THE INFLUENCE OF CERTAIN DRUGS ON BLOOD COAGULATION AND RELATED PHENOMENA*

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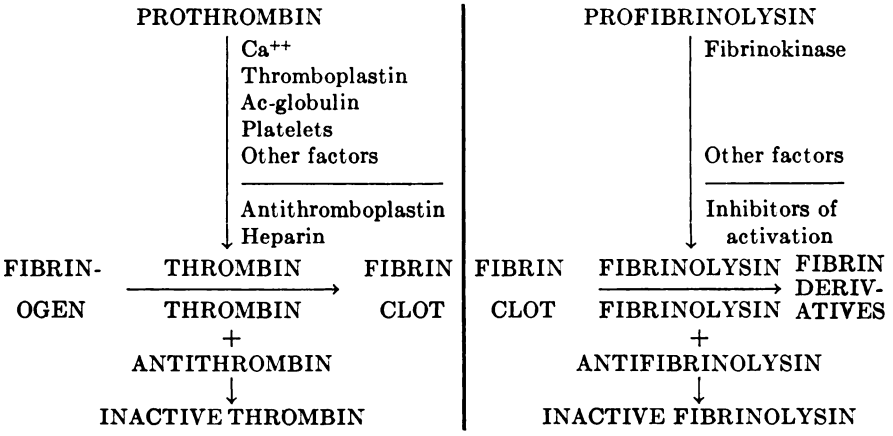
I. INTRODUCTION

In recent years there has been a widening interest in the phenomena of blood coagulation and allied metabolic processes. This is partly due to the realization that cardiovascular diseases affect human welfare as much as or more than any other general class of ailments. A large proportion of cardiovascular difficulties involve the blood coagulation mechanism either from the standpoint of hemorrhages or intravascular clots. The enormous complexity of the chemical interactions in the phenomena of coagulation has required the attention of many people trained in various disciplines. There is now the general impression that real progress is being made and many are hopeful of gaining better control over many of the diseases that involve this mechanism. To this end drugs are becoming increasingly important and a review of their effects has long been needed. It must, however, be admitted that a thorough search of the literature offers only sporadic rewards so that it is necessary to describe or suggest one or more possible effects of certain drugs when one would like to be specific and conclusive.

Blood coagulation is only a part of the physiology of hemostasis and only one of the factors which contribute to the problem of thrombosis and related phenomena. This review does not include all of these considerations which embody a large amount of literature. The aim has been to restrict the discussion to the pharmacological effects of certain drugs on those factors which are directly concerned with the physiology of blood coagulation. Only in certain instances have effects on vascular tissues been considered. The drugs reviewed are: dicumarol, compounds related to dicumarol, salicylates, methylxanthines, antibiotics, protamines, toluidine blue, the digitalis series, alpha tocopherol compounds, heparin and related substances, unrecognized nutritional factors, vitamin K, sulfonamides, adrenocorticotropic hormone, anesthetics and respiratory gases, epinephrine and acetylcholine. In addition, a number of the substances which

are a part of the blood coagulation mechanism itself have become available commercially and are used for therapeutic purposes. They may thus be regarded as drugs and are briefly discussed from the standpoint of their pharmacological action. It is not possible to discuss this topic without presupposing familiarity with the blood coagulation mechanism itself. Since this field still presents a number of conflicting viewpoints, extensively reviewed (2, 18, 19, 39, 78, 85, 88, 101, 143, 147, 165–167, 169, 197, 206, 231, 257, 268, 280, 303, 320–322, 335, 356, 369, 377, 384, 387, 398, 412, 416, 432, 433, 487, 491, 503, 514, 515, 568, 571, 583, 589, 609, 610), it seems appropriate to present very briefly the concepts which the writer has used in evaluating the literature. For essentially the same reason a brief commentary seems necessary on several of the more common methods by which clotting may be investigated and by which pharmacological effects have been evaluated. A diagram representing some of the main chemical reactions in blood coagulation is given below.



The interpretation of the above diagram is as follows: The fibrinogen of the plasma is clotted by thrombin. The latter is derived only from prothrombin by the catalytic action of calcium ions, Ac-globulin, platelets, thromboplastin (thromboplastin, Howell; thrombokinase, Morawitz; cytozyme, Fuld and Spiro; tissue fibrinogen, Wooldridge; placental toxin, in the obstetric literature; thrombozyme, Nolf) and perhaps other factors. The Ac-globulin of the plasma is probably a proenzyme and is activated by small amounts of thrombin. The platelets furnish accelerator activity. They perhaps interact with plasma to furnish thromboplastin activity and they also contribute a factor which facilitates the interaction of thrombin and fibrinogen. Thromboplastin is primarily derived from the fixed tissues such as lung, muscle and brain. A large number of other factors have been described which are said to contribute to the activation of prothrombin, but it remains to be determined whether and how many such factors exist. The inhibitors of prothrombin activation probably counterbalance the activation mechanism. Antithromboplastin is considered to be present in the plasma and other tissues. Heparin which may be released from the cells

probably acts as an inhibitor of prothrombin activation in conjunction with a plasma co-factor. In the same manner heparin probably exerts its antithrombic properties by acting in conjunction with a plasma co-factor. In addition, the plasma contains a substance, natural antithrombin, which can neutralize thrombic activity independent of heparin action. In the activation of prothrombin the following sequence of events may be suggested:

$$\text{Prothrombin} \xrightarrow{\substack{Ca^{++}\\ \text{Thromboplastin}\\ \text{Platelet Accelerator}}} \text{Thrombin} \qquad (1)$$

$$\text{Plasma Ac-globulin} \xrightarrow{\text{Thrombin}} \text{Serum Ac-globulin} \qquad (2)$$

$$\text{Prothrombin} \xrightarrow[\text{Serum Ac-globulin}]{\substack{Ca^{++}\\ \text{Thromboplastin}\\ \text{Platelet Accelerator}}} \text{Thrombin} \qquad (3)$$

In the first reaction the production of thrombin is considered to be slow; but as the small amount of thrombin activates plasma Ac-globulin the latter contributes to the acceleration of prothrombin activation. The third reaction represents, therefore, the rapid production of thrombin. The thromboplastin required for the initial production of thrombin, as represented by reaction one, is of fixed tissue origin. However, there is much evidence in the literature that the equivalent of thromboplastin may also be derived from the interaction of platelets and plasma (79, 252, 332, 339, 360, 410); this fact permits the above reactions to apply when blood is carefully drawn and not mixed with fixed tissue thromboplastin.

In addition to blood coagulation the diagram above depicts an enzyme mechanism for the dissolution of a clot. Fibrinolysin (plasmin) can dissolve a fibrin clot and produce derivatives of fibrinogen that do not respond to the action of thrombin. The fibrinolysin has its origin in plasma profibrinolysin (plasminogen). An activator of profibrinolysin is found in tissues and an inhibitor of the activation has only recently been described. Certain bacteria can also activate plasma fibrinolysin. The activity of fibrinolysin can be neutralized by antifibrinolysin which is a normal component of the plasma.

II. METHODS FOR EVALUATING BLOOD CLOTTING PHENOMENA AND EFFECTS OF PHARMACOLOGICAL AGENTS

Clotting of whole blood. The clotting time of the whole blood as generally estimated by the Lee-White method is a rough measure of the over-all clotting mechanism. It is influenced by variables introduced in drawing the blood and in doing the test. Because it is dependent upon the interaction of many factors and because of the unusually wide normal range, large abnormalities in one or more factors can be completely masked in the results of this test. For instance, the prothrombin concentration may be lowered to 20 per cent or below before

prolonged clotting time is noticed. However, valuable data can undoubtedly be obtained when the method is employed meticulously by the same individual on repeated occasions.

Prothrombin time. The prothrombin time has most frequently been employed to report clotting data. There are many variations of the general procedure in which oxalated plasma is re-calcified and simultaneously a large amount of thromboplastic material is added. The clotting time is noted. To interpret the results it is assumed that one may judge the prothrombin concentration from the clotting time. This test is influenced by anticoagulation factors, but it has been fairly well established that the effect of these is minimized when the plasma is first diluted before the test is done. The procedure makes no allowance for the determination of concentrations of prothrombin exceeding 100 per cent of normal nor is it sensitive to changes in prothrombin concentration within the range of 50 to 100 per cent. Ac-globulin and perhaps other substances are controlling factors in the speed of prothrombin conversion to thrombin; consequently the test is susceptible to variations in Ac-globulin as well as to alterations in prothrombin reactivity. Changes in the so-called prothrombin time cannot with any certainty be ascribed to prothrombin alone, but most of the conclusions in the papers reviewed are based on the prothrombin time method.

Prothrombin. In a number of studies the two-stage procedure for the *quantitative* determination of prothrombin has been employed. There is considerable evidence that it gives specific information with regard to prothrombin concentration provided Ac-globulin is present in the plasma or supplied in the technical procedure.

Other quantitative methods. Quantitative measurements of heparin and fibrinogen concentration apparently give results which are quite reliable. Quantitative methods for Ac-globulin, platelet extracts, thromboplastin, antithromboplastin fibrinolysin, antifibrinolysin and thrombin have also been described in recent years but have as yet not been employed extensively. Most measurements of antithrombin activity recorded to date probably involved multiple factors.

Evaluation of pharmacological action. The above comments are an expression of present day viewpoints concerning methods used in the study of the blood coagulation mechanism. Detailed critical evaluations have been presented elsewhere and further review at this time is not appropriate. However, because it is desirable to know what the specific effects of pharmacologic agents are, and because many of the reports in which nonspecific methods were used can only be evaluated now by saying that some sort of change occurred, or apparently did not occur, a plea must be made for thorough and competent investigations of the effects of therapeutic or toxic agents upon the individual components within the clotting mechanism *by means of methods in which the limitations are fully realized.* From the standpoint of evaluating pharmacologic action it is perhaps well to recall that alterations in the existing clotting mechanism of the living organism may take place in either the coagulant phase or the anticoagulant phase. The possibilities include alteration in the rate of physiological formulation or destruction of one or more of the significant substances participating in

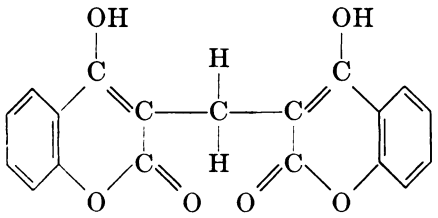
maintaining the fluid equilibrium. The same applies to anticoagulants. In the extreme, the effect may shuttle between complete removal of a coagulant and its potentiation, and between complete removal of an anticoagulant and its potentiation; of course, all combinations between these extremes are possible. Drugs may also act by entering into direct combination with a coagulant or anticoagulant or influence their release from storage depots. A brief outline of some principal mechanisms will serve to organize these viewpoints concerning the alteration of blood coagulation.

A. Coagulant effect
   1. Increased metabolic production of a coagulant.
   2. Decreased metabolic production of an anticoagulant.
   3. Direct interference with or destruction of an anticoagulant.
   4. Release of stored coagulant.
   5. Limited dilution of blood to make anticoagulants relatively less effective.

B. Anticoagulant effect
   1. Decreased metabolic production of one or more coagulants.
   2. Increased metabolic production of an anticoagulant.
   3. Direct inactivation or destruction of a coagulant.
   4. Release of stored anticoagulant.

III. 3,3′-METHYLENEBIS (4-HYDROXYCOUMARIN), DICUMAROL (BISHYDROXYCOUMARIN, U.S.P.)

Chemistry. The early history of dicumarol is associated with the observation of Schofield and Roderick (303) that the cause of a hemorrhagic and often fatal disease in cattle was the spoiled sweet clover hay on which they fed, and the demonstration by Roderick (303) that the cause of the hemorrhages was a severe reduction in the prothrombin in the circulating blood. In 1940 Link and his associates (303) not only isolated the active agent from spoiled sweet clover as a white crystalline material, but also synthesized it and described many of its metabolic effects (304, 305). This substance, 3,3′-methylenebis (4-hydroxycoumarin) has the following chemical structure:



Dicumarol is only slightly soluble in water, but forms various salts with strong alkalis that are soluble near pH 8. Its concentration in biological fluids can be measured by first extracting with heptane. After returning the drug to alkaline aqueous solution, the spectrophotometer can be used for quantitative measurements at 315 mμ (27, 503).

Metabolism. Dicumarol is effective after either oral or intravenous administration. The rate of absorption from the gastrointestinal tract is slow and varies from one individual to another and with the dosage. Six to 24 hours may be required to reach peak plasma levels. A threshold of five to ten mg. per liter of plasma must be reached before the prothrombin concentration falls, and this drop usually lags 24 to 48 hours behind the time of the peak plasma dicumarol concentration. In spite of variations in absorption there is no appreciable difference in the speed of action after administration by the intravenous as compared to the oral route. In either case, the drug is distributed to various tissues. It is found in liver, lung, and kidney, but not in appreciable amounts in the urine. Nevertheless it has been reported that the effect of the drug is prolonged and intensified by experimental renal damage (67, 449). The spleen, heart muscle and skeletal muscle contain only small amounts (503). That portion of the drug which is distributed to the plasma is almost completely bound to proteins and is removed only slowly, the time required being about 24 to 48 hours (503).

The response of different individuals to the drug varies, but seems to be the same in a given individual at different times. *Each individual is, therefore, observed when the drug is used, by analyzing the clotting status of the blood.* Individual variations in response seem not to be related to age, weight and sex, and perhaps only slightly to the diet. Interestingly, Link and his associates report that in rabbits susceptibility to dicumarol is an inherited Mendelian recessive characteristic. On continued or repeated dicumarol administration there is no development of tolerance or of increased susceptibility to the drug. Dicumarol has been given continuously for five years to rabbits (Link) and for more than three years to human beings (60) without any toxic effect.

The rapidity with which a single dose of dicumarol exerts maximal activity is a function of its quantity only when rather limited amounts of the drug are given; that is, as dosage is increased from minimal amounts, the rate of prothrombin fall increased slightly until a certain fixed slope of fall is reached, and further increase in the amount of dicumarol administered has no effect. However, the intensity of prothrombin reduction and the period required for return to normal prothrombin levels are definitely related to the amount of dicumarol administered, and are intensified by increasing amounts of the drug. If the dose is repeated on successive days, the effects will overlap and become cumulative, but the total effect will not be apparent for several days. Once a desired low therapeutic level of prothrombin has been achieved, a smaller dose of the order of 20 to 60 per cent of the initial quantity is all that is needed for maintenance of a low prothrombin concentration. Once stabilized on a maintenance dose of dicumarol, the individual requirements are remarkably constant. With common clinical therapy probably some formation of prothrombin continues and two to four days are required to reach equilibrium at about a 15 per cent level. As the effect of dicumarol subsides, the recovery of normal prothrombin levels is a somewhat slower process, requiring five to ten days (62).

The actual means by which dicumarol alters the mechanism of prothrombin formation in the liver is not known. The best estimates show that there are

about 20 mg. of prothrombin per 100 cc. of plasma (485), and there are probably no other stores available save those in the lymph. Consequently, when prothrombin production is stopped the reserves are quite limited and become depleted rapidly by unknown mechanisms. Purified prothrombin, injected intravenously into animals depleted of prothrombin by means of dicumarol, largely disappears from the blood within 24 to 48 hours (343). Dicumarol has no known direct effect on prothrombin itself, except that it tends to stabilize purified preparations *in vitro* (495) under certain conditions. It has been suggested that an altered prothrombin is produced by dicumarol, but the evidence is not conclusive because the data are capable of being interpreted in another way (290). The idea, however, has possibilities in view of the demonstration that the activity of purified prothrombin can be altered in several ways (340, 488, 493).

Attempts to gain deeper insight into the details of dicumarol metabolism have only recently been made. Lupton (319) perfused excised rat livers and showed that the livers of dicumarol-treated rats do not add prothrombin to blood perfused through them. Jaques and associates synthesized dicumarol containing C^{14} in the methylene bridge, and gave it intravenously to mice and rabbits (287, 288, 529). None of the radioactivity appeared in the expired CO_2, and no significant amounts were in the lungs. It was found in large amounts in liver, intestine, gallbladder, feces and urine. The material in the liver was identified as dicumarol itself, which shows that there is a tendency toward fixation of the drug in that organ where it probably acts to interfere with prothrombin production. The time sequence between the prothrombinopenia of the blood and fixation of C^{14} in the liver indicates a direct relationship between the presence of C^{14} in the liver and the cessation of prothrombin production. Radioactivity appeared in the urine later after injection and was confined to metabolic products of dicumarol rather than to dicumarol itself. In the mouse some C^{14} was excreted in the feces. In man it has also been found that metabolic products appear in the urine (602).

Liver. There is no liver damage produced by therapeutic or even larger doses of dicumarol. This could by no means have been anticipated, although Link (63) points out that the conclusion was already implicit in the work of Schofield and Roderick. Consequently there are many papers on the subject, but since they have been reviewed by Marple and Wright (335) and by Link (306) there is no special purpose in again marshalling the evidence for the above conclusion. There is, however, a possibility that species differences in response exist for Jansen (242) was able to produce fatty degeneration of the liver by giving large doses to rabbits; the degeneration resembled that produced by chloroform intoxication. The conclusion also depends somewhat upon what is meant by liver damage for the concept cannot be all inclusive. Fibrinogen is produced by the liver and in dogs it is possible to cause either an increase or decrease in the plasma concentration of that protein by selecting the proper dose of dicumarol (239). Likewise, Ac-globulin which is most likely produced by the liver (546) tends to be depressed when dicumarol is first administered (157); but, despite continued administration, the plasma Ac-globulin concentration returns to

normal, and its initial depression probably does not imply liver damage. It is quite possible to imagine metabolic readjustments that are reflected in temporary changes in changes in fibrinogen and Ac-globulin concentration. The cells in which the manufacture of prothrombin abruptly stops are not mechanical robots, but a kind of solution in which molecules are constructed, degraded and moved around, and perhaps the manufacture of fibrinogen and Ac-globulin is a certain kind of activity not entirely independent of prothrombin production. It is also interesting that mild trauma to the liver will cause plasma prothrombin concentration to fall (316). These viewpoints are not to be confused with the effect of liver damage, due to other causes, on the action of dicumarol (449).

New coagulation factors. The effects of dicumarol on the coagulation mechanism are apparently not restricted to prothrombin alone. The effect on antithrombin is a controversial question probably for the reasons mentioned above concerning the multiple factors which influence the antithrombin tests used to date. A group of factors are all described as being deficient in dicumarolized plasma or serum and are said to be related in some way to the activation of prothrombin. These include the following: SPCA (serum prothrombin conversion accelerator) (Alexander, 3), SPCF (serum prothrombin conversion factor) (Jacox, 240), prothrombin conversion factor (Owen and Bollman, 395), thrombotropin (Andreenko, 14), cothromboplastin (Mann and Hurn, 331), prothrombin accelerator (MacMillan, 330), proconvertin (Owren, 399), H-factor (Sorbye, 523), a new blood coagulation phase, (Copley, 119), and the factor (H-factor) in vitamin K deficient plasma which accelerates the coagulation of dicumarolized plasma (524). Possibly the aberrant behavior of Russell viper venom as thromboplastin can also be ascribed to a new factor (607). These have all been related to the conversion of prothrombin. Because further information on several of these factors is not available, comparison is hazardous, but it may be suggested that several and perhaps all of these factors are the result of different approaches to and investigations of the same phenomena. The data seem to indicate, however, that dicumarol produces an alteration in the clotting mechanism in addition to the prothrombin deficiency. Whether this alteration is a deficiency of a factor which is formed in the body separately from prothrombin or whether it is a factor derived from prothrombin in the process of thrombin formation has not been clearly established. From some of the data it is also by no means certain that the effect is not on the interaction of fibrinogen and thrombin.

Clinical application. Experience with the use of this drug has established the fact that there is definitely *a relationship between coagulability of the blood and thromboembolic phenomena and that much benefit is derived from the use of anticoagulants* (6, 32, 34, 66, 74, 75, 87, 183, 205, 212, 217–219, 282, 320, 329, 337, 338, 380, 392, 413, 414, 502, 609, 620–624). This important fact is also supported by the prior research on heparin (257, 373, 374, 521). Allen and Barker (4) have outlined the conditions in which artificially impaired coagulation of the blood is clinically of benefit, as follows: (1) thrombosis of coronary arteries, (2) thrombosis of cerebral arteries, (3) thrombosis of thrombophlebitis, (4) pulmonary embolism, (5) thrombosis of arteriosclerosis obliterans, (6) thrombosis of

thrombo-angiitis obliterans, (7) peripheral arterial embolism, and (8) venous thrombosis of non-inflammatory origin, as in polycythemia vera. Jorpes (257) has also reviewed the social and economic aspects of anticoagulant therapy.

Practical considerations. It is not the purpose of this review to discuss the practical uses of the drug. It does, however, seem appropriate to mention again that *the variable response of individuals requires that adequate control measures be instituted.* All are agreed on this point. From extensive experience several viewpoints have developed concerning the kind of analyses that should be done on plasma. Some feel that the one-stage prothrombin time on the plasma is sufficient. The chief support for this argument is the view that, although prothrombin is not measured accurately by this technic, the test reveals the over-all coagulability status of the blood and that this is precisely the information a physician needs. Others have favored the view that the prothrombin time is more properly done with the use of diluted plasma because this gives a more accurate indication concerning the concentration of prothrombin and because the inhibitors of the blood are diluted sufficiently to eliminate their influence. Since dicumarol alters prothrombin concentration it is logical that quantitative measurement of prothrombin concentration (two-stage analysis) should give a more reliable means of controlling therapy. It has also been advocated that two methods be employed simultaneously. The general viewpoint has been that the two-stage method for the determination of prothrombin concentration is too complicated and time consuming. Experience by Olwin (60) has extended over a number of years and has involved the simultaneous use of both two-stage analysis and the prothrombin time test on undiluted plasma. In his experience use of the two-stage method is not out of the question from the standpoint of convenience or cost. He found wide variations between the methods used and believes the two-stage method to be more accurate and safer, but he also obtained valuable help from the results of the simpler method.

There seems to be no definite agreement as to the exact level to which prothrombin should be reduced for best therapeutic results. Some consider that the prothrombin level should be as low as possible, but short of producing hemorrhage. The dogmatic statement (430) has been made that the hemorrhagic tendency becomes very apparent when the concentration of prothrombin falls below 10 per cent, but a review of the clinical experience indicates that this viewpoint is altogether too rigid. It has even been said that there may not be a sharp correlation between the incidence of hemorrhage and the amount of dicumarol administered or between the prothrombin time and the incidence of bleeding (423). A case of accidental over-dosage with dicumarol was studied during the recovery phase (564). There was poor correlation between the bleeding tendency and the results of prothrombin time measurements. In experimental thrombosis, with dogs as subjects, there was a tendency for thrombosis when the prothrombin level (one-stage) was above 40 per cent (367).

The problems concerned with measuring the effects of dicumarol have been numerous (35, 46, 72, 234, 235). It was difficult to find a suitable thromboplastin. There were uncertainties as to whether the results should be reported in terms

of per cent prothrombin "concentration" or in terms of clotting times. Often it was uncertain whether clotting times were being reported for diluted plasma or for undiluted plasma. Sometimes the results were adjusted in terms of control determinations and sometimes not. It has also been cautioned that a careful check of laboratory procedure might reveal errors in technic and terminology (415). It is almost surprising that all this confusion and use of empirical methods produced any definite concept at all.

Toxicity. The intravenous injection of lethal doses (dogs) resulted promptly in fatal circulatory collapse without evidence of hypoprothrombinemia (585). Splanchnic vasodilatation was observed in mice and hyperglycemia in rabbits. There was acceleration of the metabolic rate in rats and a rise of rectal temperature in both dogs and rats. Death from dicumarol uniformly occurs after a few weeks in rabbits given daily intravenous injections of 1 to 2 mg. per kg., in dogs given daily oral doses of 5 to 50 mg. per kg., and in mice and rats after ingestion of 0.01 to 1 per cent dicumarol in food (455). In other experiments in dogs with lesser amounts of dicumarol but still far larger than necessary or desirable for therapeutic purposes, the outstanding morphological changes were: (1) hemorrhages, gross or microscopic, (2) toxic lesions of small vessels, (3) acute renal glomerular swelling, and (4) toxic lymphoid tissue reaction. No necrosis of the liver or other consistent hepatic degenerative lesions were found (98). There was no effect on the extent of healing of experimental myocardial infarctions in dogs, produced by ligation of the anterior descending branch of the left coronary artery (291). In essentially similar experiments there was no effect on the electrocardiogram that could be ascribed to dicumarol (45), and no effect on the healing process or on the development of collateral circulation (65). It has been stated that the favorable effects of the anticoagulant upon coronary occlusion might be due to its action in increasing the coronary flow, but the data are not extensive (195). Capillary fragility tests show no changes when dicumarol is administered (263).

With the introduction of the drug it was inevitable that complications should be encountered from overdosage, self-medication, and other errors. The chief findings have been hemorrhage, severe headache, and bloody stools. Perhaps hematuria may be regarded as one of the first signs of impending danger from hemorrhage (91, 150, 151, 207, 302, 357, 627). A recent report based on a thorough study, but limited to one-stage prothrombin time tests, indicates that bleeding may occur when prothrombin deficiency is not great, that it may fail to occur when the prothrombin deficiency is marked, and that there is more apt to be bleeding when the deficiency has lasted for several days, the highest incidence being between the sixth and tenth day (222).

Thrombocytes. Dicumarol does not alter the thrombocyte count. It does, however, decrease the adhesiveness of platelets. The latter point was established in studies involving *in-vitro* technics (530, 619), in experimental animals (130) in which injury of the vein was produced by the technic (374) of inserting a linen thread and then crushing the intima, and by the technic (54) in which a glass seal was inserted between the carotid artery and the jugular vein.

Erythrocyte sedimentation rate. The most recent studies of this subject show that dicumarol does not alter the erythrocyte sedimentation rate (310, 605). Some earlier reports (5, 158, 414, 622) indicated that there was a definite influence which would need to be taken into account when sedimentation rate serves as a guide in diagnosis or prognosis. Five healthy male medical students were dicumarolized without influencing the sedimentation rate (403), and it appears that the evidence is decidedly in favor of the conclusion that there is no effect produced by dicumarol (280).

Reproduction. The involved situation presented during the reproductive cycle makes it somewhat difficult to obtain a clear view of the effect of dicumarol. The normal physiology of gestation itself presents some oddities which need to be kept in mind. Those who have studied the prothrombin concentration during pregnancy with the prothrombin time methods find, in general, a progressive increase in prothrombin which reaches a high peak in late pregnancy and subsides to the normal level in the early puerperium (134, 172, 175, 303, 386, 563). The same trend has been found with the two-stage assay procedure (255), but the increases in prothrombin were by no means as pronounced, thus raising the unanswered question whether the one-stage method is giving a true indication of prothrombin or whether the effect must be ascribed to some other plasma component. In the human infant the concentration of prothrombin is low at birth (80) and tends to be further depressed between the second and sixth day of life (396). With this as a background the questions which arise involve the possible effect of dicumarol on the fetus and on post-partum bleeding, and the possible transmission of dicumarol in the milk to an infant already having a low prothrombin concentration.

In experiments with dogs it was found that the prothrombin level (prothrombin time) in normal pups is lower than in adult dogs; furthermore, they are more susceptible to dicumarol than are adult dogs. The feeding of dicumarol to the mother increases the hypoprothrombinemia of the young at birth (436). In spite of the low prothrombin levels only a few of the young die of hemorrhage. In an extensive experimental study with pregnant rabbits the fetuses died *in utero* when the dose of dicumarol produced an "unsafe" level of prothrombin in the mother for as few as two days (284). When the prothrombin level of the mother was maintained at a "safe" level the newborn rabbits revealed an extreme decrease of their prothrombin with a definite tendency to hemorrhage. The mothers, on the other hand, did not experience excessive intra-partum or post-partum hemorrhage. It was found that dicumarol affects the prothrombin level of the infant to a much greater extent than that of the mother (284). Although the above data are based on animal experiments, clinicians (335, 545) advise that extreme caution is essential if and when it is necessary to use dicumarol in pregnant women. From the standpoint of puerperal thrombosis careful studies seem to lead to the conclusion that the dangers from postpartum hemorrhages are not great. In 14 patients dicumarol was given in generous therapeutic doses at the beginning of labor and the prothrombin level reached the therapeutic range in due course. The blood loss of these patients was not significantly different

from that of a control group (36). In another investigation essentially the same conclusion was reached (137).

The problem presented by dicumarol excretion in the milk was encountered many years ago. Field (175) states that Roderick suspected this effect in cattle, because a calf which appeared to be normal at birth died of hemorrhages before it was old enough to eat the spoiled sweet clover hay which was being eaten by the cow. Field conducted his own experiments with rats and concluded that dicumarol fed to the mother caused a profound hypoprothrombinemia in the pups. In another study (172) it was discovered that rats are unusually resistant to the action of dicumarol during the lactation period. It was mentioned that such animals have unusually large livers and that this might be related in some way to the resistance which these animals show. In one study with dogs there was definite indication that the milk contains a prothrombinopenic agent (436), but in another study the contrary conclusion was reached (463). Brambel and Hunter (73) and Brambel, Hunter and Fitzpatrick (76) discussed the implications for the human species, and carefully standardized prothrombin-clotting-time methods to study the prothrombin activity of the nursing infant. One-hundred and twenty-five nursing mothers received the drug in therapeutic doses and this had no effect on the infants as measured by the technics employed (73). Adamson *et al.* (1a) describe their successful use of dicumarol in pregnant patients and discuss the many factors which must be taken into account.

Miscellaneous. Dicumarol tends to prevent thrombus formation when blood is caused to sludge by experimental procedures, probably by prevention of the sludged masses of cells from becoming adherent to the endothelial lining of the blood vessel (286). Under certain experimental conditions it was found that rutin has no protective action in rats receiving dicumarol. The "defect" in the vessel walls which leads to hemorrhage as a result of dicumarol may not be the one with which rutin is concerned (108). With dicumarol the minimal requirement of calcium for optimal prothrombin time is increased (247). Animals receiving dicumarol during fever showed a tremendous increase in the prothrombin time (448). Rabbits, after dicumarol treatment, have been found to be more sensitive to intracutaneous injections of hemolytic streptococci than the control animals. It is suggested that the lack of fibrin in tissues is a factor in the spread of the infection, in contrast to the localization of the infection in the control animals (565). Clot retraction is not altered.

In some studies by Bose (69) it was concluded that the thromboplastic activity of rabbit brain is reduced by the administration of dicumarol. This conclusion was also reached in three other laboratories (370, 460, 534). Munro and Lupton (370), however, were not able to show such a reduction in activity in dog or rat brain tissue. The reviewer finds it difficult to believe that dicumarol can reduce the thromboplastic activity of rabbit brain and suggests that the data may eventually be reinterpreted.

Guinea pigs on a scurvy-producing diet respond to dicumarol with a more extensive hypoprothrombinemia and it is of longer duration than in animals on

a normal diet. The mere increase or decrease of vitamin C alone has no effect on prothrombin. It is thus considered that vitamin C has protective action against hemorrhage (42, 540). In human beings vitamin C deficiency also enhances the dicumarol effect (115) and it has been recommended that vitamin C always be administered with dicumarol (335).

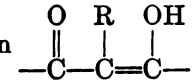
IV. COMPOUNDS RELATED TO DICUMAROL

Since dicumarol has the advantage of being equally active orally and intravenously and entails little danger of toxic side effects at therapeutic levels, search began for substances which possessed these qualities without the disadvantages of the delay in becoming effective, of the prolonged recovery period, of the need for frequently repeated testing of prothrombin levels, and of the danger of bleeding from excessive dosage. The quest for other effective compounds served the additional purpose of delimiting the portions of the 3,3′-methylenebis (4-hydroxycoumarin) molecule essential for its action on the prothrombin formation mechanism.

Link and his associates carried out an extensive program of preparing derivatives (233, 236, 531, 532, 541), degradation products (232) and analogues of dicumarol and 4-hydroxycoumarin. These were tested and evaluated on the basis of response evoked by a *single oral dose* to standardized rabbits, with prothrombin time determinations on 12.5 per cent plasma at 24, 48, and 72 hours (394). It is evident that the effect of a rapidly acting compound or one which required repeated dosage for full effectiveness could be missed under the conditions employed for the *screening* tests.

From this study it was concluded that minimal requirements for pharmacological activity are an intact 4-hydroxycoumarin residue with a hydrogen atom or carbon residue substituted on the 3 position. For maximal activity a bis arrangement is required with a 1–5 spatial relationship between the enolic hydroxyl group of 4-hydroxycoumarin and a keto group. No substance was found to be as effective as dicumarol under the conditions of the assay with selected susceptible rabbits as test animals. Furthermore, diminished prothrombin activity was found only in those analogues of 3,3′-methylenebis (4-hydroxycoumarin) and related compounds which theoretically or actually yield salicylic acid or an *o*-hydroxybenzoic acid derivative on degradation (308). For this reason and because salicylates were shown to produce hypoprothrombinemia in experimental animals (308) and man (355, 499, 501), it was postulated that dicumarol may be metabolized to salicylic acid in the body and thus induces the hypoprothrombinemic effect (303). Evidence against this hypothesis consists in the effectiveness of salicylates being only about five to ten per cent that of dicumarol (308), in the failure to find salicylates in the urine after administration of dicumarol (292), and in the fact that the hypoprothrombin effect in liver is most likely due to unchanged dicumarol (287, 288).

Similar investigation of coumarin derivatives and analogues was undertaken by Meunier and Mentzer (348–352, 354, 362) who also were unable to find a compound with activity comparable to dicumarol. Emphasizing the structural

similarity of many of these compounds to vitamin K and demonstrating that concomitant administration of the vitamin impaired their effectiveness, the above investigators proposed that dicumarol and allied compounds be called antivitamins K. Mentzer (348) suggested that the configuration  present in the active naphthoquinone, hydroxycoumarin and indandione derivatives is necessary for antivitamin K activity. Smith has emphasized that the nature of the substituent R must be defined in greater detail (512). However, Mushett and Seeler (375, 497) have demonstrated antiprothrombinemic activity on administration of 2-sulfanilamide quinoxaline, which does not bear the structure indicated above. It is, moreover, antagonized by vitamin K_1. This finding is interesting in the light of Roblin's (453) statement that not all important antagonists are structurally related to the affected metabolite. Although eventually sufficient evidence may be acquired that will show these compounds to be specifically antivitamin K, the present evidence only indicates that the effect of dicumarol and related compounds is in a general way opposed to the eventual metabolic effect of vitamin K.

Smith (511–513) has investigated various phthiocol derivatives and found that, with high dosage *in vivo*, 3-hydroxy-naphthoquinones substituted in the 2 position with a hydrocarbon chain of six or more carbon atoms induced hypoprothrombinemia in rats. Vitamin K_1 was much more effective than menadione in preventing the onset of hypoprothrombinemia. No hepatic damage was noted with these compounds.

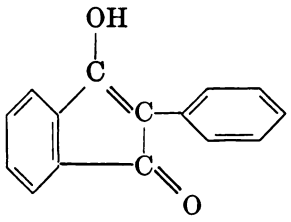
3,3'-Ethylenebis (4-hydroxycoumarin). Jansen and Jensen (243), Lehman (289), Fantl (160, 161), and Bayerle and Marx (43) also investigated substances structurally related to 3,3'-methylenebis (4-hydroxycoumarin) but found none so effective; the results, when identical compounds were investigated, are in substantial agreement with those reported by Overman *et al.* (394). Since 3,3'-ethylenebis (4-hydroxycoumarin) was found to rank closest to dicumarol in activity (about 25 per cent as active), clinical trial was made by Fantl and Nance (161) who describe a slightly more rapid action and a shorter recovery period than with dicumarol. The Australian workers (161) encountered great variations in response and a high percentage of resistance to the drug which could not be correlated with age, sex, pathological state or other treatment of the patients. Fibrinogen production was also decreased but it was not possible to ascribe this specifically to the drug because other factors may have been the cause.

Compound 63. Another substance with anticoagulant activity related to dicumarol is 2-methyl-2-methoxy-4-phenyl-5-oxodihydro-pyrano-(3,2-c) (1) benzopyran (designated No. 63) which, in rat, dog and man, appears to be two to three times more potent than dicumarol on a mg. per kg. basis (40). This 4-hydroxycoumarin was originally studied by L. D. Scheel in Link's laboratory (463a). For historical details the paper of Battle *et al.* (40) may be consulted. Optimal effects are achieved in 24–48 hours but a greater anticoagulant action

and a longer duration without hemorrhagic manifestations are claimed. No toxic lesions in the small vessels were noted, as have been reported with unduly high dicumarol doses. Vitamin K counteracts the anticoagulant action of No. 63.

Phenylindandione. Kabat, Stohlmann and Smith (266), in a study of toxic effects of indandione compounds, noted incoagulability of the blood. Because their investigations were carried out with a one-stage technic of prothrombin analysis the distinction between prothrombin and Ac-globulin changes cannot be made. Both may have been altered. Only those compounds which contained three, instead of two, ketone groups were active. These were 2-pivalyl, 2-isovaleryl, 2-propionyl, and 2-naphthoyl compounds of 1,3-indandione. 2-Pivalyl was considered to be as effective as Dicumarol. These compounds were not effectively counteracted by vitamin K (2-methyl-1,4-naphthoquinone), but in some instances and under restricted circumstances some effect was noted. Toxic effects were principally hemorrhagic with large doses, although hepatic centrolobular fatty degeneration and focal necrosis were noted. In addition to the *in-vivo* effect of these compounds, 2-isovaleryl-1,3-indandione added ***in vitro*** to plasma accelerated the deterioration of prothrombin activity.

The structure of phenylindandione is as follows:



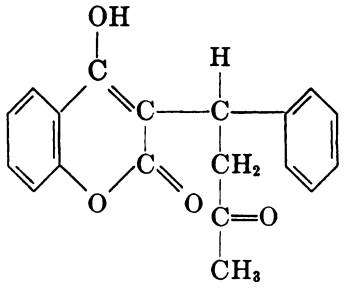
Phenylindandione was noted (353) to have a transitory effect on the prothrombin time. Soulier and Gueguen (527) further investigated this substance and later Jaques (253) and Blaustein (58, 59) and their associates also investigated its action. Phenylindandione has no effect *in vitro* and acts to lower prothrombin presumably by interfering with its formation in the liver. Ac-globulin appears not to be significantly altered, although an earlier report, corrected by the authors themselves (254), indicated a reduction in this component and several experiments of Kabat, Stohlmann and Smith (266), in which fresh serum restored coagulability to treated plasma, could be attributed to such an effect.

The rapid fall and recovery of plasma prothrombin contrast phenylindandione with dicumarol. Immediately after its administration, Jaques *et al.* (253) noted an unexplained prolonged prothrombin time lasting two to three hours. The major prolongation after a single dose begins at 10 hours, reaches a peak at about the 24th hour and returns to normal levels by the 40th hour. A single dose is much less effective than repeated administration at eight-hour intervals. On this schedule the drug is as effective as dicumarol in maintaining a prolonged prothrombin time. Recovery time after repeated dosage is of the order of 48–72 hours.

Phenylindandione is equally effective by oral and intravenous routes; it is excreted by the kidney (253). Vitamin K (2-methyl-1,4-naphthoquinone) in moderate doses does not alter the response to this drug. Individual variation in response has been noted, and resistance to it was seen in three of Blaustein's 53 patients (59). In many cases marked swings in prothrombin time values were noted.

Overdosage results in a hemorrhagic tendency, correctible within 24 hours by withdrawal of the drug. Toxic renal damage (mild fatty degeneration particularly in the loops of Henle) was noted after very large doses. Polydypsia, polyuria, dryness of the mouth and tachycardia have been noted (527) but these effects have not been confirmed (59). Therapeutic levels of the drug produce no change in erythrocytes, leukocytes, platelets, blood sugar, cholesterol, total protein, serum albumin or globulin, fibrinogen, sedimentation rate, hippuric acid formation, icteric index, van den Bergh reaction or bromsulphalein excretion.

3(α-acetonylbenzyl)-4-Hydroxycoumarin. This compound has the following structure:



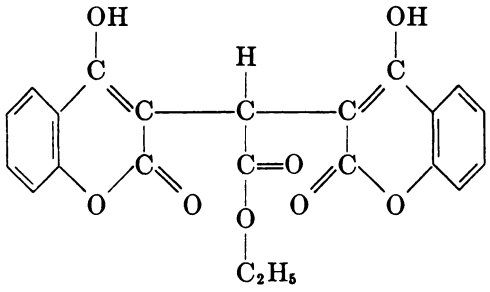
It was originally called compound 42 and is now known as warfarin (454a). This compound was one of about 300 appraised by Link's group, with the standardized rabbit assay (394). This study was later continued and the chemical was found to be of special interest because of species specificity in susceptibility to its action. It was found that the rat is especially affected and this suggested to Link and his associates (63) that the chemical might be useful in the control of rodents. In extensive field tests it has been found that the compound shows much promise as a rodenticide. The habits of this animal make it difficult to devise a particular bait which will not cause them to shy away. This apparently is not a difficulty with warfarin. Furthermore, the relatively low toxicity for other animals makes it uses in rat extermination relatively safe for those species which man desires to preserve.

3,3'-Carboxymethylene bis-(4-hydroxycoumarin) ethyl ester. In the report of Solomon (522) and associates it is stated that this compound was synthesized by Rosicky. It has been studied extensively in the laboratory and in the clinic (55, 84, 86, 139, 140, 142, 144, 192, 193, 268, 269, 271, 277, 297, 381, 426, 447, 480). The work of von Kaulla and his associates was especially thorough and

extensive. The early clinical work was by Réinis and Kubik (447), Della Santa (129) and de Nicola (381).

Like dicumarol, this drug, called tromexan, acts by impairing the capacity of the liver to produce prothrombin. It has been stated that Ac-globulin is also lowered in concentration at the beginning of administration (480), but the main effect is apparently on prothrombin (142). As compared with dicumarol the response is more rapid and the return of the prothrombin time to normal is more rapid. Some observations in human beings are tabulated in Table 1.

Pulver and von Kaulla (426) found that tromexan unites with diazotized *p*-nitraniline at pH 6.0 to form a yellow dye and this is the basis of a quantitative color reaction. In the experimental animal and in man tromexan appears in the blood about as rapidly as dicumarol after oral ingestion, but it disappears more rapidly (36 hours vs. five days) (193). When its repeated administration is stopped, the normal prothrombin time is again reached in 48 hours, and the drug is no longer in the blood in detectible quantities after 36 hours. A certain minimum blood concentration is necessary to prolong the prothrombin time. Pulver and von Kaulla (426) found that, in the rabbit at least, the drug is partially eliminated unchanged in the urine, to a large extent as inactive tromexan acid A and to some extent as tromexan acid B. The structure of tromexan is as follows:



In the absence of pre-existing hepatic disease there is no effect on liver function (83, 84, 139, 447). However, pre-existing liver damage will alter the response to the drug and renal insufficiency prolongs its action (297) but the drug causes no impairment of renal function (83, 139, 447). There is no effect on red or white blood cell count (83, 84), sedimentation rate (83, 447) or platelet count (139). A transient fall in blood fibrinogen has been recorded (139).

Tromexan has a very favorable potency/toxicity ratio. Von Kaulla and Pulver made extensive toxicity experiments (271). The oral LD_{50} after a single administration is approximately 750 mg. per kg. for mice and 1000 mg. per kg. for rabbits. After daily feeding of 100 mg. per kg. to mice death usually occurs within 26 to 51 days. At autopsy the above investigators found fatty infiltration of the liver and, less frequently, of the kidneys. In rats prolonged administration of large quantities also caused fatty infiltration of the liver. When the above toxic quantities are compared with the therapeutic doses in Table 1 it can be seen that there is a wide margin of safety.

V. SALICYLATES

The chemical similarity between salicylate and dicumarol and the discovery that salcylic acid was the primary degradation product of 3,3′-methylenebis (4-hydroxycoumarin) suggested to Link (303) that dicumarol might owe its effectiveness *in vitro* to conversion to salicylic acid. Investigation of this hypothesis (308) showed that salicylates under certain conditions produce hypoprothrombinemia. These conditions apparently were species susceptibility, limita-

TABLE 1

Effect of a single dose of tromexan on prothrombin time in normal individuals

AUTHORS AND REFERENCES	TROMEXAN DOSAGE	PROTHROMBIN-TIME			METHOD OF DETERMINATION
		Detectable fall	Maximum fall	Return to normal	
	mg.	*hrs.*	*hrs.*	*hrs.*	
Reinis and Kubnik (447)	900	1–2	12–24	48	Legler's modification of Quick's method
Della Santa (139)	Not specified	3	20–24	48	Quick's method modified by Jeanneret
Della Santa and von Kaulla (140)	300–450	Not specified	24	48	Not specified
Bickel and Della Santa (55)	600	3 hrs. (10–15% decline)	24	48	Not specified
Deutsch (142)	150–1800	6–10	30–36	36–72	Not specified
Burt *et al* (86)	1200	8 hrs. (40% decline)	8–24	Average 90% at 48 hrs.	Not specified
Lian *et al.* (297)	600–1200	2	4	9	Not specified
Burke and Wright (83)	1200–1800	12	28–36	60–72	Link-Shapiro modification of Quick's method
Solomon *et al.* (522)	1200	8	10	24	Quick's method

tion in vitamin K availability and administration of sufficiently large amounts of salicylate.

Rats, human beings and rabbits appear to be more susceptible than dogs to the hypoprothrombinemic effect of salicylate (107, 308, 442). The suggestion that this low activity in dogs is due to rapid excretion (208) was investigated by producing anuria with uranium acetate in two dogs (176). In these animals salicylates alone were still unable to produce a hypoprothrombinemia. In dogs, salicylate effects were detectable only if production of prothrombin was concomitantly reduced by dicumarol administration. In rats on a low vitamin K diet (308), however, the salicylates do reduce the prothrombin level. This vita-

min K restriction was regarded as essential to the effectiveness of the salicylates. The dog, in contrast to the rat, is an animal in which vitamin K deficiency is produced only with considerable difficulty. Thus it seems possible that the variation in species response to salicylates may be related to the state of vitamin K metabolism and storage. In rabbits (44, 307, 442) and human subjects (33, 89, 96, 138, 164, 215, 355, 397, 442, 499, 505), hypoprothrombinemia has been found without restriction of vitamin K. However, the reductions reported have generally been less than to 50 per cent of normal.

It is interesting to note that Jaques and Lepp (248) found that intravenous administration of salicylates to rabbits caused no alteration in prothrombin time, while oral administration resulted in a prolongation. After the oral administration of sulfasuxidine, the oral administration of sodium salicylate did not affect the prothrombin time. They suggest that salicylate may be converted to dicumarol or to a substance with similar prothrombinopenic properties by bacterial action in the intestinal tract. Exactly opposing results were reported by Meunier *et al.* (352) who found intravenous but not oral administration to be effective. In the rat only a time difference was noted in comparing the effectiveness of the two routes (308).

Large amounts are necessary to reduce the prothrombin, but these doses may be within the therapeutic range (111). Repeated doses are more effective than a single dose, the maximal effect being reached in about 48 hours (164, 355, 442) and followed by a gradual return toward normal prothrombin levels even under continued salicylate treatment (89, 164, 397).

The possibility that not all salicylate compounds are of equal activity may account for some of the variation in reported results. Acetylsalicylic acid, methyl salicylate, sodium salicylate and salicylic acid are the compounds most often used. Barclay (33) has reported on the administration of sodium paraaminosalicylate (PAS) and indicated that prothrombin times have a tendency to prolongation but none was below 70 per cent of normal. Link (307) observed acetylsalicylic acid to be the most potent among some 60 salicylates tested. Salicylic acid proved to have about five per cent of the activity of dicumarol. The testing methods, however, are subject to the same qualifications discussed previously regarding the evaluation of compounds chemically allied to dicumarol. Clark and Spitalny (107) found that equimolar doses of sodium salicylate, methyl salicylate and acetylsalicylic acid have about the same activity.

The metabolic state of the recipient may well have considerable effect upon the response to the salicylates. The role of vitamin K has been mentioned earlier. The prothrombinopenic action of sodium salicylate was greatly augmented by experimental hyperthermia induced by yeast injection or high environmental temperature, by hyperthyroidism and by daily alcohol administration (107). From these experiments on rats it was suggested that increased metabolic activity enhances the effect of salicylates.

The relationship to vitamin K was further probed. Link and coworkers (308) demonstrated that co-administration of synthetic vitamin K and salicylates would prevent the prothrombinopenic effects of the latter. Others observed the

same effect (172, 355, 501, 505). Approximately one mg. of menadione will counteract the prothrombin-reducing action of one gm. of acetylsalicylic acid (505). The correction of hypoprothrombinemia can be achieved without altering the plasma level of salicylic acid (629).

In discussing the mechanism of salicylate action, Field, Spero, Link and others (176, 308) have emphasized that salicylates act only when the vitamin K function is impaired and that these agents have a low affinity on a competitive inhibition basis, compared to dicumarol, for the site of vitamin K action. They believe that the effect of salicylic acid on prothrombin synthesis is at a site somewhat different from that of dicumarol. They also suggest that there may be a direct intravascular effect which produces inhibition of prothrombin.

Previously Link *et al.* (308) had indicated that there was no *in-vitro* effect of salicylates. Stefanini and Petrillo (535) showed a retardation of prothrombin time when the salicylate concentration was greater than 200 mg. per cent. A deleterious effect on purified prothrombin was noted at high salicylate concentrations by Seegers, Loomis and Vandenbelt (495). The possibility of a direct effect is not excluded.

All investigations of *in-vivo* salicylate effects were carried out by one-stage technics for the determination of "prothrombin time". The use of whole plasma testing as described by Quick (435) was found to be relatively insensitive to the degree of change produced by these compounds (303) so that 12.5 per cent plasma was most extensively employed. Because the one-stage tests utilized here are susceptible to alteration in Ac-globulin as well as prothrombin, the change produced by the salicylates cannot with certainty be ascribed to reduction in either substance or in both. The prolongation of "prothrombin time" cannot be interpolated to mean a hypoprothrombinemia. Reports of salicylate effects based on more specific methods have not yet appeared.

Salicylates in sufficient dosage and under proper metabolic conditions do interfere with the coagulation mechanism (15, 109, 116, 117, 194, 199, 308, 379, 456, 537, 575). Other pharmacologic aspects of the salicylates have been reviewed by Smith (517).

VI. METHYLXANTHINES

Interest in these compounds was stimulated by the work of Link and his associates (303). When they were isolating dicumarol it was noticed that the concentrates were far more potent when freed of purine bodies. This increase in potency was out of proportion to what was expected on the basis of simple removal of presumably inert materials. They state that the literature as early as "1896 expressed the thought that caffein evoked the development of a coagulative ferment." Further pursuit of this problem supported the conclusion (174) that the methylxanthines, caffeine, theobromine and theophylline, given orally, induce a hyperprothrombinemia in the dog, rat and rabbit. These drugs could lessen the hypoprothrombinemic effect of dicumarol, and this action was not shared by other purines and was not thought to be vitamin K-like. They suggested that the methylxanthines might exert functional stimulation of hepatic

tissue, but in a later communication Link (60) fully clarified his position by stating that this is only a suggested explanation and that the term "hyperprothrombinemia" is quite expendable. In the main their work has withstood the test of time, but there are some aspects which require further consideration.

In man, as early as 1920, Nonnenbruck and Szyszka (385) found that methylxanthines caused a definite shortening of the clotting time of the whole blood. It was suggested that they might be used to aid the hemostatic mechanism when desirable. This report caused Addicks (1) to do studies on man and the original work was confirmed. In rabbits a reduction in whole blood clotting time was also found (344). Apparently the bleeding time in rabbits is also reduced (510). In man Scherf and Schlachman (465) found the whole plasma coagulation time to be shortened. However, Blood and Patterson (64) found no change in the whole blood coagulation time in man. This disagreement makes it difficult for a reviewer, for the overwhelming evidence indicates that the whole blood clotting time *is reduced* by these drugs. Blood and Patterson do not quote the prior literature and did their tests at the end of one hour, whereas the main effect apparently comes in about three hours. Their test subjects were also not in good health. Nevertheless they have the support of another study (196) in which the capillary tube method was used for studying whole blood clotting time.

In 1945 Quick conducted experiments in dogs (434) and found no evidence that caffeine, theobromine and theophylline, fed in large doses, increase the prothrombin level of the blood. The drugs also did not antagonize the action of dicumarol. He suggested that his results differed from those of Link and associates because the latter determined prothrombin times with the use of 12.5 per cent plasma. This explanation, as will be seen later, is not adequate. In human patients receiving large therapeutic doses, the various prothrombin time methods give different results (64, 77, 196, 224, 391, 451, 465) but for the most part there are no changes observed. Positive evidence was obtained when Russell viper venom was used as thromboplastin (465). The significance of this is not clear, and it would seem to require further study. From a study conducted with a critical attitude toward methods, it was also concluded that hyperprothrombinemia is only apparent (383). If one assumes that these methods give essentially negative results with human plasma, it is necessary, in view of the results on whole blood clotting time and other experiments to be discussed below, to conclude that they are not adequate for disclosing what the methylxanthines do to the clotting mechanism. Olwin (60), who has had extensive experience with blood coagulation methods and dicumarol therapy, states categorically that human beings require much more dicumarol when they are also receiving methylxanthines.

In animal experiments Honorato (227) was able to show that the administration of caffeine, theobromine and theophylline and sodium benzoate causes an increase of his plasmatic co-factor of thromboplastin (Ac-globulin) (226). During the same year and independently, McCormick and Young (342) showed that Ac-globulin concentration shows a marked and persistent rise when aminophylline is given to dogs in large doses. They used the quantitative method for Ac-

globulin analysis devised by Ware and Seegers (596). There was no shortening of the whole blood coagulation time which is no surprise because it is normally very short in the dog. They found the prothrombin concentration as measured by two-stage methods (592, 596) to be elevated slightly, after which it was lowered somewhat below normal before returning to normal. By these more specific methods they were thus able to uncover a complex situation in which an elevated Ac-globulin concentration was the predominant feature. Their work is in agreement with that of Honorato (227). Ac-globulin is an accelerator of prothrombin activation. We can thus conclude that the methylxanthines csaue an increase in Ac-globulin concentration and thus indirectly cause prothrombin to be more effective.

It has been shown that caffeine, theophylline and theobromine increase the fibrinogen concentration in the dog and also in the rabbit. In the latter animal some related drugs were also effective (177), namely, xanthine, adenine, uric acid, guanidine and glycocyanine. The rabbit is far more susceptible than the dog to the methylxanthines.

VII. ANTIBIOTICS

The antibiotics have been investigated with respect to alteration in the coagulation mechanism. Ochsner (315) has suggested that the failure to reduce the incidence of thromboembolism in recent years, in spite of improved therapy, may be due to a deleterious effect of the antibiotics. This view has incited additional commentary (152, 315, 437). The available evidence deals primarily with penicillin.

The initial report of Moldavsky *et al.* (361) indicated a lowering of the coagulation and bleeding time following oral and intramuscular penicillin administration. Also non-retractile clots were observed. Macht, in several articles (325–327), reported a reduction in the coagulation time in rabbits, cats, and a few dogs and human beings and emphasized the "thromboplastic properties" of penicillin. Comparing amorphous and crystalline penicillins X, K, G and F, he reported a decreasing effect in that order. The evidence, in fact, for any effect of penicillin G and F is equivocal. The range of dosage used was wide and intramuscular and intravenous routes of administration did not alter the effect. Mosonyi, Palos and Komaromy (365, 366) have supported this *in-vivo* effect of penicllin in reducing the clotting time.

In contrast to the above reports are the investigations of Lewis (295) who followed the coagulation time and, in addition, measured the prothrombin time, capillary bleeding time, clot retraction, platelet count and plasma fibrinogen level. She found no effect after oral doses up to 1,500,000 units per day or single intramuscular doses up to 200,000 units. Similar results were reported after administration of 300,000 units of crystalline penicillin G in oil and beeswax, and there was no change in heparin tolerance curves (148). Hines and Kessler (220) similarly reported no changes in platelet counts or prothrombin times but did find an increased sensitivity to heparin in two patients treated with penicillin. Hypoprothrombinemia has been attributed to penicillin by Lewitus (296) and

Astrup (28), although the latter's three cases appear to have been exceptional and are regarded by the author as manifesting hypersensitivity. In summary of the *in-vivo* effects of penicillin, it appears that the antibiotic causes no major change in the coagulation mechanism within reasonable dose ranges. The possibilities must be reserved that factors not yet measured may be altered and that the various types of penicillin may differ in the responses they cause.

The *in-vitro* effects of penicillin are somewhat clearer. Lewis (295) reported no change up to 500 units per cc. of blood. Fleming and Fish (181) found that concentrations over 3000 units per cc. retarded clot formation and retraction. This inhibiting effect of a very high penicillin concentration has been confirmed by Menghini (347), Mosonyi *et al.* (365) and Strausz (539). Mosonyi (366) suggests that this effect is due to an alteration in fibrinogen, involving —SH groups.

Streptomycin has also been implicated by Macht (325–327) for its "thromboplastic properties," that is, the coagulation time was shortened following administration of the antibiotic. Overman and Wright (390) found a coagulant action under special *in-vitro* conditions. Opposing results (97, 378) demonstrated a retarding effect *in vitro* and no effect *in vivo*. An additional report on intravenous streptomycin administration to rabbits, guinea pigs and cats showed no change in blood coagulation time up to 1 mg. per kg., a decrease in coagulation time after 5 to 40 mg. per kg., and an increase after 50 mg. per kg. (149).

Aureomycin has been investigated by Harned *et al.* (211), Ross *et al.* (459), Lasser *et al.* (317), Shapse and Wright (507) and Herrell (214), who found no significant alteration in coagulation time, prothrombin time, bleeding time, or *in-vitro* heparin tolerance test. Two reports which are contradictory partially disagree with these findings. While agreeing that the prothrombin times are not appreciably changed, Macht and Farkas (328) maintain that the coagulation time is markedly shortened; Galt and Hunter (189) maintain that it is prolonged.

Unfortunately our knowledge of the effects produced by antibiotics on the coagulation process has progressed very little. Most of the reports in favor of an effect do not convince the critical reader. It is plausible that the antibiotics may alter the clotting mechanism, but specifically which mechanism is involved? The matter warrants more thorough investigation.

VIII. PROTAMINES

The action of protamines on the blood coagulation mechanism is complex and it is perhaps profitable to discuss their nature first. In biological materials they are associated with nucleic acids (283) as salt-like compounds. As prepared in the chemical laboratory these proteins have strongly basic properties due to their high arginine content. A preparation of salmine, derived from salmon sperm, contains 58.9 per cent arginine (163). The normal pH of the blood is below the isoelectric point of protamine, and thus the protamines have a charge opposite to that of the plasma proteins themselves.

In 1900 Thompson (562) studied several protamine preparations and found

that their intravenous administration may cause a drop in blood pressure, prolongation of the clotting time of the blood, and a reduction in the leucocyte count. After high doses death resulted. In later work Jaques (246) showed that the fall in blood pressure was due to dilatation in the arterioles of muscles. He also found that salmine added to blood in silicone-treated containers caused thrombocytopenia but not leucopenia. Attempts to produce local reactions in the skin at the site of intracutaneous injection were negative. It was also not possible to produce hypersensitivity to protamine in the guinea pig (276).

Shelly, Hodgkins and Visscher (508) studied the toxicity of protamine in the guinea pig and the rat; administration by the intravenous route produced death at a dose level of 6 to 12 mg. per 100 gm. Intraperitoneal injection delayed the lethal effect. When protamine was added to whole blood in the test tube, the cells of the human being, dog, cat and rat were agglutinated. Perfusion of blood through isolated organs was blocked by protamine probably because of embolic vascular phenomena. Other investigations (581) showed the median lethal subcutaneous dose in mice and rabbits to be 200 to 300 mg. per kg. Other untoward effects include respiratory distress, muscular rigidity, marked malaise, polyuria, hyperglycemia, and hemorrhages in the thyroid, lungs and kidneys. Oxygen consumption of minced rat liver is reduced by protamine (446).

The addition of sufficient protamine to blood plasma causes fibrinogen to precipitate (376). This precipitate is soluble in 3 per cent sodium chloride solution. Much of the fibrinogen lyses after incubation of the precipitate. This is probably due to the fact that the precipitate contains, besides fibrinogen, practically all the profibrinolysin of the plasma (203). The precipitation of fibrinogen from human plasma by protamine is also a reversible process for another reason (162). Protaminase, an enzyme present in the plasma of a number of animals, will digest the precipitate. Protamine is also thought to act on prothrombin in test tube experiments (168) but apparently this view has not been put to a test by the use of purified prothrombin. Phosphatides, such as cephalin, form water-insoluble salts with protamine (100). In this manner a lipoprotein such as thromboplastin would be inactived by protamine; Tocantins (572) was able to show that an appropriate amount of protamine could be added to plasma to give an effect which seemed antithromboplastic. He also found that protamine, in certain concentrations, exaggerates the antithromboplastic and anticephalin activity of hemophilic plasma, but not to the same degree as that of normal plasma.

The work of Chargaff and Olson (102) showed that an anticoagulant such as heparin can be inactivated *in vivo* with protamine. This is owing to the combination of the basic protein with the acidic heparin to form a stable salt. This reaction has been studied extensively by many others (245). The action of protamine sulfate, when given intravenously to the heparinized patient or animal, returns the clotting time to normal almost immediately. For use in man protamine is freed of pyrogens (262). The practical use of protamine in the neutralization of heparin developed quite rapidly, despite the natural hesitation stemming from the available knowledge concerning its toxicity (51, 125, 251, 261, 407, 444). The material is given slowly by the intravenous route. A typical commercial

preparation consists of a water-clear, one per cent solution of salmine sulfate. One mg. of standard heparin (Connaught Laboratories) is neutralized by 1.28 mg. of protamine, but the figures in the literature for neutralizing a given quantity of heparin *in vivo* are not consistent, probably because heparin itself shows variations in composition. Jorpes, in pioneer work, for example, states that 60 mg. will neutralize 100 mg. of heparin *in vivo* in the human being and that 40 to 50 mg. will neutralize 75 mg. of heparin. The quantity of protamine required for neutralization in the heparinized patient diminishes rapidly with the elapse of time after the prior administration of heparin. If administered immediately after heparin, the amount of protamine required is approximately mg. per mg. that of the amount of heparin, whereas 30 minutes later often less than one half the dose of protamine is sufficient to neutralize the effect of heparin (6a, 6b). The duration of the antiheparin action of protamine sulfate is about two hours. The last traces of heparin are removed very slowly (548).

The quantity of protamine which may be given safely and with best results would presumably depend upon the amount of anticoagulant requiring neutralization. Consequently, and for lack of extensive experience, only examples can be cited. Allen and associates (10) have given 150 mg. intravenously over a period of several hours as the initial dose. In addition 50 mg. were given intramuscularly every four to six hours. Occasionally pain at the site of intramuscular injection has been reported. In cases of leukemia Hill (216) has administered as much as 250 mg. dissolved in fresh blood.

A protamine titration method has been devised for a rough estimate of heparin or heparin-like anticoagulants in the blood. This becomes of interest in conditions such as total body exposure to ionizing radiation (7, 11, 12, 598), the bleeding tendency of thrombocytopenic purpura (9, 408) and the leukemias (573), where heparin or heparin-like materials might be involved. The therapeutic use of protamine, while advocated by some, would seem to require further study, for the results of others have been disappointing. The above named conditions seem to be accompanied by an increase in plasma concentration of heparin or of compounds not easily distinguished from heparin. By contrast normal plasma apparently is almost free of heparin.

IX. TOLUIDINE BLUE

This dye was used by Jorpes and associates (257) to locate the physiological occurrence of heparin in the mast cells. Toluidine blue (dimethyl-orthomethylthionin) shows only slight metachromasia with heparin test tube experiments (250). Some samples of the dye, usually old, show considerable metachromasia. A shift in the absorption spectrum of toluidine blue occurs, and in this respect it is then indistinguishable from azure A (250). Variations in the dye from one sample to another have apparently been encountered quite frequently. Like proteins the dye combines with heparin to form a stable compound. Quantitative measurements show that the reaction follows the mass law (245).

The properties of toluidine blue make it of interest in blood coagulation problems in much the same manner as described for protamine above (7, 9, 10, 12,

225, 230, 250, 407, 408). Protamine will rapidly neutralize an overdose of intravenously injected heparin. Toluidine blue is useless in this respect because of its slow action in neutralizing heparin. On the other hand, the antiheparin effect of toluidine blue, even though slow, can be detected for nearly 24 hours after an initial injection of 4 mg. per kg. in dogs. Allen and associates (9) state that the dye, dissolved as a one per cent solution in 250 to 500 cc. of sodium chloride solution and sterilized by filtration, is well tolerated by human beings in the dose range 1 to 4 mg. per kg. The solution was given slowly intravenously. The toxicity of toluidine blue has apparently not been studied extensively. In dogs but not in man it is strongly hemolytic and causes leucocytosis and thrombocytosis. In man the urine and stool become highly colored and remain so for 36 to 48 hours after a single intravenous dose of 2.0 to 2.5 mg. per kg. The dye is not active when given by mouth (9).

A curious and as yet ill-defined clotting disturbance occurs in patients with certain bleeding disorders associated with, if not caused by, a number of pathologic states. This disordered clotting mechanism is characterized by a prolonged clotting time, a slow rate of fibrin formation, an increased "protamine titration" and clinical evidence of abnormal bleeding (6a, 6b). In some instances, evidence of a clotting inhibitor can be detected. In some respects this inhibitor resembles heparin but in other respects it appears singularly different, with the result that no conclusion as to its nature can be reached at present. The evidence which suggests its heparin-like nature is based solely on the fact that bleeding in many of the patients with the above described syndrome improves after the intravenous administration of toluidine blue, 4 to 5 mg. per kg., for two to four days. The syndrome has been observed in certain cases of menorrhagia, upon estrogen withdrawal in man and dog, in some patients with leukemia, in some patients and dogs exposed to lethal dosages of ionizing irradiation, and as a complication of aminopterin therapy. Some of these conditions are associated with thrombocytopenia, but platelet deficiency alone does not appear responsible for the particular pattern of events described. While toluidine blue is a useful agent in properly selected patients in aiding the control of bleeding, its mode of action in these instances is not known.

X. THE CARDIAC GLYCOSIDES

It is especially difficult to organize the conflicting literature on the effect of digitalis on blood coagulation. The main trend seems to indicate, almost without exception, that there is an accelerating effect of digitalis on blood coagulation when the rabbit is used as the experimental animal. The same is apparently true of the effect of digitalis in the cat, but the experiments with this particular species are limited. This applies also to the dog. With regard to the human being, it is difficult to escape the conclusion that the clinical evidence indicates a predisposition towards thrombosis when digitalis is used. Laboratory work done on human blood is, however, conflicting. There are some trends toward negative results when the criterion of measurement is the prothrombin time or whole blood clotting time. Evidence of hypercoagulability seems to be obtained when

extreme care is taken with the technic of determining whole blood clotting time. These generalizations will be examined in more detail.

Apparently the first observation was that of Tanaka (557) who showed that strophanthin accelerates the coagulability of rabbit blood. In work by Field *et al.* (177), lanatoside C and digitoxin administered to rats and rabbits had an accelerating effect on coagulation. There was, however, no effect on the prothrombin time of whole or diluted plasma and there was no antagonistic effect on dicumarol. In another study tincture of digitalis (5 ml. per kg.), digitoxin (0.5 mg. per kg.) and strophanthin (0.1 mg. per kg.) injected into rabbits accelerated the coagulation time of the plasma. It was suggested that the accelerating effect of these drugs is due to the mobilization of prothrombin from the liver because hepatectomy in dogs prevents the effect (542). In another report of the reduction of the coagulation time of rabbits' blood by digitalis, the number of animals was small and the results were not especially striking (603). In the rabbit and the cat digitalis was found to have a pronounced effect in decreasing the coagulation time of the blood (424), and it is recorded by Honorato and Lopetegui (229) that in experiments with rabbits digitalin has a definite effect on blood coagulation. They also record that ouabain, *Digitalis lanata* glycosides, cholic acid or coumarone shortened the prothrombin time as determined on diluted plasma. Honorato and Gomez (228) produced experimental thrombosis in rabbits very readily after pretreatment with digitalis glycosides. A small dose of dicumarol prevented this quite readily. Honorato and associates believe that the decreased prothrombin time observed with diluted plasma may be explained on the basis that the plasmatic co-factor of thromboplastin is increased in concentration. It has already been suggested above that Ac-globulin and the plasmatic co-factor of thromboplastin may be one and the same substance. Consequently it may be that an increase in Ac-globulin concentration of rabbit plasma is caused by the glycosides of the digitalis series.

In experiments in cats, digitalis seemed to shorten the coagulation time of the blood (323). Other experiments by Macht tend toward the same conclusions (324, 326).

Observations on human beings are equivocal. In answer to the title of an article, "Is digitalis indicated in mycocardial infarction?", it is stated that digitalis administered alone for congestive heart failure associated with auricular fibrillation and myocardial infarction would seem to be contraindicated (16). In another study (424) therapeutic doses of digitalis in patients with complicated cardiac decompensation did not significantly affect the coagulation time of blood but it was suggested that, in cases of cardiac decompensation complicated by devitalized tissue due to infarction or trauma, the administration of digitalis may increase the danger of thrombus formation and embolism. It was suggested that this is due to the release by digitalis of an intracellular clot promoting factor—perhaps thromboplastin. Peters, Guyther and Brambel (414) found that digitalization for congestive heart failure in patients with coronary thrombosis increases the incidence of thromboembolic complications and that the hazard could be nullified by dicumarolization of such patients. de Takats *et al.* (554)

point out that it has always been a source of speculation why patients whose auricles have fibrillated for many years suddenly begin to throw emboli, and they state that no attention has been paid to the coagulation system of the blood and that digitalis is received by many such patients. They found heparin to be less effective in the presence of digitalis and while, as they say, there are no data as to the mechanism it must mean that somewhere in the coagulation system digitalis opposes heparin. They also cite a case in which digitalis showed an antagonistic action toward dicumarol. Additional comments by other investigators urge that more careful consideration be given to the possible effects of digitalis on blood clotting (237).

Pere (411) reviewed the literature extensively and made a thorough attempt to ascertain the nature of the influence of digitalis on blood coagulation in studies in human patients. Like many others he investigated the validity of the whole blood coagulation time in man and included a study of the influence of CO_2 and O_2 tension and of venous stasis. The absolute values of blood coagulation time may differ from one person to another, but significant information can be obtained when the determinations are all made by the same person and with careful technic. This idea would seem to be substantiated by those who have developed extraordinary technical skill. Of course, it is another matter when the species being studied has a very short coagulation time. Digitalis shortened the coagulation time in patients with congestive heart failure. On the other hand this did not occur in normal healthy control subjects. Strophanthin shortened the coagulation time in patients with congestive heart failure more markedly than did digitalis; in control subjects no shortening was observed. The mercurial diuretic, novurit (trimethyl-cyclopentane-carbonic-acid-allyl-amide-methoxy-carbonic-acid) and 0.05 gm. of theophylline, also reduced the coagulability time greatly in patients with cardiac insufficiency. In general it seemed that all the drugs that cause rapid disappearance of edema accelerated the coagulation time in cases of congestive heart failure. In control subjects only novurit caused an acceleration of coagulation in which case diuresis was associated with the phenomenon. The prothrombin time failed to show any changes after digitalis, strophanthin or novurit, either in control subjects or in patients with congestive heart failure. The same was true for fibrinogen. In patients with congestive heart failure digitalis increased the daily volume of urine on an average of two-fold, strophanthin by nearly three-fold and novurit by about four-fold. It is suggested that the effect of the drugs is, therefore, not specific but due to hemo-concentration. It is further suggested that as hemo-concentration increases tissue fluid is drawn into the circulating blood and that this increases the thromboplastin content of the blood and so shortens coagulation time. These suggestions extend beyond the limits of the observations, for one must question whether thromboplastin enters the circulation in this manner. It is also known from the work of Tocantins that slight dilution of normal blood in test tubes favors coagulation, most likely because dilution places the inhibitors of coagulation at a greater disadvantage than the activators. Certainly Pere's fine work has indicated possibilities not adequately considered previously.

A number of other workers who have studied the effect of digitalis on human beings have come to the general conclusion that there is no significant effect. In one study, ten patients given digitalis showed no significant alteration in the coagulability of the blood (520). Sutton (544) found no change by the Lee-White method, in the heparin tolerance curve or in the prothrombin times in subjects given digitalis. Likewise Cathcart and Blood (99) found no statistically significant changes in either clotting time or prothrombin time following the administration of digitoxin, lanatoside C or digitalis to patients having congestive heart failure or to normal subjects. Levin and Ruskin (293) also came to similar conclusions.

Moses found no change in the clotting time and no change in response to heparin following intravenous administration of digtalis (364). Almost completely negative experiments with dogs are recorded by Ramsey, Pinschmidt and Haag (441) with the exception that there was a decrease in coagulation time under certain conditions of anesthesia (441). In other reports some patients showed a shortening of the coagulation time and in some it was prolonged (237, 556).

XI. ALPHA TOCOPHEROL COMPOUNDS

Naphthotocopherol was synthesized by Tishler and Evans (570) and was found to have both vitamin E and slight vitamin K activity. Its vitamin K activity was ascertained by bioassay in chicks. In a study of inhibition by structural analogs of metabolites, Woolley (617) found that alpha tocopherol quinone produced manifestations in mice related to those seen in tocopherol deficiency. The administration of vitamin K prevented the resorption of the embryos and the excessive vaginal bleeding which accompanied the resorption process. While the hemorrhagic manifestations produced by alpha tocopherol quinone could be reversed by vitamin K, estimations of the prothrombin time were essentially the same as for the controls so that a decrease in prothrombin concentration probably was not one of the factors associated with the hemorrhage. The author (617) points to the structural similarity of alpha tocopherol, alpha tocopherol quinone and vitamin K_1. Descriptions of vitamin E deficiency have usually included mention of hemorrhagic manifestations (336). Except in chicks on a diet which was probably deficient in vitamin E, hyaline, capillary thrombi were found in and about the necrotic areas of the cerebellum. These thrombi were almost invariably found within and about degenerated areas but it was not possible to present proof that the capillary thrombosis was a primary cause of the ensuing necrosis. It is possible that the thrombi were caused by thromboplastin from the degenerating zones (406, 611).

A number of organic chemicals have a powerful inhibitory effect on the thrombin-fibrinogen reaction (170), while others have a potentiating effect. Among these are gum acacia, phenol, *o*-cresol and *p*-cresol (190). Alpha tocopherol phosphate is one of the compounds which inhibits the action of thrombin. This occurs to a certain extent at a concentration in which the tocopherols are found in normal human serum, which is said to be about 1 mg. per cent. This antithrombic effect of alpha tocopherol could be demonstrated *in vitro* and *in vivo*

(628). With purified reagents the inhibition of thrombin activity in the test tube can be reversed by the addition of calcium because calcium forms an insoluble compound with alpha tocopherol phosphate (273). Kay and associates have devised an antithrombin test which is not based on fundamental relationships and is, therefore, an empirical test (275). They present data to show that there is a thrombosing tendency when this test shows low "antithrombin" values. Furthermore, the administration of alpha tocopherol phosphate with calcium gluconate to patients causes the "antithrombin" value to be increased. The group of patients which received alpha tocopherol phosphate with calcium gluconate had a far lower incidence of thrombosis than a comparable group not so treated (274). They therefore advocate the use of this drug to deal with the problems presented by thrombo-embolic phenomena. It is not easy to decide whether the test tube experiments correlate with the clinical observations. At first one would be inclined to assume a direct connection but *the evidence is incomplete.* The evidence presented by Kay and associates has not been met with much enthusiasm (63) and it remains to be seen whether the clinical conclusions (274) can be confirmed. Overman (63) tried several antithrombin tests and could find no response with various doses of alpha tocopherol in animal experiments.

In another study (272) alpha tocopherol phosphate and inositol phosphatide were shown to have antithromboplastic activity. The tests consisted of producing generalized thrombosis in mice by injection of thromboplastin. After thromboplastin was first incubated with monosidium alpha tocopherol phosphate or inositol phosphatide the mixture could be injected and the clotting effect of thromboplastin was not observed. Under certain conditions *in-vivo* experiments were also successful in that large doses furnished protection against thromboplastin. Fibrinogen counteracts the effects of each of these substances in test tube experiments.

XII. HEPARIN AND RELATED SUBSTANCES

The story of heparin has been summarized in a monograph by Jorpes (257) and it does not seem necessary to review historical aspects such as its discovery, isolation, industrial production, and role in the development of anticoagulant therapy. It does seem appropriate, however, to review recent literature and attempt to cover the pertinent points concerning its chemical nature, mode of action, rationale for methods of administration, and heparin substitutes.

Chemical nature. The commercial products which are available are obtained from animal tissues. In these tissues the anticoagulant is located in the mast cells (257, 606) found mainly in the connective tissue near capillaries and in the walls of blood vessels. The heparin material is found with the amorphous microsome material occupying the "intergranular" cytoplasm (264, 547). From here the native material has been obtained in the form of a heparin-lipid-protein complex. The protein part has an amino acid pattern similar to that of the histones (547). Since heparin as such has not been found in appreciable quantity in the blood it may be that the lipid-protein complex is all that remains when and if the whole native complex is released into the blood stream. In any case the

commercial heparin isolated from lungs or liver is devoid of the lipid-protein complex. It is obtained as crystalline material, either as the barium or sodium salt. It is a mucoitin polysulfuric acid which has a molecular weight of 17,000 (256). The repeating unit in the molecule is a disaccharide and consists of glucosamine (257) and glucuronic acid (612, 614, 615) joined through chemical linkage and containing the sulfuric acid as ester. Alternative structures for the general architecture of the molecule are proposed (615). Mild acidity causes the heparin molecule to lose its anticoagulant activity. The rate of loss in activity is proportional to the rate of appearance of a free amino group in the molecule. It is believed that its nitrogen linkage is essential for its biological activity (613). Heparin has a strong negative charge and when added to plasma a new electrophoretic component, the C component, appears and migrates with a mobility between that of heparin and albumin (104). It may be that all heparin preparations are more or less inhomogeneous in that they may possess one, two or three sulfate groups for each disaccharide unit (260).

A unit of heparin is defined as 1/130 mg. of a potent sodium salt of heparin supplied to the National Institute of Medical Research, London, by Best and associates of Toronto. It is the Provisional International Standard and is equivalent to one U.S.P. (XIV) unit. In the United States commercial preparations are adjusted to a potency of 100 units per mg. In Sweden it is standardized to contain 80 units per mg.

Mechanism of action. Heparin is effective at two points in the chemical mechanism of blood coagulation. Firstly it blocks the activation of prothrombin. Actual measurements show that heparinized blood contains its full quota of prothrombin. To block the activation of this prothrombin a plasma cofactor is required, for heparin will not block the activation of purified prothrombin (17, 81, 82, 429). The nature of the co-factor is not known, except that it is probably a protein. Secondly, heparin has an effect as an antithrombin. When added to plasma heparin causes plasma to develop powerful antithrombin properties (431). When heparin is mixed with purified thrombin this powerful inhibition is not observed and it is, therefore, believed that plasma furnishes a co-factor which acts in conjunction with heparin to give the antithrombin effect. It could be that this co-factor is the C component (104) or the protein-lipid complex associated with heparin in the native state (457) or that these two are the same, but the answer is not known. It has been shown, however, that this antithrombin effect of heparin does not involve destruction of thrombin (20, 278). The mechanism is apparently an interference with the ability of thrombin to clot fibrinogen. Heparin thus acts as an anticoagulant whose effect is superimposed on the blood clotting mechanism whereas dicumarol acts on physiological mechanisms to interfere with the production of prothrombin.

From the work of Copley and Robb (120, 121) it became known that heparin affects the platelets. When added to dog blood *in vitro* it produces a platelet decrease; *in vivo* this same effect is transitory and there may be a subsequent increase above the original. In mice single or repeated subcutaneous injections did not result in a significant change in platelet count. Fidlar and Jaques (171)

also found a brief thrombocytopenia in dogs and man, and the fall recurred with repeated intravenous injections. There was some variation from one heparin lot to another. In another study it is concluded that the agglutination of platelets is of little moment in the use of the drug (439), with the possible exception that thrombin might cause platelets to agglutinate, in which case heparin would be an agent to prevent agglutination indirectly via the mechanisms described above, namely, by blocking prothrombin activation and by acting as antithrombin.

In small amounts heparin accelerates blood coagulation (154, 178). Thereafter, with higher concentrations, the effects of the above described mechanisms are observed. Upon intravenous injection of heparin, the clotting time is prolonged in proportion to the dose (53, 249). A linear relation is found between dose and the logarithm of the clotting time obtained. With moderate doses five to ten minutes are required for the clotting time to reach a maximum and thereafter the heparin effect becomes dissipated in a few hours, the duration of action being proportional to the dose (53, 244). The drug is probably inactivated by an enzyme. Also its excretion by the kidney has been considered. This route of elimination may not be important (52) for the excretion by the kidney may be limited (122, 257). The excreted material may be a derivative of heparin, called uroheparin (53). It is said to be far less active than heparin itself. In one experiment Jaques and Ricker (249) found no difference in the rate of disappearance of heparin in normal and nephrectomized dogs. On the other hand, in rabbits 25 to 50 per cent of injected heparin was excreted in the urine by glomerular filtration and tubular secretion (421). Deposits were found in the kidney, liver, spleen and lung of the rabbits. Also carinamide (4′-carboxyphenylmethanesulfonanilide), which affects certain renal transport mechanisms responsible for tubular secretion, has been reported to influence the duration of heparin action in man (341). From the practical standpoint it was suggested that the use of carinamide might reduce the quantity of heparin required by 50 per cent (341).

Administration. Heparin is not effective by oral administration. The various methods of administration have taken into account its fundamental pharmacological properties described above. Large doses are not toxic, nor do they affect wound healing (47, 258), and the only danger is that of hemorrhage. The rapid neutralization of heparin action by protamine or toluidine blue, as described below, provides a valuable safety measure. The possible danger of hemorrhage has prompted the use of minimal dosage, but Jorpes (257) has emphasized the need for quantities adequate to prevent thrombosis. The intravenous drip method employed by the pioneering Toronto group attempted to match the slow disappearance of heparin. This method entailed inconveniences and involved large fluid volumes (251, 372). In Sweden (257) intermittent intravenous doses were given, for example, 50 + 50 + 50 + 100 mg. or 75 + 75 + 75 + 125 mg. per day at 8, 12, 16 and 20 hours, respectively. For details the reader should consult Jorpes (258, 259). Strong aqueous solutions have been given by the intramuscular route (533, 587). A repository form of heparin has been used and consists in purified heparin compounded with gelatin-dextrose-acetic acid (450). It caused

pain at the site of injection, but this objectionable feature has been eliminated by appropriate adjustment of pH (312). It prolongs the action of heparin effectively (156, 198, 202, 223, 311, 313, 314, 368). The disadvantage of unpredictability has been recorded, but as an average the full effect of the drug is observed at five hours, high effectiveness for the next 20 hours, and significant elevation of the coagulation time for 50 hours. With the addition of vasoconstrictors such as epinephrine and ephedrine the coagulation time is prolonged by the eighth hour, remains at that high level for about 40 hours, and returns to normal at about 70 hours. The use of heparin is widely discussed in the literature and some additional practical views not previously cited are given (41, 281, 309, 333, 334, 452, 552).

Heparin substitutes. The fact that commercial heparin of animal tissue origin is a disaccharide containing sulfate suggested the possibility of making synthetic polysaccharide sulfuric acids. Esters of cellulose, chitin, pectic acid, glycogen, starch, gum arabic and yeast nucleic acid were prepared (22, 23, 24, 37, 48, 49, 270). They were all found to possess heparin-like activity but were far less potent than heparin. Pharmacological studies (26, 418, 419, 420) showed the cellulose and starch sulfuric acid esters to be the most toxic and chitin the least (26); the chitin material had the highest anticoagulant potency (419). The main difficulty encountered was platelet agglutination after intravenous injection (418, 420). Cellulose and starch sulfuric acid esters precipitate fibrinogen and clump the platelets with the result that emboli are produced and later infarcts develop in the lung and kidney (420).

The results with another synthetic compound were far more encouraging. A polysulfuric ester of polyanhydromanuronic acid, called Paritol, has an intravenous LD_{50} for mice of 1898 mg. per kg. as compared with 1590 to 2000 mg. per kg. for heparin. Its anticoagulant potency is about $\frac{1}{7}$ that of heparin (525, 526), but the duration of action is prolonged as compared with heparin. About 30 per cent of an intravenous dose is recovered in the urine, but its action is not prolonged by bilateral nephrectomy, splenectomy, hepatectomy, injections into the bone marrow, or blockade of the reticuloendothelial system with India ink. Sorenson and Wright (525) reported evidence of satisfactory maintenance of anticoagulant therapy with Paritol. Three of 35 patients showed reactions. Two of these reactions were mild and the other was severe; however, the authors feel that further studies are definitely indicated.

Bartholomew and Barker (38) found, in a series of ten subjects, that protamine sulfate neutralizes the effect of Paritol. For example, the coagulation time of whole venous blood which had been markedly prolonged by the administration of 300 mg. of Paritol returned rather rapidly to normal when 200 mg. of protamine sulfate were given intravenously within one hour after the Paritol. Similar effects were noted with 100 mg. given four hours or 50 mg. given eight hours after the administration of Paritol.

XIII. UNRECOGNIZED NUTRITIONAL FACTORS

Warner and Owen (595) found that patients with Addisonian pernicious anemia in relapse have a prothrombin level between 40 and 65 per cent of normal. This

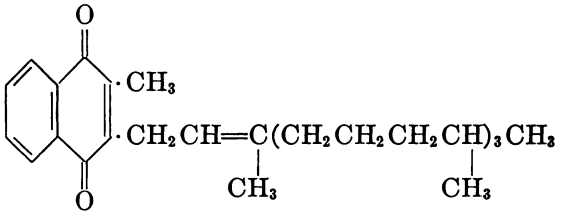
hypoprothrombinemia is not rectified by large doses of vitamin K but there is a prompt response when specific liver therapy is instituted (595). In further pursuit of this problem Owren (401) found that crystalline vitamin B_{12} or folic acid or both may cause the red blood cell count to return to normal, but the hypoprothrombinemia remains in the range of 60 to 90 per cent of normal. It was also found that the macrocytes are flatter, increased in diameter and more resistant to hemolysis in hypotonic saline solutions. On the latter basis the macrocytes could be separated from the other cells, and it was found that their hemoglobin is of the fetal type as shown by the rate of alkali denaturation. A substance found in crude liver extract returns these aberrations as well as the hypoprothrombinemia to normal. The substance in liver may correct the hypoprothrombinemia and fetal type hemoglobin without altering the red blood cell count. Apparently the liver factor is generally concerned with protein metabolism and the hypoprothrombinemia is only one of several ways to detect the deficiency.

Folic acid is also concerned with one of the proteins of the clotting mechanism. Macrocytic anemia in chicks, resulting from folic acid deficiency, is accompanied by an increase in plasma antifibrinolysin activity. Crystalline folic acid prevents this marked increase (204).

XIV. VITAMIN K

Previous reviews (18, 78, 88, 92, 133, 147, 303, 335, 432, 461, 594) cover most of the field up to present times. Thayer (558) asserts that over 600 papers have been written on the effectiveness of vitamin K in preventing hemorrhages in the new born and it is hardly appropriate to take up this subject or the others covered by the above references. However, at this time it seems appropriate to recall the basic facts and to discuss the relationship of vitamin K to hypervitaminosis A, to hyperprothrombinemia and to antagonistic drugs.

Vitamin K deficiency causes a bleeding tendency because vitamin K is necessary for the synthesis of prothrombin by the liver (132). This mechanism requires a normal functioning liver and in fact a vitamin K tolerance test (578) is a useful liver function test. The vitamin is not a part of the prothrombin molecule itself (495) for it cannot be found in purified prothrombin preparations. There are many compounds possessing K activity and most of them are naphthoquinones. Some of those most commonly available are the following:



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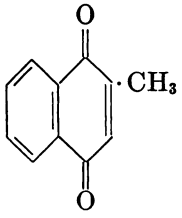

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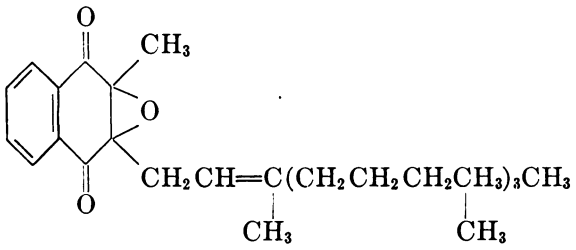

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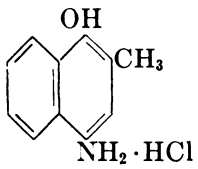

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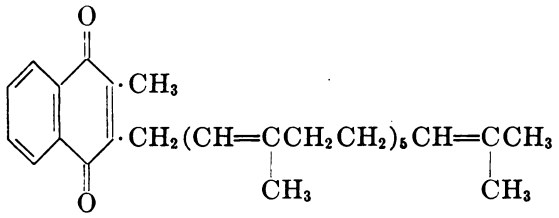

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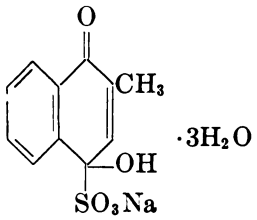

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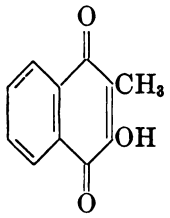

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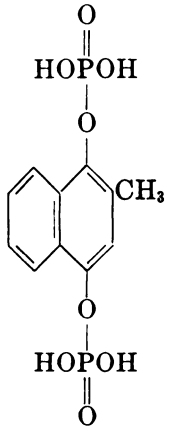

(8)

(1) Vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone). Fat-soluble, natural vitamin; melting point, −20°C; 1000 Thayer-Doisy units per mg.; route of administration: orally, intramuscularly in oil, intravenously in emulsion.

(2) Vitamin K_1 Oxide (2-methyl-3-phytyl-1,4-naphthoquinone-2,3 oxide). Fat-soluble; route of administration: intravenously in emulsion.

(3) Vitamin K_2 (2-methyl-3-difarnesyl-1,4-naphthoquinone). Fat-soluble, natural vitamin; melting point, 53.5–54.5°C; 600 Thayer-Doisy units per mg.

(4) Phthiocol; Vitamin K_3 (2-methyl-3-hydroxy-1,4-naphthoquinone). Slightly water-soluble; sodium salt is water-soluble; melting point, 173–174°C; 2

Thayer-Doisy units per mg.; route of administration: intravenously as sodium salt.

(5) Menadione; Vitamin K_4 (2-methyl-1,4-naphthoquinone). Fat-soluble; slightly water-soluble; 1050 Thayer-Doisy units per mg.; route of administration: orally in oil, intramuscularly in oil, intravenously in emulsion.

(6) Synkamin; Vitamin K_5 (2-methyl-4-amino-1-naphthol hydrochloride). Water-soluble; 1000 Thayer-Doisy units per mg.; route of administration: orally, subcutaneously, intramuscularly, intravenously.

(7) Menadione sodium bisulfite. Water-soluble; route of administration: intravenously.

(8) Synkavite (2-methyl-1,4-naphthohydroquinone diphosphoric acid ester). Water-soluble; route of administration: intravenously.

Further information on these compounds can be obtained from the reviews of Brinkhous (78) and Almquist (13).

Dicumarol-Vitamin K antagonism. This controversial subject had its beginning in an erroneous report by Quick (430) stating that vitamin K does not counteract the effects of toxic sweet clover hay in rabbits (430). Link's (303) work soon indicated another conclusion and clinical as well as experimental reports dealt with the questions of adequate dosage of the vitamin as compared with the amount of dicumarol being given, sensitivity of the patient to dicumarol, and the effectiveness of water-soluble forms of vitamin K as compared with K_1 or K_1 oxide (70, 115, 126, 136, 241, 318, 438, 504). It does not seem necessary to take up each of these studies individually for the main facts are contained in the most recent reports (62, 358, 506). Miller, Harvey and Finch (358) worked with rats, dogs and man and made an attempt to overcome criticisms which could be made of other reports. The water-soluble vitamin K preparations had no antagonistic effect, under the conditions of their experiments. Vitamin K_1 and K_1 oxide were very effective in decreasing the prothrombin time. To this Shapiro, Wiener and Simson (506) add the observation that a single large dose of water-soluble vitamin K may be adequate to correct hypoprothrombinemia in man when there are relatively low concentrations of dicumarol in the plasma for a short duration of time. Overman, Sorensen and Wright (393) point out that the conclusions of Miller, Harvey and Finch (358) tend to be misleading. Under the conditions of their (358) experiments vitamin K and dicumarol were administered simultaneously, a condition which should not occur in the clinical use of dicumarol. Under clinical conditions Overman, Sorensen and Wright (393) did find water-soluble vitamin K effective in counteracting excessively prolonged prothrombin times due to dicumarol. Patients with persistently high plasma concentration of dicumarol may require repeated large doses to bring the prothrombin time to a safe level and control its excessive prolongation. Since the water-soluble forms fail under certain conditions and are required in repeated doses it can only be concluded that K_1 and K_1 oxide are comparatively more effective. The word *effective* is used instead of *superior* because the solubility characteristics of the fat-soluble preparations may make them more slowly

disposed of than water-soluble forms and thus permit them to exhibit a more sustained effect. They may not necessarily function better in the actual chemical interactions. The massive doses of the vitamin required are stressed in most of the papers.

In hypervitaminosis A the most characteristic lesions are skeletal fractures and hemorrhages, the latter being predominant in adult animals. In rats overdosage of vitamin A is associated with a prolonged prothrombin time, and this effect can be counteracted by the administration of vitamin K (2-methyl-3-phytyl-1,4-naphthoquinone) (299). It was also found possible to use water-soluble forms of vitamin K for the same purpose (588).

Field and Link (173) have reviewed the question whether vitamin K can produce high prothrombin levels in the blood. Their own experiments with the dog, rabbit and rat showed that a definite hyperprothrombinemia can be produced and is readily detected with the use of the 12.5 per cent plasma method for testing prothrombin time. In man large doses of K (menadione) produced similar effects (577). Where the increase was demonstrable, it continued for brief periods only, lasting 24 to 48 hours.

XV. SULFONAMIDES

When fed to rats given a synthetic ration, sulfaguanidine produces a hypoprothrombinemia as measured by the 12.5 per cent plasma method for testing prothrombin time (57). This prothrombin deficiency can be corrected by feeding *p*-aminobenzoic acid, liver extract or vitamin K. The observations are thought to be explained adequately on the basis of bacterial synthesis of vitamin K in the intestinal tract. The *p*-aminobenzoic acid and liver extract probably act by nullifying the effect of sulfaguanidine, and the bacteria of the intestine then flourish and produce vitamin K. Succinylsulfathiazole was fed in the same manner and with the same results. In another study sulfathiazole, fed at a one per cent level in the ration, also caused rats to develop a prothrombin deficiency which could be corrected by vitamin K (71).

Some of the sulfonamides also have a direct effect on blood coagulation. With purified prothrombin preparations certain sulfonamides inhibit while others accelerate the autocatalytic activation of prothrombin in 25 per cent sodium citrate solution (484, 493).

XVI. ADRENOCORTICOTROPIC HORMONE

In a series of patients the whole blood clotting time was found to be shortened as was also a heparin-retarded coagulation time of venous blood (124). In further detailed studies no changes of significance were found in the prothrombin time or in the protamine titration tests. The authors consider the hypercoagulability of the blood caused by ACTH or 11-dehydro-17-hydroxycorticosterone (cortisone) of importance to the patient's health. In another study (518) it was found that the adrenal cortex seems to exert an effect on a number of constituents of the clotting mechanism, and that the response is not the same from one patient to another. From their work it is evident that further studies are required. These

authors (518) also cite the work of Dougherty and Dougherty which showed changes in the mast cells of experimental animals receiving 11-dehydro-17-hydroxycorticosterone (cortisone); as discussed elsewhere in this review these cells are concerned with heparin metabolism.

XVII. ANESTHETICS AND RESPIRATORY GASES

That chloroform anesthesia produces injury to the liver of animals has been known for a long time. Whipple and Hurwitz (604) were able to confirm a prior observation and establish the fact that the fibrinogen of plasma practically disappears with the advance of the liver necrosis after chloroform intoxication. The liver repair process is rapid and the blood fibrinogen values may return to normal in approximately a week. In a further extension of this work by Smith, Warner and Brinkhous (516) it was found that plasma prothrombin concentration also decreases after deep chloroform anesthesia. Furthermore, it was possible to show that prothrombin may disappear without a marked drop in fibrinogen levels, thus indicating that the liver is more susceptible to changes influencing prothrombin production than to those affecting fibrinogen production. Oxygen given in excess with the chloroform does not minimize the drop in prothrombin concentration (129). In man chloroform anesthesia also causes a fall in the prothrombin level. That these effects of chloroform are indeed on the liver and do not operate by some other mechanism is substantiated by the work of Uvnäs (580). The occlusion of the arterial blood supply to the liver causes a rapid fall in prothrombin concentration. The occlusion was performed either by ligating the hepatic vessels in cats and rabbits or by making a modified Eck fistula in cats. Partial hepatectomy in rats also causes the prothrombin concentration to drop (593). Acute hepatic damage caused by chloroform intoxication also causes plasma Ac-globulin concentration to decrease (546). Its concentration returns to normal more rapidly than prothrombin. It is thus established that prothrombin concentration decreases more readily than fibrinogen concentration and the position of Ac-globulin with respect to these two still needs to be established. Owren (400) finds that, in the course of development of a liver disease, the plasma prothrombin level falls sooner and more severely than the factor V (Ac-globulin) level.

Attention has been directed toward the question whether common anesthetic agents cause sufficient liver injury to produce a postoperative fall in prothrombin concentration. Apparently the changes which commonly gain the physician's attention can be ascribed to indirect effects in patients with obstructive jaundice or biliary fistulas (129), for neither ether nor cyclopropane causes a drop in prothrombin concentration. In all but one patient no prothrombin reduction appeared with the following: diethyl ether, divinyl ether, ethylene-oxygen, nitrous oxide-oxygen and tribromoethanol, and procaine and nupercaine spinal anesthesia (8). In another study no changes in prothrombin were found with local anesthesia; but with nitrous oxide plus ether there was a tendency for the prothrombin index to drop and this became more marked on the fourth day after operation. The figures were statistically significant (68).

Another possibility is an increase in prothrombin concentration. Levy and Conroy (294) made careful prothrombin time studies when the patients entered the hospital, before ether anesthesia and during deep anesthesia. At this latter time the presumptive test for prothrombin showed an increase in concentration. This did not occur with spinal anesthesia. Another observer records a slight reduction in coagulation time of the whole blood (482), and the same was found by Searls who also found an increase in platelet count (481). Most of the evidence seems to indicate hypercoagulability of the blood, but this is not a profound change and there is no way of knowing from the present literature which constituent(s) of the blood is responsible. Perhaps shifts in blood volume can account for the differences. For example, it has been known for a long time that post-hemorrhagic blood is hypercoagulable, a constant finding even if the adrenals or intestines are excluded in the experiments (201). Perhaps the dilution of the hemorrhagic blood alters the balance between activators and inhibitors of prothrombin so as to favor the activators. This possibility was beautifully demonstrated by Tocantins (574) and such shifts could easily be the basis for the effect of ether anesthesia. Mendenhall (346) also found that ether anesthesia lowers the clotting time of whole blood and showed that the effect is mediated wholly through the action upon the adrenal glands, because the effect was not obtained after adrenalectomy.

It is stated that pentobarbital sodium lowers the clotting power of the blood (294), and in another report that the clotting time is shortened (29). In experiments with pigeons and cats (371) the coagulation time of the blood was shortened during the first few hours of barbital anesthesia, and returned to normal between 20 and 48 hours. This change went parallel with the blood sugar values and it is suggested that the adrenal glands are involved.

Pere (411) has reviewed the literature concerning the effect of the respiratory gases O_2 and CO_2. Apparently a decrease in CO_2 tension favors clotting and an increase in CO_2 tension has the opposite effect. In a special kind of "antithrombin test" arterial blood was found to inactivate thrombin more readily than venous blood; it is therefore reasoned that arterial blood clots less effectively (405) than venous blood with its heavier charge of CO_2. Pere's own experiments on venous stasis showed that clotting was first accelerated and then retarded. Quite evidently these various experiments involve different mechanisms and it is not possible to make a generalization about the effects of CO_2 or O_2. In asphyxia experiments with dogs there were no significant differences in the clotting times of blood specimens removed before, during and after periods of severe asphyxia (422). Some additional experiments on this subject are recorded, but the reviewer has had access only to the abstracts (155, 371, 404, 479). Quastel and Recker (428) look at the problem from another viewpoint. An extract of the muscle of a limb rendered anoxemic by application of a tourniquet brings about the clotting of blood at a much more rapid rate than an extract of normal healthy muscle. When rat tissue slices were allowed to incubate in the Warburg apparatus under aerobic and anaerobic conditions the latter yielded the highest concentration of clot-accelerating material. This may be a factor in shock, but in any case

it shows that tissues undergo changes under anaerobic conditions which are concerned with blood coagulation.

XVIII. EPINEPHRINE

In experimental studies of the sugar content and the extravascular coagulation of the blood after administration of epinephrine, Vosburgh and Richards (584) found in every case that the time of coagulation is lessened. The dimunition was equal in some cases to $\frac{4}{5}$ of the control coagulation time. They suspected a relationship between blood coagulation and the pancreas. In further pursuit of this problem Cannon and Mendenhall (94) devised a graphic method for recording the coagulation time of whole blood, thus placing more reliance on this method as against ordinary test tube experiments. Epinephrine injected in large doses intravenously and in large doses subcutaneously shortened the coagulation time to $\frac{1}{2}$ or $\frac{1}{3}$ the control duration. When the blood was confined anterior to the diaphragm or when the intestine or liver was removed epinephrine in small doses did not cause rapid clotting (93). It was suggested that epinephrine accelerated the clotting process by stimulating the liver to greater activity, by disturbing some factor or factors in coagulation. Stimulation of the splanchnic nerves (95) resulted immediately or after a brief delay in shortening the coagulation time of blood. The period of rapid clotting lasted from 10 to 30 minutes and the effects were less marked with repeated stimulation. The removal of the adrenal gland on one side nullified the effect of splanchnic stimulation on that side whereas stimulation on the other side was still effective. Stimulating the nerves supplying the liver and intestine did not hasten clotting and it was concluded that the adrenal gland is directly involved. In further work Cannon and Mendenhall (96) concluded that emotional excitement is associated with very rapid clotting of blood and that the blood clotting time is prolonged when the splanchnic nerves are cut. They cite the fact that cats differ widely in their responses to excitement in the laboratory. Some, especially young males, become furious; others especially elderly females, take the experiment quite calmly. This difference of attitude was used to test the effects of emotions on blood clotting. Substantially the same ideas are expressed by Selye in discussing the general adaptation syndrome and he cites further original experiments (500). Link also found that epinephrine hastened the restoration of normal prothrombin time when given to animals about to bleed from excessive doses of dicumarol (303). The experimental demonstration of this fact involved care in getting the animals adapted to laboratory surroundings. The work of Bradfield (200) explains the epinephrine effect as being due to an increase in the amount of prothrombin in the circulating blood, but the data presented do not permit the conclusion that prothrombin is the specific substance involved because methods for the quantitative determination of prothrombin were not available at the time his work was done. However, Uvnäs (580) reports that there is an increase in plasma prothrombin levels (prothrombin time) when cats are given intravenous injections of epinephrine and after electrical stimulation of the splanchnic nerve. This could, of course, still involve factors other than prothrombin. In a thorough study of the entire problem with the use of the prothrombin time

method with diluted (12.5 per cent) plasma, Wakim, Fink and Chen (586) come to the following conclusion: The effects of different doses of epinephrine administered to dogs or rats intravenously, intramuscularly, and subcutaneously justify the conclusions that the drug produces no significant change in the prothrombin time of diluted plasma. In dogs made hypoprothrombinemic by dicumarol the effects of epinephrine are variable and inconsistent and, therefore, probably insignificant. The fact that this *variability* was encountered would tend to support the general view that stress states are factors to be considered and these were probably not ruled out in the experiments of Wakim, Fink and Chen. At this point it perhaps needs to be recalled, as already mentioned above, that the hypercoagulability of post-hemorrhagic blood is due to causes other than those involving epinephrine.

de Takats devised a test of the clotting mechanism referred to as the heparin tolerance test. It is essentially a simple test wherein a small amount of heparin is injected intravenously and the coagulation times are then determined at ten-minute intervals for about 40 minutes (550, 553). Epinephrine increases the heparin tolerance. In other words, a hypercoagulable effect counteracts the anticoagulant response to heparin (551). By using the same test Moses (364) found no significant or consistent change in five human subjects following the administration of 1 mg. of epinephrine intravenously. Perhaps the fact that the changes were inconsistent is of importance for reasons inferred above. The statement is made by Moses (364) that epinephrine reportedly hastens coagulation of blood but such reports have not been generally confirmed. The facts in the literature are to the contrary.

There are papers which indicate that the effect of epinephrine is due to the activity of certain of its oxidation products produced in the tissues. The chief product is thought to be adrenochrome. The hemostatic effect of this substance is currently being studied (30, 31, 141, 179, 180, 425, 457, 458, 519).

Another effect of epinephrine is concerned with fibrinolysis (56). Epinephrine was injected subcutaneously in human volunteers, blood samples were taken within an hour, and fibrinolysis was allowed to proceed for 24 hours. In general control samples did not lyse whereas the experimental samples lysed extensively. *In vitro*, epinephrine did not lyse the blood, so that the effect must have been mediated via physiological processes and these most likely involved the activator of profibrinolysin in the manner outlined in the introductory remarks to this review. It is quite possible that the fibrinolysis associated with exercise, fear, trauma and some pathological states results indirectly from the stimulation of epinephrine secretion. However, another possibility is that epinephrine is not to be regarded as having a very specific role, for it has been found that parenteral administration of procaine or even sodium chloride solution causes lysis of blood samples (267).

XIX. ACETYLCHOLINE

de Takats (551) expresses the view that there can be no doubt that the adrenergic and cholinergic components of the autonomic nervous system exert an effect on the clotting mechanism. Puharich and Goetzl (424), in a review of the

literature, develop the same ideas and even point to the suggestion that a center for the regulation of blood coagulation may exist in the diencephalon. It is suggested (551) that parasympathomimetic drugs such as methacholine and neostigmine tend to constrict the hepatic veins and prevent a steady flow of prothrombin from the liver, and in this way contribute to the hypocoagulability of the blood. de Takats points out that other possibilities must be considered. It seems to the reviewer that more data are required to determine which substances and/or physiological mechanisms are involved. As an example of the complexity of the situation the work of Soulier and Koupernik (528) shows that fibrinolysis takes place regularly in blood withdrawn between the second and tenth minute after acetylcholine-induced shock. This most likely involves the fibrinolysin activation system.

XX. BLOOD COAGULATION COMPONENTS

The advances in our knowledge of blood coagulation have been made, in part, as the result of methods devised for preparing some of the important components in purified form. Heparin is an example, and has already been discussed from the standpoint of its uses and pharmacological action. Some of the others are also of importance and, therefore, need to be discussed.

Thrombin. The methods used for the industrial production of thrombin are essentially the same as those employed in its production for research purposes (21, 113, 114, 369, 483, 489, 491, 492, 494, 495, 543). A unit of thrombin is that amount which will cause one cc. of standardized fibrinogen solution to clot in 15 seconds. Standards are maintained in the United States by the National Institutes of Health, and the World Health Organization anticipates the use of this standard.

Following the introduction of thrombin as a hemostatic agent (496, 597) its extensive use (50, 90, 106, 110, 123, 127, 128, 131, 159, 184–187, 213, 238, 298, 300, 301, 409, 417, 427, 440, 443, 445, 454, 538, 559, 566, 567, 601, 625, 626) required further knowledge concerning its pharmacological properties. It has been found, as one would expect, that the intravenous injection of thrombin causes deposition of fibrin and the many dangerous consequences of such deposition. Apparently the intravenous injection of minute amounts of thrombin causes an increase in the defensive mechanisms of the organism (191) and may shorten the bleeding time (555). This is obviously a dangerous way in which to shorten the bleeding time. Typical responses to the injection of strong thrombin solutions are those reported by Astrup and Volkert (25). Rapid injection into the femoral vein causes most of the animals to die in one to two minutes in convulsions. The animals may survive for a matter of minutes and develop a large embolus in the pulmonary artery. When larger doses are injected intramuscularly or subcutaneously there is no gross response and the animals survive without any apparent serious consequences. The same has been observed in rats (597). In rabbit experiments (25) no response was seen to intraperitoneal injections but massive doses in rats (597) seemed to cause extreme discomfort, inasmuch as the animals rolled and tossed about. On examination of the blood it was found to

be partially and in some instances completely defibrinated. Apparently there is absorption of thrombin from the peritoneal cavity when such tremendous quantities are given. This has also been observed by Zucker (63). Injection into such vital organs as the liver may cause sudden death (491). There is a drop in blood pressure when thrombin is given intravenously; this is due to its clotting power, for the effect can be abolished by preliminary heparinization (549).

Under proper conditions and with a certain amount of luck it is possible to administer thrombin intravenously and completely defibrinate the animal (265, 382). Jürgens and Studer (265) completely defibrinated many animals in this manner. They then identified fibrin in the lung, the kidney and the liver. They also found that these fibrin deposits do not remain there very long after the thrombin infusion. Some mechanism for their removal must therefore exist. When the animal is completely defibrinated there is danger of bleeding. It is interesting that a massive dose of thrombin causes changes in the vascular tree which actually favors bleeding. It seems as though thrombin alters the capillary permeability. The above authors also record a tremendous drop in the leucocyte count after thrombin infusion. The active thrombin is neutralized in due course by the normal antithrombin of the plasma. The return of the fibrinogen concentration to normal values takes place over a short period of about three to four hours.

The practical use of thrombin is confined to topical application to bleeding surfaces and the above untoward pharmacological effects are avoided entirely. However, thrombin is a protein and its use, when it is prepared from bovine material, has raised the question whether antigenic properties are of importance. There is no difficulty in demonstrating the antigenicity of thrombin preparations when the appropriate procedures are employed to elicit an antigen-antibody reaction (135). In contrast, the proper medical use of bovine thrombin has in no instance produced an antigenic reaction. In a direct consideration of this question Light (298) used bovine thrombin surgically to control hemostasis during brain operations in monkeys. They did not develop either serum precipitins or any local tissue reaction in the brain (Arthus phenomenon). In 15 patients precipitin tests, done from 10 to 94 days following the neurosurgical use of bovine thrombin as a hemostatic agent, yielded no positive reactions. On the basis of extensive clinical experience Tidrick (105) has also found no undesirable results from the use of thrombin. None of his patients has shown any evidence of absorption of thrombin or of local thrombosis of vessels (567). Even with the use of fibrin-thrombin films in skin transplants (566) there were no reactions observed; moreover, in this work *bovine fibrinogen* was used successfully as a source of fibrinogen for the surface of the transplants.

Prothrombin. The use of prothrombin in experimental work has not been extensive nor has the substance been introduced for practical use. The possibility of restoring prothrombin deficiencies is self-evident but so far it has not been possible to furnish the material in the required quantities. In dogs the hypoprothrombinemia produced by dicumarol could be corrected by the intravenous administration of a prothrombin preparation (343). The normal prothrombin

levels of the blood produced by intravenous injection are of short duration. After about 24–48 hours the prothrombin values are again found to be very low, which is in accordance with the general finding that prothrombin disappears from the blood rather rapidly when the stores are not replenished by a normally functioning liver. The experiment of McGinty, Seegers, Pfeiffer and Loew (343) has recently been confirmed in all respects (536).

Streptokinase-streptodornase. The bacterial product, streptokinase, is capable of activating profibrinolysin and has been prepared (105) in combination with streptococcal desoxyribonuclease (streptodornase). In clinical studies (509, 569) intrapleural injections of these substances caused intrapleural fibrinolytic and proteolytic changes, the effects of which were self-limiting after each injection. Toxic manifestations from the injections were limited to transient febrile reactions with general malaise and a local outpouring of leucocytes which lasted for a few days. The use of these substances by Tillett and Sherry (569) has indicated the importance of streptokinase and streptodornase in influencing the course of certain exudative disease.

Thromboplastin. (*a*) *General remarks.* Crude preparations from organs of different species can be prepared easily and some are available commercially as laboratory reagents. Partial purification can be accomplished by taking advantage not only of the usual precipitation methods but also of the high molecular weight which permits differential sedimentation of thromboplastin by high speed centrifugation (103). The thromboplastin which can thus be obtained is not to be confused with other accelerators of coagulation, such as platelet factors (485). Topical application of thromboplastin has been suggested for local hemostasis (118). For this purpose it is wholly dependent for its action upon the available prothrombin of the blood. The value of its subcutaneous or intramuscular administration in traumatic or other hemorrhagic conditions (579) is debatable. The suggestion that thromboplastin be given intravascularly for the treatment of hemophilia, anaphylactic shock or other types of shock (118) can only be regarded with skepticism; this route of administration would appear to be most hazardous according to our present knowledge.

Purely pharmacological studies of intravascular coagulation by injection of "tissue fibrinogen" (tissue extracts) were reported by Wooldridge in 1886 (616); these were extended in the studies of Mellanby (345) who, however, used snake venoms instead of thromboplastin. Hanzlik and Karsner (209) injected lung extract thromboplastin as one of various agents during a study of intravenous therapy in relation to anaphylactoid phenomena. They were not interested in differences between the effects of the intravascular injection of lung extract and those of "the otherwise inert" agents: agar, kaolin, India ink, ricin, or intravenous medications, all of which caused severe reactions in their animals, including death and/or acute pulmonary changes, among which were peribronchial edema, embolism and thrombosis (209).

(*b*) *Experimental pathology and relationship to pregnancy.* The remaining pharmacological studies have been the by-product of experimental pathology. Several of these have been in relation to toxemia of pregnancy (145, 146, 462, 466–477),

one was incidental to virus studies (560, 561) and one was in relation to cerebrovascular accidents (221, 608). The toxemia investigations have several times led to the conclusion that the placental "toxin" under consideration was thromboplastin and that its action was that of intravascular coagulation. This was also concluded by the earlier German workers (146, 466, 582) who recognized thrombi in the liver and in other organs. The Japanese workers likewise found that the placental "toxin" of Obata (388) was a tissue kinase of coagulation which resulted in pulmonary and other thrombi and could be promptly lethal. Meanwhile Dieckmann (145) had carried out investigations on the experimental production of liver necrosis, in which he was influenced by the concept of Mills (359) that the tissue factor of coagulation was a "tissue fibrinogen" in the sense of a kind of plasma fibrinogen. More recently, in a series of experiments, Schneider (467–477) concluded that the potent agent of extracts from the placenta and from the decidua is thromboplastin in the modern sense of that term. Intravenous injection resulted in vascular occlusions which had a predilection for the lesser circulation (473–476, 486, 490). These occlusions had a characteristic fibrin structure (473–476, 486, 490) which suggested that they had been built up by a process of "fibrin embolism" (475).

Mice (470) and rabbits (473) are two to three times more sensitive during late pregnancy than normal animals. Pregnant dogs are also more sensitive as shown by the fact that a dose of tissue extract which is not lethal for non-pregnant dogs is promptly lethal late in pregnancy (608).

(*c*) *Effect on circulation.* In experimental animals, the markedly effective route of administration is intravascular because this permits activation of the blood coagulation mechanism. The result may be prompt death caused by vascular occlusion. Sublethal doses cause a variety of pathological disorders consequent upon intravascular coagulation and these can be varied to some degree by the manner of injection. Whether physiologic disorders of a more elusive nature result from administration by other routes apparently remains to be determined, although some qualitative comparisons have been made (210, 471). The greater portion of the circulating blood becomes solid only if the intravenous dose is enormous. Intravascular injection of large doses may also cause severe reactions without death, but then the blood will no longer clot. This is because the fibrinogen is almost quantitatively deposited from the circulation (188). As a result the animals are temporarily refractory to further injection of the tissue extract (188, 345, 467). If the fibrinogen is restored artificially to the blood *in vitro* (345) or *in vivo* (188) the sensitivity to further additions of thromboplastin is restored. If a large dose of thromboplastin is given slowly or if repeated small doses are given, it is possible to render the animal completely refractory to further injection of even massive doses (188, 467, 471). If the animal is permitted to restore its own fibrinogen, nearly full sensitivity returns within one day or slightly longer (467), and it is probable that the fibrinogen restoration is accomplished in the same time (475) or even in less time.

If the injection is a lethal one, death in coma or convulsions may occur in a very few minutes. If essentially complete defibrination is brought about, this in

itself does not result in hemorrhage, nor may there be outwardly visible evidence of the profound disorder of the coagulation mechanism. However, if bleeding is initiated by trauma or by surgery, fatal hemorrhage may ensue because of failure of the hemostatic mechanism (474, 475, 486, 490).

Among the secondary complications, death occasionally occurs after a delay. Rarely, violent premonitory convulsions have been observed in mice within a few minutes or hours (471), presumably as a result of an embolus to a vital area. In dogs, "thrombosis" of the portal circulation has been observed to cause delayed death (471, 608). After repeated injections, neurologic signs and even eventual death may result from massive hemorrhage in vital cerebral areas (608). These effects seem to be rare following single injections and it is to be wondered (476) whether they may be due to a combined effect of focal necrosis from an earlier injection (221) and the stress of one or more later injections (608).

Because of the tendency for immediate fibrin deposition within the lesser arterial circulation following injection of thromboplastin, there may be a resultant tendency for the development of an acute cor pulmonale (476). However, judging from the rarity of delayed deaths, it is unlikely that death is often caused in this manner by a single injection, without an underlying pathologic process in addition. Possibily as a result of fibrin deposition, parenchymal lesions may develop in various organs of surviving animals. An example is liver necrosis which in the mouse is focal in distribution (469) and in the rabbit it is midzonal (473). Degenerative kidney lesions (478) may be a result of fibrin deposition or perhaps they result secondarily from the hemolysis which occurs with extensive intravascular coagulation.

The prompt death that is so readily caused by injection of thromboplastin is probably due to peripheral circulatory failure (472). *In-vivo* microscopic observations (472, 561) show that the circulation ceases although the respiration and the heart beat continues for a short time. This cessation of blood flow also occurs with the sublethal reactions, but in these cases the pulsating circulation is soon reestablished. Microscopic, *in-vivo* observations (472, 561) may reveal a momentary shower of arteriolar emboli before the blood flow stops in the peripheral vasculature. It is possible that the chief site of occlusion is within the lesser circulation, for it is there that fibrin occlusions are found to be widely disseminated upon microscopic examination of the tissue sections. In fact, one group of investigators finds that "thrombi" within the central nervous system are rare, although minute perivascular hemorrhages therein are frequent (608). Hence, the evidence points toward occlusion of the lesser circulation as the cause of death. This view is supported in another way; in the organs of animals recently dead of thromboplastin injection, there is splanchnic pooling of the blood, including massive engorgement of the liver with distention of its capsule (469, 476). Furthermore, the occlusion of the lesser circulation appears to be aggravated by constriction of the pulmonary artery (279). This combined evidence points to the possibility that the minute hemorrhages and necrosis in the central nervous system of survivors (221, 608) may be as much on the basis of anoxia

from temporary circulatory failure as on the basis of direct local obstruction by fibrin deposition. Similarly, an apparent failure of cardiac and respiratory centers may be secondarily due to anoxia caused by occlusion of the lesser circulation.

(*d*) *Nature of fibrin deposits.* The fibrin deposits are chiefly intrarterial, are widely disseminated, especially in the lung (473, 474), and are of a characteristic type (473, 474, 486, 490) which suggests the mechanism of their deposition as one of "fibrin embolism" (475). The term *fibrin embolism* means that the deposits are built up by oriented deposits of myriads of fibrin elements from the pulsating circulation (475). These vascular plugs are not mere blood clots such as occur in a test tube. The best display occurs when large doses of thromboplastin are given; the fibrin plugs are massive and dense although still essentially microscopic. They appear as cords of fibrin occluding the pulmonary arteries of all sizes (473–477, 486, 490). The fibrin elements of such occlusions are oriented parallel to the axis of the vessels, and are densely arranged. This is best seen in longitudinal section. There are few erythrocytes, and these are trapped in columns within the fibrin matrix. There are relatively many leucocytes. These give a clue as to the manner of deposition of the fibrin, for some of their nuclei are stretched in the same axis as the fibrin, as though deposited within fibrin masses under tension; this distortion is sometimes so extreme that a single nucleus may extend as a beaded filament beyond the limits of an entire high power field of the microscope. In cross sections, many of these fibrin occlusions have a minute central core of clotted blood, and from this it may be wondered whether, during the process of deposition of the fibrin from the moving stream, the cross-sectional area of flow ultimately became so small and the flow so retarded that coagulation of the remaining central core occurred without differential deposition of fibrin (476).

When large but less concentrated doses of thromboplastin are injected, the fibrin occlusions may be less dense. At the opposite end of the scale, if the dose is small, there may still be fibrin deposition; the deposit is then more loosely arranged and less well oriented, but has within it such an unusually large number of leucocytes as to give the appearance of a white cell thrombus (476). The above results follow upon single injections. In contrast to the characteristic fibrin deposit, another type of deposit has been reported, and demonstrated with excellent color photography (608). It is laminated, and the laminations are of different ages, corresponding probably to the different times of the repeated thromboplastin injections.

Fibrinogen. This protein becomes the fibrin clot in the blood coagulation process. It has been made available by the methods of plasma protein separation of Cohn and associates (112). The preparation contains the protein in concentrated, dry form and is thus stable for long periods. Its usefulness in human cases of afibrinogenemia has been reported (363, 599, 600). A person of average body weight can be expected to have about seven to ten grams of fibrinogen in the circulation. If this is completely depleted the hemostatic mechanism can be restored by returning the fibrinogen artificially. It is, however, not necessary to have complete restoration. In one instance three grams of fibrinogen and 3000

cc. of blood were administered effectively (600). Two to six grams are recommended by Weiner, Reid and Roby (599). It is of interest that afibrinogenemia is of concern in obstetrical practice (134, 363, 402, 475, 599, 600); apparently placenta abruptio or partial placenta abruptio may first cause intravascular coagulation (475) and, when this is sufficiently extensive, the fibrinogen of the plasma may be completely depleted.

XXI. SUMMARY

The evaluation of the pharmacological actions of drugs on blood coagulation requires a fundamental knowledge of the blood clotting mechanism and the use of analytical technics whose limitations are realized. Many of the data from the papers considered in this review were obtained before much of our current fundamental information was available and before the technics which can be employed today had been devised. As a consequence, a number of these papers are devoid of definitive merit. Therefore, an attempt was made to interpret the vast amount of conflicting data in terms of modern concepts, and to indicate the need and the possibilities for further studies of the influence of drugs on blood coagulation and related phenomena.

3,3′-Methylenebis (4-hydroxycoumarin) or dicumarol decreases the prothrombin concentration of plasma. Its action is probably in the liver, and it may be that the drug itself rather than one of its metabolic derivatives causes the effect. Dicumarol produces a transitory drop in plasma Ac-globulin concentration, and probably affects the plasma concentration of one or more of the new blood coagulation factors which have recently been discovered. Dicumarol is secreted in milk but perhaps not in sufficient quantity to be of therapeutic significance. There is no liver damage caused by the drug. In this respect, and apparently in all other respects also, the metabolic action is completely reversible. Massive doses of vitamin K can greatly accelerate recovery from the effects of dicumarol. This generalization includes the action of both the water-soluble and the fat-soluble forms of the vitamin. The intravenous administration of purified prothrombin restores the prothrombin concentration of the blood to the normal level.

Several compounds related to dicumarol produce essentially the same qualitative effects as produced by dicumarol itself. Quantitative differences are, however, found. These pertain to the rapidity with which the prothrombin concentration falls when the compound is administered and the rapidity with which the prothrombin concentration returns to normal when the compound is withdrawn. Species differences in response to compounds related to dicumarol have been described; for example, rats are especially sensitive to warfarin and, therefore, it is a valuable rodenticide.

In large quantities the salicylates may produce a hypoprothrombinemia, and this effect is quite readily prevented or reversed by vitamin K. The methylxanthines, given in relatively large doses, cause the blood to be hypercoagulable. One of the contributing factors in this effect is an increase in plasma Ac-globulin concentration.

The antibiotics penicillin, streptomycin, and aureomycin have been reported to produce hypercoagulable blood. The validity of this conclusion must be questioned, since there is no satisfactory knowledge about their action on blood coagulation.

When injected intravenously, protamine rapidly neutralizes the effect of injected heparin. The protamine itself disappears from the blood within a few hours. *In vitro*, protamine precipitates fibrinogen and profibrinolysin. In contrast to protamine the antiheparin effect of toluidine blue develops slowly but can still be detected 24 hours after an initial injection.

The bulk of evidence indicates that the cardiac glycosides produce hypercoagulable blood. This might be due to an increase in plasma Ac-globulin concentration. It has been stated that alpha tocopherol, by its action on plasma antithrombin, lowers the incidence of thrombosis. The validity of this claim rests largely upon clinical observations. Laboratory data do not supply an adequate theoretical basis to support the claim made for the effect of alpha tocopherol.

Heparin produces its anticoagulant action *in vivo* immediately. It has a twofold effect on the blood clotting mechanism. It blocks the activation of prothrombin and probably interferes with the action of thrombin on fibrinogen. Its duration of action, as measured by clotting effects, is limited to several hours. However, respository forms of heparin greatly prolong the action. The chief difficulty with synthetic heparin-like compounds has been their toxicity; however, a polysulfuric acid ester of polyanhydromanuronic acid has qualified for clinical trial.

There are certain nutritional factors which influence the coagulability of the blood, independently of vitamin K action. The sulfonamides, however, produce their effect by eliminating the intestinal bacteria which produce vitamin K.

From the available literature it is not possible to reach a conclusion concerning the effect of the adrenocorticotropic hormone. Probably hypercoagulability is one of its most important side effects. With the exception of chloroform, the anesthetics and respiratory gases do not produce profound effects. Deep chloroform anesthesia causes a decrease in the prothrombin, Ac-globulin and fibrinogen concentrations of the blood. This is the result of liver damage.

Under appropriate conditions epinephrine produces hypercoagulability of the blood. Acetylcholine, on the other hand, probably has the opposite effect and can apparently alter the blood so that fibrinolysis occurs in blood taken from the veins after acetylcholine-induced shock.

Some of the blood coagulation components have been prepared in concentrated form and their pharmacological effects have been studied in some detail, though not adequately as yet. Among these are thrombin, prothrombin, streptokinase-streptodornase, and thromboplastin.

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