. The dried brain is scraped off and stored in a well-stoppered bottle. Its thrombokinase activity does not appear to deteriorate with keeping. Immediately before use, 2 g. of the dried brain emulsified in 10 ccm. of physiological saline solution, and incubated for 15 minutes at 37° C., after which it is filtered through gauze to remove coarse fragments.

The calcium solution consists of M/40 calcium chloride in distilled water. It should be made /

made up in bulk and stored for a few days before use.

For the actual estimation, 0.1 ccm. of blood plasma and 0.1 ccm. of the brain emulsion are pipetted into a clean dry test-tube. 0.1 ccm. of calcium chloride solution is then added, and the tube is immediately immersed in a water bath at 37° C., where it is shaken vigorously for a few moments and then rocked gently until the mixture coagulates. The time in seconds (measured by stop-watch) from the moment of adding the calcium to the moment of coagulation constitutes the prothrombin time, and by comparing this with the clotting time of a control plasma (which should always be estimated at each sitting) the prothrombin index may be obtained.

Significance of Quick's Prothrombin Test.

It is clear that the estimation described above is a test of some factor other than thrombokinase and calcium concerned in the elaboration of thrombin; and on Morawitz's theory, the missing factor can only be prothrombin. The possibility /

possibility must be considered, however, that Morawitz's theory is incomplete, in which case Quick's test may have some other significance. For example, an abnormal finding might be due to an excess of anti-prothrombin or anti-thrombin, rather than to a deficiency of prothrombin.

To determine these questions the blood in jaundice was compared with normal blood to which a solution of Heparin (anti-prothrombin or anti-thrombin) had been added, as in the following experiments.

(1) Quick's prothrombin test was carried out, using normal blood plasma to which varying amounts of a 0.5 per cent. solution of Heparin (Roche) had been added. The results seen in the following Table were obtained:

TABLE.

<u>Mixture.</u>	<u>Clotting Time.</u>
Plasma only	30 secs.
Plasma + 0.01 ccm. Heparin	46 "
Plasma + 0.05 " "	56 "
Plasma + 0.1 " "	∞

This /

This experiment thus showed that a lengthening of the clotting time as estimated in Quick's test does not specifically indicate a reduction of prothrombin, for it may be produced by the addition of anti-prothrombin or anti-thrombin.

(2) Quick's prothrombin test was carried out on plasma obtained from a healthy subject before and after the administration of 20,000 units Heparin (Roche) intravenously. The initial coagulation time as estimated by Quick's method was 30 seconds; the coagulation time 15 minutes after the administration was raised to 42 seconds.

This experiment thus confirmed the previous finding that Quick's test is not specific for prothrombin deficiency.

(3) It was thought that further information might be obtained by noting the effect of adding Protamine to blood.

It has been established (Chargaff and Olson⁽¹⁵⁾) that although Protamine inhibits clotting in normal blood, Protamine added to blood rendered incoagulable by means of Heparin has the reverse effect /

effect of promoting clotting. This effect is believed to be due to the formation of an insoluble compound of Protamine with Heparin.

It appeared that this observation might be used to test the significance of Quick's method as applied to jaundice. If, as Quick assumed, the faulty coagulation of jaundiced blood is due to a deficiency of prothrombin, the addition of Protamine should have little effect; if, on the other hand, the faulty coagulation is due to an excess of anti-prothrombin or anti-thrombin, the addition of Protamine might be expected to precipitate the anti-substance and thus accelerate clotting.

(a) The first step was to confirm the observation that Protamine antagonises the anti-coagulant effect of Heparin.

Accordingly, to 0.1 ccm. of oxalated normal plasma were added equal amounts of brain-thrombokinase emulsion, calcium chloride solution, and 0.5 per cent. Heparin solution. Three such tubes were set up; in all of them the Heparin completely inhibited coagulation. Three similar tubes /

tubes were then set up and to each was added 0.1 ccm. of 0.025 per cent. Protamine solution; clotting took place in 70 seconds, 90 seconds, 85 seconds, respectively.

(b) The next step was to determine if the addition of Protamine to plasma from a jaundiced patient with bleeding tendency would have a similar effect in promoting coagulation.

To 0.1 ccm. of oxalated plasma from such a patient, (Case 27), were added equal amounts of brain-thrombokinese, calcium chloride and physiological saline solution (i.e., Quick's prothrombin test was carried out with the addition of 0.1 ccm. of physiological saline). Three such tubes were set up; they underwent coagulation in 52, 53 and 55 seconds respectively. In three similar mixtures in which the physiological saline was replaced by 0.1 ccm. of 0.025 per cent. Protamine, coagulation occurred in 63, 60 and 63 seconds respectively.

This experiment indicates that Protamine does not accelerate but rather delays clotting in /

in jaundiced blood. It therefore gives no support to the view that the delayed clotting in jaundice might be due to anti-prothrombin or anti-thrombin. It confirms that Quick's test is, as claimed, a method of estimating the prothrombin content of the blood.

Reliability of Quick's Prothrombin Test.

Unlike the ordinary methods of estimating the coagulation time, Quick's prothrombin test is a highly satisfactory one to work. It is reasonably simple to carry out, the end-point is exact, and the figures obtained in normal controls are notably consistent.

The Charts 17 and 18 show the results obtained in two series of control estimations on plasma from non-jaundiced patients suffering from a variety of acute and chronic diseases (excluding diseases of the biliary tract). It will be seen that the normal range lies within narrow limits. This is especially seen in the second series, which /

which consists of observations gained after we had had some experience of the test. In this series, the normal "prothrombin time" lay between 24 and 35 seconds, whilst in 36 of the 54 cases it lay within the narrow range of 28 to 32 seconds. These figures compare strikingly with those obtained in prothrombin-deficient bloods, where the "prothrombin time" may be 70 or 100 seconds or more.

It should be noted that the figures given above are of estimations using two samples of brain, and that the normal range in the two series differs considerably. Different brains vary greatly in their thrombokinase content and the normal figure for each brain must therefore be estimated before applying the test. Since, however, a single brain suffices for from 200 to 300 estimations, this involves no special difficulty.

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