PROTHROMBIN AND VITAMIN K:

A REVIEW OF THIS SUBJECT INCLUDING A NEW SIMPLIFIED METHOD OF ESTIMATING PROTHROMBIN IN CAPILLARY BLOOD.

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by

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In the last few years great strides have been made in the study of the mechanism of the coagulation of blood. Much of the knowledge that has accumulated has found valuable clinical application in the classification, diagnosis and treatment of the haemorrhagic states. In particular, work on the role of the plasma prothrombin in the blood coagulation, and on the synthesis of prothrombin from a new accessory food factor called Vitamin K, has done much to promote an understanding and basis of treatment of a group of haemorrhagic conditions of much importance to the physician, surgeon and obstetrician in their respective clinical fields. It is proposed here to review the development of these advances, to discuss their significance and applications, and lastly to deal with the various technical questions in connection with this subject, including a new and simple technique for prothrombin estimation which can bring a hitherto academic investigation within the scope of any medical man.

It is now generally agreed that the clotting process of blood occurs in two distinct stages: first, the formation of thrombin from prothrombin in the presence of thromboplastin and calcium; and secondly the conversion of fibrinogen to fibrin by the thrombin. Thus four primary factors are necessary for the clotting of blood namely, prothrombin, thromboplastin, calcium and fibrinogen, and obviously a deficiency of any one of these/
these will inhibit or delay the coagulation of the blood and thereby result in a haemorrhagic state. It would seem that of these four factors the prothrombin is the one which is most commonly reduced to a level at which interference with blood coagulation occurs. Of the other factors, calcium and fibrinogen appear to have a wide margin of safety before their reduction has a significant influence on the clotting time. At present it is not possible to name any clinical or experimental bleeding condition that can be attributed to a deficiency of calcium, and fibrinogenopaenia of sufficient severity to cause bleeding is found mainly in conditions in which there is an associated hypoprothrombinaemia as a more potent cause of a haemorrhagic tendency. An insufficiency of the available supply of thromboplastin in the blood would appear to be the cause of haemophilia, the fault lying in the undue stability of the platelets, but apart from this important disease, thromboplastin deficiency as a cause of bleeding does not seem to be of much significance.

Prothrombin is the precursor of thrombin, the active coagulating enzyme. It is believed to have the nature of a proenzyme and is closely associated with the serum globulin. It is inactivated by heating for \( \frac{1}{2} \) hour at 56°C and can be removed from the plasma by adsorbing agents such as magnesium hydroxide. The methods for estimating prothrombin will be described/
described later, but two findings disclosed by studies on the prothrombin content of normal blood must be mentioned. First the prothrombin level in normal individuals is remarkably constant, and second, the fact that this level is greatly in excess of the physiological requirements for blood coagulation. It has been shown by Quick, Stanley-Brown and Bancroft (1935) that 80% of the prothrombin can be lost before the coagulation time is appreciably prolonged; and clinically several workers, e.g. Quick (1938) have found that serious haemorrhage does not occur until the prothrombin in the plasma falls below 20%.

The origin of the plasma prothrombin was until recently a matter of some doubt. About a decade ago it was widely held that prothrombin is formed in the bone marrow, probably in the megakaryocytes, and is released into the plasma from the platelets. In experiments with thoroughly washed platelets, however, Eagle (1935) and Ferguson (1936) were unable to uphold this view, as they could find only extremely minute traces of prothrombin in washed platelets. Furthermore, the finding of normal amounts of prothrombin in the plasma of patients with a marked reduction in the number of circulating platelets, such as in cases of thrombocytopaenic purpura and aplastic anaemia, is additional evidence that the platelets are not the source of prothrombin.

Recent work on the origin of prothrombin has confirmed the earlier view put forward by Nolf in 1908 that it is formed in the/
the liver. It has been shown by many workers e.g. Smith, Warner and Brinkhous (1937), Warren and Rhoads (1939), that experimental injury to, or removal of, the liver, results in a profound decrease in plasma prothrombin, and that regeneration of the liver following injury or partial removal results in a return of the prothrombin to normal levels. From this it may be concluded that the liver is essential for the maintenance of a normal plasma prothrombin level, and that when the liver is partially excised or is injured by poisons, infection or tumour growth, the level of the plasma prothrombin falls.

The existence of an accessory food factor or vitamin connected with the process of blood coagulation was first suspected as a result of experiments on chicks done by Dam of Copenhagen in 1929. In the course of studies on cholesterol metabolism, newly hatched chicks were placed on a fat-free diet. After several weeks these chicks developed spontaneous haemorrhages into the skin, mucous membranes and other parts of the body. Similar findings were noted by other workers and it was considered originally that the haemorrhagic syndrome was due to scurvy, despite earlier reports that chicks are able to synthesise vitamin C. However, in later studies, scurvy as well as other known vitamin deficiencies were ruled out and it was demonstrated that a new fat-soluble vitamin is essential for the prevention of this bleeding tendency. The name for this vitamin or antihaemorrhagic/
antihaemorrhagic factor was suggested by Dam in 1935 as vitamin K, standing for the Danish "Koagulations-Vitamin;" and this name has been adopted generally.

Subsequent studies by Dam and his colleagues and by other workers, notably by Almquist and Stokstad in America showed that the fat-soluble vitamin K is present in large amounts in certain green vegetables such as alfalfa, spinach and kale. It was also shown that the vitamin can be produced by bacterial action and is synthesized by the bacteria in the lower portion of the intestine. Absorption from the lower intestine is minimal in the chick, but in mammals where such absorption does occur, vitamin K is present in sufficient amounts to prevent deficiency except in cases of faulty absorption. It was later shown in rats by Greaves and Schmidt (1937) and in man by Warner, Brinkhous and Smith (1938) that the presence of bile salts is necessary in the intestine before absorption of the vitamin K can occur.

Following the recognition of the haemorrhagic chick disease as a nutritional disorder, the relationship of this new dietary factor to blood clotting was soon established. It was observed that chicks with the haemorrhagic disease have a prolonged clotting time and Schonheyder (1936) suggested that the bleeding tendency was the result of a hypoprothrombinaemia. Shortly after, Dam, Schonheyder and Tage-Hansen (1936) found that they could/
could not isolate prothrombin from the blood of chicks kept on a vitamin-K poor diet, and a few months later Quick (1937) showed that the prothrombin began to diminish 4 days after removing vitamin K from the diet, and could be reduced to less than 10% of normal in less than 3 weeks. A distinct haemorrhagic tendency appeared when the low prothrombin levels were reached. Both the low prothrombin and the bleeding tendency were cured by adding 2% alfalfa meal to the diet. These results definitely demonstrated that vitamin K is necessary for the synthesis of prothrombin in the chick. In mammals the production of the vitamin by bacterial activity in the intestine renders a nutritional deficiency difficult to obtain, and a vitamin K deficiency develops only when absorption of this compound from the intestine is interrupted. Faulty absorption occurs when bile is excluded from the intestine as in the experimental biliary fistulas in rats described by Greaves and Schmidt (1937), or in dogs, (Hawkins and Brinkhous (1936)) or in cases of obstructive jaundice in human subjects. Such human cases were first observed to have a severe diminution of plasma prothrombin by Quick, Stanley-Brown and Bancroft in 1935, and these findings were later corroborated by Brinkhous, Smith and Warner (1938) and by Snell and other workers of the Mayo Clinic. These investigators demonstrated the efficacy of treating this deficiency condition with vitamin K and bile salts.

The above observations establish the fact that vitamin K is/
is essential for the formation of prothrombin in the liver. It has not yet been determined whether the vitamin enters chemically into the formation of prothrombin, or whether it merely keeps certain tissues in a normal healthy state of activity essential for the synthesis of prothrombin.

A great deal of work has been done towards the isolation and chemical synthesis of vitamin K. There are many natural sources of the vitamin among which alfalfa and spinach are particularly rich in their content. Other plants such as cabbage, kale, and cauliflower also have a high natural content. Vitamin K is also present abundantly in bacteria and is apparently synthesised and retained within the bacterium during growth. The formation of the vitamin in the intestine of mammals is thus explained as being dependent on bacterial activity; and in newborn infants a vitamin K deficiency causing a haemorrhagic tendency may occur before the taking of food establishes a bacterial intestinal flora. The vitamin is widely distributed in moderate amounts throughout the tissues of the animal body, and there does not appear to be any one organ which stores the vitamin in any great quantity.

Early in the study of vitamin K it was observed that the antihaemorrhagic factor is extractable from natural sources by fat solvents such as petroleum ether.

Following the discovery of Almquist and Stokstad in 1935 that/
that alfalfa meal is an excellent source of the vitamin, this material has been used extensively in the preparation of concentrates of the vitamin. In such concentrates the vitamin resists heating up to 120°C for 24 hours, but is destroyed by oxidising agents and certain other chemicals, and by exposure to sunlight or artificial light for a few hours. The first practically pure vitamin K preparation isolated from alfalfa was reported by Dam and his co-workers in January 1939. This was a clear yellow oil having approximately 75,000 times the antihaemorrhagic activity of dried alfalfa. In the Spring of 1939, it became apparent that more than one compound possessed vitamin K activity. MacCorquodale (1939)' and McKee and co-workers isolated two distinctly different compounds with very high vitamin activity from alfalfa meal and from putrefying fish meal respectively. They called the vitamin from alfalfa vitamin $K_1$ and the vitamin from fish meal vitamin $K_2$. The potency of vitamin $K_2$ is 60% of that of vitamin $K_1$.

Analyses of the oily alfalfa concentrate isolated by Dam and his associates, lead, in a surprisingly short time, to the final identification of the chemical structure of the vitamin and to the laboratory synthesis of the compound. In September 1939 the synthesis of vitamin $K_1$ was reported practically simultaneously by three separate groups of investigators, - Almquist and Klose (1939)$^1$, MacCorquodale and associates (1939)$^2$ and/
and by Fieser. The natural and synthetic products had the same physical and chemical properties and approximately the same antihæmorrhagic activity. The structure of the vitamin K₁ isolated from alfalfa was shown to be 2 methyl-3-phytyl-1, 4-naphthoquinone. Vitamin K₂ obtained from fish meal has since been shown by Binkley, McKee, Thayer and Doisy (1940) to be a 2 methyl-1, 4-naphthoquinone. Since these findings a large number of similar synthetic chemical preparations which are basically 1, 4 naphthoquinones or hydroquinones, have been investigated for vitamin K activity by many workers and many have shown a high degree of activity. Several potent preparations of the vitamin, both concentrates and synthetic forms, are now being marketed by the leading drug manufacturers.

The methods for the assay of vitamin K preparations are based on the prevention or cure of vitamin K deficiency in chicks. The criteria used for the detection of the disease have varied with different investigators and with the same investigation from time to time. Of the preventive methods of assay that of Almquist, Mecchi and Klose (1938) is an example, whereas of the many curative procedures for assay those devised by Schonheyder (1936) by Dam and Glavind (1938), Thayer, Doisy et al (1939) and Ansbacher (1939) are among those most frequently employed. In general the various units are expressions of the amounts of vitamin K required to correct an altered blood clotting time or prothrombin level in a chick in a standard time.

Having/
Having reviewed the development of our knowledge regarding the role of vitamin K in blood coagulation, it is possible to indicate the practical applications of these findings and to consider the clinical conditions in which they are likely to prove of value for diagnostic or therapeutic purposes. It is logical to remember the normal cycle of events in the vitamin K-prothrombin-coagulation process and to deal with the clinical disorders in the sequence which represents interference with the physiological mechanism at its different stages. It is important to bear in mind the fact that whereas in all these conditions there may be a haemorrhagic tendency resulting from a diminished plasma prothrombin and therefore a prolonged coagulation time, the clinical signs of bleeding do not usually occur until the prothrombin sinks below 20% of its normal level (Quick 1938).

Because of the synthesis of vitamin K by bacterial activity in the intestine, a reduction of plasma prothrombin in adults is most unlikely to occur as a result of dietary deficiency alone. A report of simple nutritional deficiency of vitamin K in man has however been made by Kark and Lozner (1939). They observed 4 patients with diminished plasma prothrombin levels due apparently to a simple dietary deficiency. Three of the patients had been eating diets practically devoid of fruits and green vegetables for a period of one to nine years. These cases had scurvy/
scurvy in addition. The fourth patient, a chronic alcoholic, had pellagra and subclinical scurvy as well as a low plasma prothrombin. Vitamin K, without bile salts, was given by mouth to each of these patients, and on the day following this therapy the plasma prothrombin level had returned to normal or nearly normal.

The tendency to undue bleeding in the newborn has been recognised for centuries, but the explanation of this haemorrhagic state and the question of its prevention and cure have but recently become apparent in the light of the work on prothrombin and vitamin K which formed a new approach to the study of this disease. In 1937, Brinkhous, Smith and Warner found a low plasma prothrombin in a case of haemorrhagic disease of the newborn, and since then several workers have studied the prothrombin content of the blood of newborn infants: (Waddell and Guerry 1939, Nygaard 1939, Quick and Grossman 1940, Kato and Poncher 1940). Their reports show that the prothrombin content of infants' blood is nearly at the same level at birth as that of the adult. It often falls abruptly, however, in the first three days of life and may reach dangerously low levels, but thereafter it is restored spontaneously and usually promptly to a normal level. Since the food intake in such an infant is minimal during this period, the suggestion is made that the recovery of the prothrombin concentration is brought about by the/
the establishment of a bacterial flora in the intestine and that this initiates the synthesis of vitamin K which becomes available to the infant for the production of prothrombin. The cause of the temporary prothrombin deficiency seems to be due to an inadequate storage of prothrombin or of vitamin K in the foetus and the physiological demands after birth promptly exhaust the available prothrombin. Any delay in the restoration of the prothrombin level will result in an exceedingly low prothrombin concentration in the blood and in the clinical manifestations of external or internal bleeding. The condition is clearly due to a lack of vitamin K, for not only can the prothrombin be promptly restored to normal by giving the baby a concentrate of this vitamin, but also the serious fall in concentration can be prevented by the oral administration of this accessory food factor. (Waddell and Guerry 1939). Macpherson, McCallum and Haultain (1940) have used synthetic vitamin K analogues, naphthoquinone derivatives, in the treatment of this condition in the newborn. They have further shown that the administration of such substances to the mother before delivery, will prevent the haemorrhagic tendency in the child, and will minimise the risk of cerebral haemorrhage in the child in cases of difficult or instrumental delivery. Vitamin K or its analogues are now being widely used by obstetricians for such purposes, and this constitutes a striking therapeutic advance in the treatment of what/
what was until quite recently a serious and baffling condition. The haemorrhagic tendency in patients with chronic obstructive jaundice has been recognised for many years, and has for long been a source of difficulty in the surgical treatment of such cases. Until recently, however, there was no evidence which pointed conclusively to the clotting defect responsible for this haemorrhagic diathesis. The occurrence of a bleeding tendency in patients having a chronic biliary fistula but without jaundice, was not so generally appreciated, and it was only after Hawkins and Whipple (1935) pointed out a similar condition in dogs with biliary fistulas, that attention was drawn to this type of case. The first studies of plasma prothrombin in patients with obstructed jaundice were reported in 1935 by Quick, Stanley-Brown and Bancroft who found a severe diminution of prothrombin in certain cases. In 1938 these findings were corroborated by Brinkhous, Smith and Warner and by Snell and other workers at the Mayo clinic. Both groups of investigators found that the prothrombin defect and the haemorrhagic tendency in these cases could be remedied by treatment with vitamin K and bile salts. Since then, several workers, e.g. Olson and Menzel (1939) and Stewart (1939) have reported on cases of obstructive jaundice and biliary fistula in which the haemorrhagic diathesis was successfully treated with bile and a vitamin K concentrate. This work, which was recognised to be of much practical/
practical value in the surgery of obstructive jaundice, was confirmed in Edinburgh by Illingworth (1939). The intramuscular injection of the synthetic naphthoquinone preparations in these cases has been reported by Almquist and Klose (1939)\(^2\), Macfie, Bacharach and Chance (1939) and by Reid (1941), and has been found effective in the prevention or cure of the bleeding tendency. If the vitamin K preparation is thus injected, bile salts are not necessary, as their function is only to ensure absorption of the vitamin from the intestine following oral administration.

The tendency for cases of obstructive jaundice to bleed within the few days after operation has been repeatedly noticed, although these cases may have shown no evidence of excessive bleeding at the time of operation. This condition has been shown to be caused by a progressive decline in the plasma prothrombin which occurs during the early postoperative days and which may result in the appearance of a haemorrhagic tendency between the first and fourth days after operation. Studies of this condition have been made by Butt, Snell and Osterberg (1938 and 1939) and by Brinkhous, Smith and Warner (1938). Treatment with vitamin K is effective in preventing or curing this postoperative bleeding tendency. Many suggestions have been put forward to account for the postoperative fall in prothrombin. Amongst these are: (1) increased consumption of prothrombin due/
due to haemorrhage and exudate formation, without adequate reserves of prothrombin or vitamin K. (2) A low bile salt output by the liver resulting in consequent malabsorption of vitamin K for a period after the obstruction has been relieved and (3) liver damage by anaesthesia, infection or operative trauma, and decreased production of prothrombin by the liver.

In 1938, Fanconi reported six cases of sprue who showed a bleeding tendency and a prolonged coagulation time, with a normal calcium and fibrinogen content of the blood. He suggested that the bleeding tendency in this disease is due to the faulty absorption of fats and vitamin K. It is probable that a low plasma prothrombin concentration will also be found in various other conditions in which one might expect inadequate absorption of fat soluble vitamins. Clinical confirmation of this supposition has been provided by Clark, Dixon, Butt and Snell (1939) who observed a deficiency of plasma prothrombin in sprue, intestinal polyposis, chronic ulcerative colitis, gastrocolic fistula and other conditions.

A tendency to bleed often occurs in severe liver injury, and is frequently observed in cases of acute yellow atrophy. In these conditions the fibrinogen in the blood is often greatly diminished and it has been generally assumed that this accounted for the haemorrhagic condition. Experiments by Smith, Warner and Brinkhous (1937) and by Quick (1938) showed that in dogs subjected/
subjected to prolonged chloroform anaesthesia, a marked drop in prothrombin occurred which was far more abrupt and more severe than the decrease in fibrinogen. In view of these findings it seems reasonable to postulate that in extensive parenchymatous damage of the liver, the main cause of bleeding is the reduced concentration of prothrombin, and as mentioned before, there is much evidence to show that the liver is essential for the formation of prothrombin.

The effect of liver damage on the prothrombin content of the blood is of considerable significance in the jaundiced patient. Treatment of cases with diminished prothrombin by vitamin K therapy shows that there are great variations in the response, for while some will react promptly, others respond only sluggishly. It would appear therefore that correct utilisation of vitamin K is a necessary factor as well as an adequate supply and good absorption of this accessory food substance. As the liver is concerned in the conversion of vitamin K into prothrombin, it follows that if liver function is seriously impaired, the synthesis of prothrombin will be decreased even though the intake and absorption of the vitamin is adequate to meet normal metabolic demands. Thus if impairment of liver function occurs more vitamin K will be required to compensate for the faulty utilisation. In a jaundiced patient therefore, a diminished plasma prothrombin may result from either inadequate absorption or from faulty utilisation and/
and in many cases both factors are probably present. Reports on the finding of hypoprothrombinaemia in cases of acute hepatitis in cirrhosis of the liver and in other forms of liver damage have been made by many authors including Scanlon et al (1939), Pohle and Stewart (1939), Wilson (1939), Butt et al (1940) and Reid (1941). Suggestions have been made that the amount of prothrombin in the blood may be used to assess liver function, e.g. Fullerton (1940). Wilson (1940) has reported a relation between the hippuric acid liver function test and the blood-prothrombin levels. Reid (1941) suggests that the failure of naphthoquinone preparations to increase the subnormal amounts of prothrombin in the blood in patients suffering from chronic hepatic disease such as cirrhosis, may prove to be a valuable index of liver inefficiency. This matter is worthy of further investigation as it is almost generally agreed that the present available tests of liver function yield little or no information of value that cannot be gained from clinical examination.

The determination of the plasma prothrombin content is now recognised as a necessary step in the investigation of the haemorrhagic states in general and is of particular value in those conditions which have just been described. We must now review the methods of prothrombin estimation which are in use and a new simplified method of determination will be described. It must be remembered that relatively little is known of the chemical composition of prothrombin and it has never been isolated/
isolated in pure form. The only means of recognising this clotting factor is by its capacity to form thrombin; and hence any method for the quantitative determination of prothrombin must be an assay based upon thrombin formation.

The first practicable method for prothrombin determination was outlined in 1934 by Warner, Brinkhous and Smith. This method utilises the biphasic nature of the clotting reaction and therefore is done in two stages. In the first stage defibrinated plasma is mixed with an optimal amount of calcium and an excess of thromboplastin. This results in the conversion of all the prothrombin to thrombin. In the second, or clotting stage, the amount of thrombin so formed is measured by the time required for the clotting of a standard fibrinogen solution. The result is expressed in units of prothrombin, one unit being defined as the quantity which when converted to thrombin, will cause the clotting of 1 cc. of a fibrinogen solution under standard conditions. Normal human plasma contains approximately 300 units of prothrombin per cc. when so estimated. A modification of this two-stage method has recently been used by Stewart & Rourke (1939) in the study of prothrombin deficiency in cases of obstructive jaundice and they have apparently obtained reliable results by this procedure.

The second method of prothrombin estimation entails only one stage in procedure, and most of the work done on prothrombin during/
during recent years has employed this type of investigation. The method devised by Quick in 1935 was the first one-stage procedure and has become the best known and most widely adopted technique for prothrombin estimation. It is dependent on the observation that the clotting time of oxalated plasma, when mixed with an excess of thromboplastin and an optimum amount of calcium, can be employed as a direct measure of the prothrombin content of the plasma. This accelerated clotting time, determined after the addition of an excess of thromboplastin and a fixed amount of calcium to oxalated plasma at 37.4°C, is called the prothrombin time, and in normal human plasma varies between 10 and 25 seconds depending on the activity of the thromboplastin used. 4.5 cc. of oxalated venous blood is required for the test and a standard thromboplastin solution is prepared from dehydrated rabbit brain. If plasma is diluted to give prothrombin concentrations between 5 and 100 per cent of normal, a curve can be constructed to express the relationship between prothrombin time and prothrombin concentration (Quick 1938). If this is done it will be seen that the prothrombin time for plasmas containing as little as 60 per cent prothrombin is nearly the same as for normal plasma. For this reason a normal prothrombin time cannot be interpreted to mean that the plasma contains 100 per cent prothrombin. Moreover, if prothrombin times are done not only on the whole plasma but also on the plasma/
plasma after diluting two or three times, a diminution in the prothrombin level can be determined more accurately (Magarth, 1938, Quick and Grossman 1939, and Fullerton 1940).

Pohle and Stewart (1939) have suggested a modification of Quick's method in which a series of prothrombin tests are done with each plasma, using varying amounts of calcium. They found that the addition of an optimal amount of calcium gave the shortest prothrombin time. Quick (1939) obtained similar results, but believes that this modification is unnecessary, however.

The preparation of the thromboplastin solution in Quick's test is associated with certain disadvantages as pointed out by Fullerton (1940). The tissue extracts are not always of the same potency when prepared, and normal figures must therefore be established for each batch. Also, the extracts gradually lose their potency making control tests necessary, and fresh extracts have to be made frequently. Furthermore, the technical difficulties in making a suitable tissue extract are alone sufficient to restrict the extent to which the test is used. Fullerton therefore suggested the use of a commercial preparation of Russell-viper venom (e.g. "Stypven") as a source of thromboplastin in Quick's test and this modification would appear to have simplified greatly the technique of this method of prothrombin estimation.
A simplified test for prothrombin which can be carried out at the bedside, was devised by Smith, Ziffren, Owen and Hoffman (1939). It consists in mixing 1 cc. of freshly drawn whole blood with a fixed large amount of thromboplastin and determining the clotting time. A fresh tissue extract prepared from rabbit lung is employed as the source of thromboplastin, and there is no oxalation and recalcification. A control test on normal blood is carried out and the results of the test are expressed in the form of a percentage of clotting activity as compared with this normal. This method has been used by Macpherson, McCallum and Haultain (1940) in studies of prothrombin in the newborn.

In all the methods of prothrombin estimation so far described, venous blood must be withdrawn from the patient. In obese or collapsed adult patients the localisation of a suitable vein may prove difficult; and in infants where the blood is usually obtained from the anterior fontanelle, this procedure may seem unjustifiable to the clinician in view of the haemorrhagic tendency liable to be present in cases to be studied. Accordingly, if the prothrombin in capillary blood could be estimated in a simple and satisfactory manner, the scope and application of such an investigation would obviously be extended.

A test for estimating the prothrombin content of capillary whole/
whole blood was described by Kato (1940). The test is performed in a watch-glass and the capillary blood and other ingredients used are measured with a special microhaemopipette designed by Kato (1938). A fresh solution of rabbit brain extract is used as a source of thromboplastin and this solution is standardised so as to produce a clotting time of approximately 20 seconds. This test has been carried out in hundreds of cases in adults and especially in newborn infants, and has yielded results comparable to those obtained on venous blood by Quick's method. Kato (1940), Kato and Poncher (1940).

A new method of estimating the prothrombin in capillary whole blood has been devised and recently reported by Innes and Davidson (1941). The aim in introducing yet another method of prothrombin estimation, has been to provide an accurate but essentially simple method that can be carried out in a few minutes without requiring much technical skill and without the necessity for elaborate apparatus or for the preparation of special tissue extracts.

It is believed that in the following test this aim has been accomplished, and that if this method is used, any medical man can investigate the plasma prothrombin level of a case without the need for specialised laboratory assistance.

**Method and Materials.**

A *standard* white blood cell counting pipette is used to measure/
measure the various ingredients. It is better to have a number of such pipettes, but if only one is employed it must be cleaned thoroughly with saline after measuring each ingredient. A solution of crystalline sodium oxalate (13.40 grammes per litre) is drawn up into the pipette and a quantity equal to one division of the pipette is blown into a clean watch glass. The skin is punctured so that the blood flows freely without squeezing. In infants the heel is the most suitable site. Blood is drawn up to the ninth division and then transferred to the watch glass. Thorough mixing of blood and oxalate solution is effected by means of a small glass stirring rod. The snake venom used is Russell viper venom, supplied by Messrs Burroughs Wellcome and Co. under the name of Stypven. The solution is prepared by adding 1 ccm of distilled water to 0.1 mgm of the venom in dry form. A freshly prepared solution is best, as the efficacy of the venom in solution decreases after a few days. Stypven solution and a solution of anhydrous calcium chloride (2.775 grammes per litre) are in turn sucked up into the pipette to the tenth division and are mixed with the oxalated blood in the watch glass. Following the addition of the calcium chloride solution, visible fibrin formation occurs on the average within 25–35 seconds as timed with a stop watch. The change is best seen against a white background. The test is repeated on a normal person at the same time under the same environmental conditions.
conditions, and the prothrombin index of the patient's blood is expressed as a percentage of this normal.

Thus Prothrombin Index = \[
\frac{\text{Normal Prothrombin Time}}{\text{Patient's Prothrombin Time}} \times 100.
\]

With practice, a high degree of accuracy can be obtained in assessing the coagulation time. Thus, repeated estimations done on the same individual under standard conditions show a deviation of only 1 or 2 seconds.

As already mentioned, it has been shown by Quick (1938) and by Fullerton (1940) that a marked lengthening of the coagulation time occurs only when the level of the prothrombin in the blood falls to 20 per cent of normal or less. By diluting the blood of both the patient and the control to 20 per cent with normal saline, minor differences in the coagulation time become more apparent. If it is desired to adapt this modification to the present technique, a quantity of normal saline equal to 40 divisions of the white cell pipette are added to the 10 divisions of oxalated blood present in the watch glass. After mixing thoroughly, a quantity of this mixture equal to 10 divisions is transferred to a second clean watch glass in which the test is completed. It should be noted that the effect of such dilution makes it somewhat more difficult to see the onset of fibrin formation.

This capillary prothrombin test is held to have certain important advantages when compared with that of Kato. These are:
are:-

1. The use of a white blood cell counting pipette obviates the need for obtaining the special microhaemopipette designed by Kato (1938).

2. The use of snake venom as a source of thrombokinase as described by Fullerton (1940), which eliminates the necessity for preparing a brain extract as used by Kato.

3. The carrying out of the test on a normal control person, at the same time and under the same environmental conditions, is felt by us to be a fundamental part of the method. In Kato's method no control on normal blood is done, reliance being placed on standardising the solution of thrombo-plastin so as to produce a clotting time of approximately 20 seconds. The control eliminates the effect of minor variations in the solutions used and the changes in the clotting time which result from variations in temperature and humidity. In practice we use our own blood as a control, and have found that the clotting time of undiluted normal blood may be as short as 20-25 seconds in the heated nursery of the maternity pavilion and as long as 35-45 seconds in the cooler general hospital wards in winter time.

The new capillary test described above has been carried out on a large series of normal persons, newborn infants, and patients with obstructive jaundice and liver damage, and has been found to give results comparable to those obtained by Quick's and other methods.

In concluding this essay, one feels that it is desirable to indicate the part which the results of studies on prothrombin and vitamin K may be expected to play when viewed from the broad standpoint of clinical application and treatment value as/
as a whole. The work on this subject, which has nearly all been done in the last few years, has yielded results of great value in the understanding and treatment of certain haemorrhagic states. In particular it has shown that the bleeding tendency in obstructive jaundice and in haemorrhagic disease of the newborn can be prevented and cured by administration of vitamin K. A similar bleeding diathesis in certain malabsorptive conditions has likewise been shown to be due to vitamin K deficiency and to be amenable to treatment with this substance. Estimation of the prothrombin content of the blood has come to be recognised as an essential step in the investigation of a patient with a bleeding tendency, as providing the only real indication for vitamin K therapy, and as a possible test of liver function. Apart from these most important applications, however, the value of the prothrombin - vitamin K question would appear to be limited. In all new fields of research yielding spectacular results, there is a tendency for clinicians to fail to realise the limitations of the findings and to indulge in the irrational use of any new remedy described in many conditions outwith the scope of its applications. Thus it is felt that today many cases of haemorrhagic states are being given vitamin K therapy, just because of the bleeding tendency and quite without justification such as a demonstrable low blood prothrombin. It follows that in such circumstances the existence of a simple, rapid,
rapid test for prothrombin determination will be of much value in the investigation and treatment of these haemorrhagic conditions. It is hoped that the new simplified method for estimating prothrombin in capillary blood will help the subject of prothrombin and vitamin K to establish its real place amongst the advances in clinical science of our times.

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