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THE ANTI-COAGULANTS

Heparin and the Dicoumarin-3, 3′ Methylene-Bis-(4-Hydroxycoumarin)*

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THE problem of inhibition of coagulation has been the subject of numerous and frequently conflicting reports during the past two decades. From this material pertinent findings will be briefly reviewed in order to present a background for the consideration of Heparin and the Dicoumarin-3, 3′ Methylene-Bis-(4-Hydroxycoumarin). The conclusions suggested should probably be considered as representing current opinion rather than final truth.

Prothrombin, a protein normally present in the blood plasma in a concentration of approximately 40 mg. per cent,[1] is quantitatively converted into thrombin by the action of thrombokinase and ionic calcium.

Prothrombin is the component chiefly affected by 3, 3′ methylene-bis-(4-hydroxycoumarin),[2] a clotting inhibitor which will be discussed at length later. We shall, therefore, dwell upon it briefly. The liver is

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the chief source of prothrombin.[3,4,5,6] Vitamin K appears to be important in its formation.

Hypoprothrombinemia may occur in conditions associated with insufficient vitamin K intake, absorption and utilization. Reduction in prothrombin concentration to below 35 per cent of normal, will, in many instances, produce a hemorrhagic state. Avitaminosis K on the basis of dietary insufficiency rarely occurs, possibly due to the capacity of the intestinal flora to synthesize the vitamin when such a deficiency exists.[7] Absorption of vitamin K is contingent upon an adequate concentration of bile salts in the gastrointestinal tract,[8,9] hence obstructive jaundice and biliary fistulae are frequent causes of hypoprothrombinemia. Diminished prothrombin concentration occurs in ulcerative colitis, sprue, intestinal obstruction and polyposis,[10,11,12,13] due to interference with absorption of vitamin K through the intestinal wall.

Prothrombin deficit despite adequate vitamin K intake and absorption may occur in hepatic derangements.[13,14,15,16,17,18] Hepatic intoxications (due to carbon tetrachloride,[19] chloroform,[3] and phosphorus), hepatectomy[5] and extensive liver damage on other bases[13,17,18] result in a hypoprothrombinemia in no way influenced by the administration of large doses of vitamin K. DeLor and Reinhart[20] observed prothrombin to be universally diminished when liver function fell below 50 per cent, as determined by the hippuric acid method.

Calcium, as ionic calcium, hastens the conversion of prothrombin to thrombin.[21] For calcium deficiency to significantly inhibit coagulation, however, serum calcium would have to fall below the lowest recorded level compatible with life.[22]

Thrombokinase is a lipoprotein closely allied to cephalin. In combination with calcium it is considered by Eagle[23] to constitute a proteolytic enzyme analogous to trypsin, which reacts with prothrombin to form thrombin. Crude or crystalline trypsin[24] and proteolytic snake venoms[25] can be substituted for calcium-platelet or calcium-tissue-extract mixture in the activation of prothrombin in vitro. The view that activation of prothrombin involves proteolysis would seem to be supported by these reactions. Thrombokinase is present in all tissues; the lung, platelets, and brain being its richest sources. Thrombokinase affects only the velocity of the prothrombin to thrombin conversion not the quantity of the thrombin derived from prothrombin.[26,42] Heparin antagonizes thrombokinase,[27] blood remaining fluid despite the presence

of large quantities of circulating prothrombin and fibrinogen, only as long as the thrombokinase-heparin balance is maintained in favor of heparin, and in all probability certain other anti-coagulants about which little is known.

Thrombin, a protein, is probably a hydrolytic product of prothrombin. Schmidt[28,29] and Eagle[30] believe it to be a proteolytic enzyme which converts fibrinogen into fibrin. Thrombin is capable of converting 2000 times its weight of fibrinogen into fibrin.[30] It may be replaced in the conversion of fibrinogen to fibrin by proteolytic enzymes such as papain[24] and numerous snake venoms (Crotalus adamantus, Crotalus terrificus, Crotalus horridus, Bothrops nummifera, etc.).[25] No apparent stoichiometric relationship exists between thrombin and fibrinogen.

Fibrinogen is a globulin-type protein of special solubilities. It is produced exclusively in the liver. Except in very severe forms of liver disease, the fibrinogen concentration in the blood is maintained at a normal level of 0.2 to 0.3 mg. per cent. Its conversion from the physical state of a disperse hydrosol to a quasi-crystalline fibrin gel is the *sine qua non* of natural blood clotting.

In vivo, anti-coagulants may be arbitrarily grouped in five classes:

1. Substances in nature which have anti-coagulant activity when added to blood; for example, hirudin, an anti-thrombin[31,32] and anti-kinase,[33] derived from the buccal gland of the leech, and snake venoms of the cobra venom type.

2. Anti-coagulant substances isolated from blood or tissue, e.g., heparin.

3. The physiological anti-coagulant presumed to be present because of delayed coagulation of the blood. This is believed by some to be heparin.[34] Others contend that it is an anti-thrombin distinct from heparin.

4. Substances which, when ingested, either depress formation of one of the components of the coagulation mechanism or render it inactive, e.g., dicoumarin.

5. Synthetic substances, which, when added to blood, inhibit coagulation; e.g., the diazo dyes (Chicago blue, chlorazol fast pink, sodium thiosulfate, trypan blue), germanin and liquoid.[35,36,37,41,50]

Since it is beyond the scope of this paper to discuss in detail the mechanism of, and the clinical results obtained with each of these substances, the major portion of the discussion will be devoted to heparin,

and dicoumarin, a recently isolated clot inhibitor which has been the subject of investigation in the Department of Medicine of the New York Post-Graduate Medical School and Hospital during the past year.

The limitations of hirudin which render its clinical use impracticable are its low potency, as compared to heparin, and its limited supply.

The synthetic in vivo anti-coagulants are similar to heparin in their mode of action.[38] With the exception of chlorazol fast pink, a very narrow margin exists between the therapeutically effective and the toxic dose. The anti-coagulant activity of chlorazol fast pink has been found to be 10 to 15 times less than that of heparin.

Heparin

Heparin is a strongly acidic compound containing mucoitin polysulfuric acid, acetic and glycuronic acid plus a base glucosamine.[39] It inhibits the coagulation of blood in vitro or in vivo by retarding the conversion of prothrombin to thrombin, and by direct antithrombic activity.[42] Heparin arises in the mast cells of Ehrlich[40] which are found chiefly in the vicinity of the finer blood vessels.[40] Jorpes[34] concluded from the location of the mast cells that they constitute a hormonal system feeding heparin to the blood. The liver capsule and the lung are the richest reservoirs of heparin in the body. Appreciable amounts have also been extracted from the subcutaneous tissue and the blood vessels. Histologically, heparin inclusions in the mast cells may be detected by the characteristic metachromatic stain which they yield with toluidine blue.

Ferguson[42] has demonstrated that heparin possesses two distinct actions in the first phase of the clotting mechanism. First, it retards the rate of prothrombin conversion to an extent which is inversely proportional to the amount of the thrombokinase present. Second, there is an effect on the amount (effectiveness) of the thrombin formed.[43] Heparin apparently not only inhibits coagulation by retarding the formation of thrombin, but also by acting as an anti-thrombin.[22,44,45] Quick,[46] Brinkhous,[47] Jaques and Mustard[48] have demonstrated that heparin is only active in the presence of a co-factor which is part of the albumin fraction. In their opinion, heparin combines with and enhances the action of normal plasma anti-thrombin. Trypsin[49] and thrombokinase[27] directly antagonize heparin, coagulation being prevented unless an excess of these substances is present. Many clot inhibitors resemble heparin

in their mode of action and in the presence of sulfur in the molecules. For example, sulfo-cellulose, hermophenyl, liquoid, germanin, diazo dyes, cystine, and sodium thiosulfate, like heparin, exhibit an anti-coagulative activity proportional to their sulfur content.[38,39,40,50] Lindgren and Wilander[51] and Hedenius[52] observed that with heparin the coagulation time may be maintained at optimal levels of twenty to thirty minutes without influencing the bleeding time as determined by the Ivy method. Best[53] demonstrated, by means of a glass cell technique, that platelet agglutination, as well as fibrin deposition, was inhibited by heparinization. Subsequently, Solandt and Best[54] presented evidence that very extensive injury to the arteries and veins never resulted in a maximal stimulus to platelet agglutination. The amount of heparin required to prevent platelet thrombi in vivo was found to be much smaller than that required to prevent the process in a glass cell. Salmine-sulfuric acid, a protamine, neutralizes the effect of twice the amount of heparin in vitro and in vivo. An intravenous injection of this protamine will immediately check any undesirable bleeding produced by heparin.[55,56]

Preparations: The products available for clinical use are:

1. Heparin—Lederle 10 cc. vial—10 mg./cc.-1100 Toronto u/cc.
2. Liquemin—Roche Organon 10 cc. vial—10 mg./cc.-1100 Toronto u/cc.
3. Solution of heparin—Connaught Laboratories 10 cc. vial—10 mg./cc.-1100 Toronto u/cc.

These products are at present so standardized that each cc. keeps 5000 cc. of plasma in vitro in a liquid state for four hours at 37° C.

Methods: The methods of heparinization in use today are local, regional and general. Local and regional heparinization are used chiefly in vascular surgery in order to confine the anti-coagulant effect to the operative area. In the treatment of thrombosis and thrombo-embolic conditions, general heparinization is the method of choice. This may be achieved by either of two techniques: (1) By the continuous intravenous drip; and (2) by multiple intravenous injection. In the first, advocated by Best and Murray and utilized widely in this country, the appropriate amount of heparin is added to an infusion which is permitted to flow continuously throughout the course of therapy into the selected vein. The choice of diluent may be modified by the individual case, heparin being equally active in five per cent glucose solution,

normal saline, or Ringer's solution. Should fluid restriction be imperative, the entire daily dose may be given in as little as 800 to 1000 cc. The amount of heparin required to secure an arbitrary optimal prolongation of coagulation time, namely, twenty to thirty minutes, varies according to the response of the individual patient. Twenty to thirty mg. per hour usually suffice to maintain this level. Variation in response in different patients and in the same patients from day to day will of necessity modify the dose. In our opinion coagulation times in excess of forty minutes are hazardous. Maintenance of a coagulation time of twenty to thirty minutes is insured by repeated estimations of the venous blood clotting time at four hour intervals by means of the Lee-White two tube method. The intermittent intravenous injection method has been used almost exclusively by Crafoord,[57] Jorpes,[34] and Lindgren and Wilander[51] since 1936. These observers have been using doses of from 50 to 75 mg. three times daily at 8 A.M., noon and 4 P.M. with an evening dose of 100 to 125 mg. at 8 P.M. Appropriate adjustments in the dosage are made as indicated by the coagulation time obtained. The marked fluctuations in coagulation time encountered with this method render dosage estimation problematical. Dosage should theoretically be so regulated that the coagulation time never falls below 15 minutes, but this is very difficult to achieve with this technique. The disadvantage of multiple venipuncture may be obviated by employing a specially constructed needle designed by Olovson.[58] This needle is allowed to remain in situ throughout the course of therapy. It is supported by an aliform plate which is fixed to the arm by adhesive tape and is provided with a detachable cap with a rubber membrane through which the injections may be made even while the patient is sleeping. Coagulation has never been observed in the needle during the course of treatment.[59]

Certain theoretical disadvantages are inherent in this method of heparin administration. Chief among these is the impossibility of maintaining the more or less constant elevation of coagulation time secured with the continuous intravenous drip. Secondly, the coagulation time immediately following injection of undiluted heparin attains levels, which may be hazardous, of one hour or more. Subsequent to the initial abrupt rise, there is a steady fall over a four hour interval to as low as eight to ten minutes in some cases. The foregoing drawbacks notwithstanding, these authors have reported satisfactory results with the intermittent intravenous injection technique.

Heparin has also been administered by the subcutaneous route. The magnitude of the doses required to obtain the desired levels with this method renders it impracticable.

Because of the voluminous available literature concerning the indications for the therapeutic and prophylactic use of heparin, we shall confine ourselves to a brief summary of recent clinical and experimental developments. Regional and general heparinization have proved invaluable in maintaining vessel patency following arteriotomy for the removal of thrombi and emboli. Murray,[60] Lam,[61] Lindgren and Wilander[51] have reported successful embolectomies with this technique. Pratt, of our service, has obtained similar results with heparin in six embolectomies and in twelve other instances of vascular surgery, hitherto usually unsuccessful because of the rapidity with which the repaired vessel became obstructed by thrombi, during the postoperative period. In mesenteric thrombosis, with a mortality rate of from 85 to 95 per cent, heparin was employed successfully by Murray[60,62] in six cases. Ravdin[63] reported comparable results in two instances. Holmin and Ploman[64] demonstrated that heparinization of patients with thrombosis of the central retinal vein appreciably reduced the incidence of blindness following this disease. This observation has been confirmed by Boström and Olsson,[65] Rea[66] and other ophthalmologists. Whipple[67] and Murray[68] observed that postoperative heparinization will prevent the portal thrombosis which frequently follows splenectomy for Banti's syndrome and familial jaundice. Recovery in patients with massive pulmonary embolization appears to be influenced by heparin therapy. The authors have successfully treated five such patients in whom the initial prognosis was extremely grave. Murray,[68] Ravdin,[63] Clason,[69] Rosenqvist[59] and Priestley[70] have reported similar experiences, observing likewise a marked diminution in morbidity as well as mortality. The fact that spontaneous recovery does occur in approximately 12.2 per cent of cases of pulmonary embolization, according to Barker's[71] statistics, renders observation of a large series desirable before any conclusion can be drawn as to the efficacy of this form of therapy. The treatment of subacute bacterial endocarditis with heparin and sulfonamide derivatives has yielded encouraging results in a few of the cases reported by Kelson and White.[72] In the case reported by Dockeray and Kawerau,[73] which terminated in a fatal cerebral hemorrhage, heparin was only of transient value. Other reports[74,75,76,77] which ap-

pear in the literature reveal that fatal cerebral hemorrhage following embolization not infrequently occurs in patients receiving combined heparin and sulfanilamide therapy for subacute bacterial endocarditis. The value of this combined therapy is yet to be determined. It has failed in nine cases on our service.

The value of heparin therapy in coronary thrombosis is still speculative. Solandt and Best[78] showed that preliminary heparinization will prevent thrombosis of coronary arteries injected with sodium ricinoleate. This work seems to indicate that if administered early, and in adequate dosage, heparin may minimize or inhibit propagation of the thrombus. At present, however, the prevention of mural thrombi seems to be its sole virtue in this disease.

Lyons,[79] Schall,[80] Ershler and Blaisdell[81] have reported the successful treatment of cavernous sinus thrombosis by combining heparin with chemotherapy.

Marked improvement in a single case of thrombosis of the posterior inferior cerebellar artery (Wallenberg syndrome) following heparinization has been reported by Magnusson.[82]

In a series of 627 patients reported by Crafoord,[57,83] 325 who had received heparin prophylactically during the postoperative period showed a complete absence of thrombo-embolic phenomena. The remaining 302 patients subjected to comparable operative procedures, who had not received the anti-coagulant, showed an incidence of thrombo-embolic complications of approximately 9 per cent. (This figure is considerably greater than that encountered in this country.) Nine of this latter group died. Diagnosis was confirmed by autopsy. Similar observations supporting the efficacy of heparin as a prophylactic against thrombosis following obstetric and gynecologic procedures have been reported by Wetterdal,[84] Leissner[85] and others.

The numerous variations in the clinical picture accompanying thrombophlebitis, and the frequently insidious onset of this condition account in some measure for failure of its early detection. Most cases, if diagnosed in their incipiency, respond favorably to heparinization. Localization of the thrombus to its original site, accompanied by marked diminution in embolic complications, morbidity and mortality, are the advantages reported by numerous observers. On the other hand, the difficulty inherent in its use, together with other objections to be discussed later, have led many groups to confine its use

in thrombophlebitis to those patients who have suffered one pulmonary embolus. Venography in suspected cases, according to Bauer's[86] recent report, gives promise of affording a means of earlier and more certain recognition of incipient thrombophlebitis.

An interesting recent development in heparin therapy, while not related to the cardiovascular system, is worthy of mention. This is the prevention of adhesions by instillation of heparin into the peritoneal cavity of rabbits subjected to contamination and trauma.[87] In dogs, the recurrence of divided adhesions was 6 times greater in the non-heparinized group as compared to that in heparinized animals. Wright and Hinton[88] have tried this in man with apparently good results. Definite conclusions as to the efficacy of intraperitoneal heparinization in man cannot yet be drawn inasmuch as no opportunity to reëxamine the abdominal cavity has presented itself. Some danger of hemorrhage following closure does exist, when the initial contraction of the small vessels wears off three to four hours postoperatively; hence routine use of heparin intraperitoneally is not to be recommended at this time.

There exists some difference of opinion as to how long heparin therapy should be continued. Following vascular surgical procedures in which the lumen is kept patent by heparinization, the site of arterial suture has been found experimentally[68] to be healed after 72 hours. Murray[68] and Lindgren[51] prefer to continue heparinization postoperatively until the patient is out of bed. In thrombophlebitis Bauer[86] discontinues therapy after five days in abortive cases; in certain instances he has withheld heparin as soon as the temperature and pulse returned to normal. Hedenius[52] proposed four days as the minimum effective period of therapy. In our experience such periods have frequently proven too short. In the majority of cases of thrombophlebitis the intravascular inflammatory process persists despite inhibition of thrombus propagation. We feel, therefore, that in order to minimize the tendency to relapse the administration of heparin in quantities adequate to maintain coagulation time at optimum levels should be continued over a period of from twelve to fourteen days. Withdrawal of heparin is followed by a so-called negative phase during which the blood exhibits increased coaguability. For this reason heparin should never be discontinued abruptly but rather by a successive slow diminution in dosage. On our service the following criteria have been adopted as evidence of cessation of the intravascular process in thrombophlebitis: Return of

temperature and pulse to normal for one week; complete disappearance of local tenderness, pain, and erythema along the course of the involved vessel over a similar period of time; the sedimentation rate and leukocyte count, if elevated, should likewise return to and maintain a normal level for one week; reduction of edema to a minimum is desirable. Strict adherence to the above routine has reduced, but not eliminated, the incidence of reactivation of the acute process after discontinuing heparin. As a rule, the briefer the period of heparinization the greater the possibility of recurrence. Reactivation of acute thrombophlebitis may definitely occur following heparinization. In an experience involving the heparinization of approximately 100 patients, the following examples have been encountered. Three patients in whom heparin therapy was maintained for two to five days following satisfactory clinical evidence of subsidence, showed reactivation shortly after cessation. Two others had recurrences after heparinization for ten days; two more showed reactivation of the process following fourteen days of heparin therapy, one of whom suffered a second recurrence following an additional fourteen days of treatment. We have also seen two instances of non-fatal pulmonary embolization while heparin therapy was maintaining the clotting time at levels of twenty-six to thirty minutes.

Complications: In 315 cases reported by Murray and Best[68] four developed hematomata of the wound. Priestley, Essex and Barker[70] observed transient hematuria in several of their forty-five patients. Ershler and Blaisdell[81] and Witts[76] reported the occurrence of massive hematuria following heparinization. Lam[61] in his series of thirty cases noted evidence of a hemorrhagic diathesis in four patients. Kelson,[72] Fletcher,[75] Friedman,[77] Witts[76] and Miller[74] have reported deaths as the result of cerebral hemorrhage following treatment of subacute bacterial endocarditis with heparin and sulfonamide drugs.

Although heparin is a valuable agent in anti-coagulant therapy, it does have certain disadvantages. Chief among these are:

1. Difficulty in administration with its attendant discomfort to the patient.
2. Costliness: Fifteen to twenty dollars worth of heparin is required daily in the average case.
3. Prolonged administration.
4. Occasional failures.

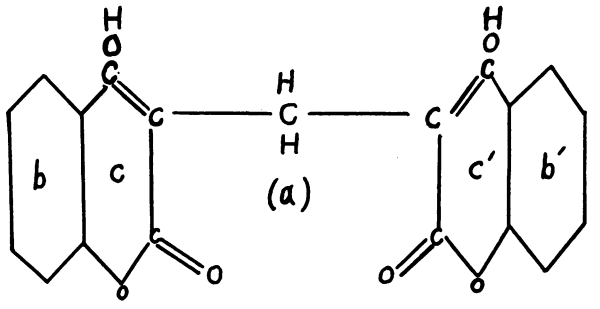

Fig. 1—Structural formula for dicoumarin 3, 3'-methylene-bis-(4-hydroxycoumarin).

Added to these are the dangers inherent in all types of anti-coagulant therapy, unless scrupulous control is observed.

Dicoumarin*

The isolation and synthesis of the dicoumarin 3, 3' methylene-bis-(4-hydroxycoumarin) by Link[89] and his co-workers in 1940 provided a new and valuable substance for workers interested in the field of anti-coagulants. Rather exhaustive studies in which this substance has been administered to rabbits, dogs, and other animals have been reported by Link[89] and his co-workers, and by Bingham, Meyer and Pohle.[106] As may be anticipated from a consideration of the structural formula shown in Figure 1, 150 substitution and degradation products of 3, 3' methylene-bis-(4-hydroxycoumarin) have been isolated by Link's group,[90] more than 40 of which show anti-coagulant properties as measured by coagulation and prothrombin studies.

Dicoumarin 3, 3' methylene-bis-(4-hydroxycoumarin), when fed to susceptible animals, diminishes the clotting power of the blood and, in some cases, produces fatal hemorrhage. In nature, this compound occurs in spoiled sweet clover silage. It was for this reason that Roderick[96,97,98] and Schofield[94,95] used the name "sweet clover disease" to describe the hemorrhagic diathesis resulting in cattle from the ingestion of spoiled sweet clover hay. The coagulating power of the blood can be restored by discontinuing the administration of the hemorrhagic agent, or by injection of serum or whole blood freshly drawn from

* The Dicoumarin used in this study was supplied through the courtesy of Dr. Karl Paul Link, University of Wisconsin, and Dr. Y. Subbarow of the Lederle Laboratories.

cattle. Quick[99] confirmed Roderick's[97] observation that the coagulation defect in animals with sweet clover disease was due to a hypoprothrombinemia, the mechanism of which was, and still is, unknown.

Link[89,90,91,92,93] and his co-workers completed the isolation and synthesis of this dicoumarin in April 1940. The pharmacological activity of 3, 3′ methylene-bis-(4-hydroxycoumarin) has been established in animals by Link,[105] Bingham,[106] and Butt[107] with their associates. Link and his group have demonstrated that within certain limits, increasing the size of a single dose increases the degree and prolongs the time of reaction in standardized susceptible rabbits. These observers likewise demonstrated that dogs survived large single doses, whereas in some cases repeated divided doses aggregating less than the large single dose, produced toxicity and death. Link[109] suggests incomplete absorption from the gastrointestinal tract as an explanation of this variation in response.

Postmortem studies on animals that had succumbed to hemorrhage produced by the ingestion of spoiled sweet clover, were first made by Roderick[96] who noted that hemorrhage may occur in any part of the body, but most frequently at points of stress, in the subcutaneous and intermuscular fasciae. No changes in the blood vessels which could explain the hemorrhagic tendency were observed. Hemorrhage was, however, easily produced prior to death by surgical incision or by trauma. In October 1941, Bingham, Meyer and Pohle[106] reported the results of similar studies on twenty-five dogs which had received hemorrhagic doses of the synthetic dicoumarin. Their observations revealed that hemorrhage had occurred in the subcutaneous and intermuscular fasciae in all the animals and that gross gastrointestinal hemorrhage and bleeding into the pleural spaces and pulmonary parenchyma were frequently observed. Marked dilatation of capillaries, small arteries and veins was also noted. No lesions of the vessel walls were found which could explain the mechanism whereby the blood was extravasated. Another interesting observation made by these authors was the absence of significant gross or microscopic evidence of parenchymatous disease of the liver, kidney or other organs. Occasional moderate hydropic degeneration of the liver was observed, but nothing more. Hydropic degeneration is known to occur post mortem, depending upon the time which has elapsed between death and tissue fixation. Roderick had previously reported liver damage as an occasional pathologic finding

in cattle which died from this disease.

Studies on the response to 3, 3′ methylene-bis-(4-hydroxycoumarin) administered orally to human subjects have been reported by Butt, Allen and Bollman,[107] Bingham, Meyer and Pohle[106] and the authors. Bingham[106] and his co-workers have also used the disodium salt of the dicoumarin intravenously. Characteristic prolongation of the prothrombin and coagulation times, similar to those observed in animals, have been noted, by all workers. The present report on the effects of the dicoumarin 3, 3′ methylene-bis-(4-hydroxycoumarin) includes a description of the hemorrhagic complications which were encountered in our series in man, but which neither Bingham et al[106] nor Butt et al[107] have previously mentioned. An attempt has been made, therefore, to ascertain the nature of this variation in response.

Our objectives have been to study the reactions produced in man by this substance, the mechanism involved therein and to determine the minimum effective dose required in humans to produce an effect on the coagulation time comparable to that of heparin. In this the prothrombin reduction plays an important role. To date this dicoumarin has been administered to thirty-one patients. Detailed studies regarding twenty of these patients have been analyzed and embodied in this report. The age of the patients varied from 22 to 83 years. The distribution according to admission diagnosis is as follows:

Thromboangiitis obliterans	2
Rheumatic cardiovascular disease	2
Thrombophlebitis	4
Arteriosclerosis obliterans	12

Prior to administration of the medication, a thorough history was taken and a complete physical examination done in order to eliminate preëxisting hemorrhagic diathesis, and to avoid the possibility of causing uncontrollable bleeding from peptic ulcers, fibromyomata or other hitherto quiescent foci of potential hemorrhage.

Blood counts, including red and white cell counts with differential and hemoglobin determinations, were done on all cases before administration of the drug and at weekly intervals thereafter until prothrombin time returned to normal. Because of the wide variations obtained in platelet determinations during the control period and the feeling among hematologists that most platelet counts using available techniques are at best inaccurate, this procedure was discontinued after a trial.

Capillary fragility tests, using the method described by Wright and Lilienfeld,[108] were performed prior to administration of the dicoumarin, during the course of therapy, when prothrombin time exceeded 35 seconds, and when and if hemorrhagic manifestations appeared.

Sedimentation rates were determined by the Westergren[110] method prior to administration of the drug and at weekly intervals thereafter.

Routine urinalysis was performed three times weekly during the control period and during administration of the dicoumarin.

An attempt to estimate renal status was made on the basis of urea clearance tests, blood urea and non-protein nitrogen ratio and renal concentration tests (2-hour method described by Mosenthal).[117] These were done prior to administration of the medication with the object of reducing the possibility of retention toxicity, such as that encountered at times with other types of chemotherapy.

An evaluation of the *hepatic status* was made on the basis of the bromsulfalein test (Magath and Snell modification),[116] plasma proteins, cholesterol ester ratio and cephalin flocculation test.

Gastric fractional analysis, using parenteral histamine, was done once during the period of observation, following the suggestion of Link[109] that gastric anacidity might hasten absorption of the dicoumarin.

Prothrombin time was determined daily, using the Fullerton[111] modification of the Quick technique. Normal plasma with this method gave values of 20 seconds plus or minus 2. Daily controls performed simultaneously served as a check on the activity of the thromboplastic substance used.

Coagulation time was estimated by the Lee-White[115] two tube method with normals ranging between 5.5 and 7.5 minutes.

A three day control period preceded the administration of the drug in all cases. During this time base line coagulation and prothrombin times were ascertained.

Dosage: At the outset of our experiments no data on the dosage of this dicoumarin in humans were available. Anticipating the possibility of hemorrhagic complications, we began with doses which, on the body weight basis, were but a fraction of those which had produced prolongation of coagulation and prothrombin time in animals. Five patients received daily single, oral doses of 100 mg. for seven days followed by doses of 200 mg. at daily intervals for periods varying from four to eleven days. Of this group two patients received a total of 1500

mg. over an eleven day period; one patient a total of 1700 mg. over a twelve day period; and two a total of 2900 mg. over a period of eighteen days. Four patients received single oral doses of 200 mg. at twenty-four hour intervals for periods varying from four to eleven days. Total doses in this group were as follows: 800 mg., 1200 mg., 1800 mg., and 2200 mg.

Three patients received a series of four 300 mg. doses at twenty-four hour intervals and one patient a series of five 300 mg. doses, totaling 1200 and 1500 mg. respectively. The remaining patients received an initial dose of 600 mg. of the dicoumarin, followed in all but one case by daily doses of 200-300 mg. for periods varying from five to eight days. The amount of the dicoumarin administered to these patients total 600, 2200, 1800, 1800, 1500, 1500 and 2400 mg. The dose of the dicoumarin was administered in all instances at twenty-four hour intervals. Total dose of the body weight basis varied from 7.8 to 45.9 mg./kg.

Responses in the form of prolongation of the prothrombin and coagulation time varying widely in degree and duration were observed in patients receiving different doses and in different patients receiving the same total dose. We shall briefly outline the result of an analysis of the coagulation and prothrombin time curves obtained.

The initial effect manifested itself as a significant prolongation of prothrombin time which occurred in from one to five days, average 3.2 days. A change equivalent to three times the standard deviation for accuracy of the method was considered significant.

The maximal effect, namely, the greatest increase in prothrombin time following the first administration of dicoumarin, occurred in from one to twenty days, with an approximate average of thirteen days.

The coagulation time at the period of maximal prolongation varied from eight to thirty-three minutes, averaging thirteen minutes.

The maximal prolongation of prothrombin time ranged from twenty-six to seventy seconds, with an average of forty-seven seconds.

The total duration of effect, or time lapsing between the initial rise in the prothrombin time and its return to control level, was two to twenty-six days, averaging 11.2 days.

The duration of effect, after discontinuing the administration of dicoumarin, varied between one and twenty-three days, average 11.2 days. As a rule the degree and duration of prolongation of coagulation

TABLE I

SUMMARY OF FINDINGS ON PATIENTS IN WHOM A HEMORRHAGIC SYNDROME WAS PRODUCED BY DICOUMARIN

Case No.	Age	Total Dose		Duration of Therapy	Day on Which Hemorrhage Appeared	Proth Time During Hemorrhage	Coag Time (Lee-White) During Hemorrhagic Episode	Duration of Hemorr. Manifest.	Treatment	Signs and Symptoms
		gm.	mg/kg							
1	25	1.5g	27.2	11 days	16	54-66 sec	12-17 min	4 days	Withdrawal of Dicoumarin	(1) Gingival bleeding (2) Weakness—Vertigo
4	37	1.7g	27.7	12 days	14	34-53 sec	15-16 min	7 days	(1) Transfusion T (2) Vitamin K.C.P. (3) Nicotinic Acid (4) Supportive	(1) Spontaneous hemorrhage from wound (2) Weakness (3) Gingival hemorrhage, sublingual ecchymosis (4) Hematuria (5) Subconjunctival hemorrhage (6) Hematemesis (7) Purpura
5	45	2.9g	36.4	18 days	19	40-70 sec	16-33 min	5 days	(1) Transfusion T (2) Vitamin K.C.P. (3) Nicotinic Acid (4) Supportive	(1) Spontaneous bleeding from wound (2) Weakness (3) Purpura over trunk and extremities (4) Hematuria (5) Pharyngeal & Sublingual ecchymoses (6) Subconjunctival & Gingival hemorrhage
6	60	2.2g	38.1	11 days	12	34-48 sec	14-18 min	8 days	Withdrawal of Dicoumarin	(1) Spontaneous bleeding from wound (2) Purpura over trunk and extremities (3) Weakness and lassitude (4) Microscopic hematuria
9	60	1.2g	18.6	6 days	12	48-56 sec	12.5-22 min	10 days	(1) Transfusion T (2) Vitamin K.C.P. (3) Nicotinic Acid (4) Supportive	(1) Hematuria (2) Hematemesis (3) Sublingual ecchymosis (4) Epistaxis (5) Gingival hemorrhage (6) Weakness
10	67	1.2g	15.5	4 days	12	40-42 sec	11.5-13.5 min	5 days	(1) Vitamin K.C.P. (2) Nicotinic Acid (3) Supportive	(1) Subconjunctival hemorrhage (2) Weakness—Vertigo (3) Epistaxis
16	69	1.8g	28.5	5 days	16	38.5-53.5 sec	22-26 min	4 days	(1) Transfusion T (2) Vitamin K.C.P. (3) Nicotinic Acid	(1) Syncope (2) Hematemesis (3) Purpura over trunk & extremities
19	54	1.5g	23.6	5 days	5	38-40 sec	10-17 min	4 days	(1) Transfusion T (2) Vitamin C.P. (3) Nicotinic Acid	(1) Hematemesis (2) Weakness

and prothrombin time varied directly with the magnitude of the dose and the period of time over which it was administered.

Complications: Various manifestations of a hemorrhagic tendency made their appearance in eight of the twenty patients in this series. They were as follows: Purpura 4; sublingual ecchymosis 3; conjunctival hemorrhage 3; gingival hemorrhage 4; epistaxis 2; hematuria 3; spontaneous bleeding at wound site 3; vertigo 2; hematemesis 4; weakness and lassitude 7. There were variations in extent, intensity and duration of these signs and symptoms.

Treatment of the hemorrhagic tendency: Two patients received only supportive therapy, in order to evaluate the efficacy of the various therapeutic agents employed. Five patients received daily doses of 6.4 to 12.8 mg. of 2 methyl 1, -4 naphthohydroquinone 3 sodium sulfonate, and 300 mg. of cevitamic acid parenterally in addition to 150 mg. of nicotinic acid and the grated peel of four oranges daily by the oral route. One patient received only cevitamic acid, nicotinic acid and vitamin P in the above doses. Five patients received transfusions of 250-500 cc. of whole citrated blood.

Effect of vitamins K, C, P and Nicotinic acid.[112] Synthetic vitamin K substitute in doses substantially greater than those proven to be therapeutically effective in hypoprothrombinemias due to other causes, were ineffective in lowering the prothrombin time or controlling hemorrhage produced by the administration of the dicoumarin. A further report on the effect of vitamin K substitutes on the hypoprothrombinemia induced by administration of a 3, 3′ methylene-bis-(4-hydroxycoumarin) will be embodied in a paper now in preparation. Vitamins C and P and nicotinic acid likewise exerted no detectable influence on the hemorrhagic tendency.

Effect of Transfusion. In patients showing hemorrhagic complications transfusion produced varying results. In two instances four day old bank blood was found to be ineffectual in the control of hemorrhage. Transfusion with fresh whole blood resulted in a fall in prothrombin time to a point below the hemorrhagic level, with complete cessation of hemorrhage in one case. Four other patients with hemorrhagic complications showed a temporary fall in prothrombin time with diminution in the severity of hemorrhages following transfusion. In these cases there was a subsequent prolongation of prothrombin time with recurrence of spontaneous hemorrhage requiring repetition of transfusion before control conditions were restored (cf. Table I).

TABLE II

INDICATING LACK OF RELATIONSHIP OF AGE, WEIGHT AND DOSE OF DICOUMARIN TO HEMORRHAGIC COMPLICATIONS IN 20 PATIENTS

HEMORRHAGIC GROUP						NON-HEMORRHAGIC GROUP					
Case No.	*Age yr.*	*Body Wt. Kg*	*Total Dose*		*Duration of Therapy Days*	*Case No.*	*Age yr.*	*Body Wt. Kg*	*Total Dose*		*Duration of Therapy Days*
			gm.	*mg/kg*					*gm.*	*mg/kg*	
1	25	55.0	1.5	27.2	11	2	53	73.6	2.9	39.4	18
4	37	61.3	1.7	27.7	12	3	43	85.9	1.5	17.4	9
5	45	79.5	2.9	36.4	18	7	69	51.8	1.8	34.7	9
6	60	57.7	2.2	38.1	11	8	70	68.6	.8	11.6	4
9	60	64.5	1.2	18.6	6	11	68	75.4	1.2	15.9	4
10	67	77.2	1.2	15.5	4	12	63	76 3	.6	7.8	1
16	69	63.1	1.8	28.5	5	13	68	74.5	2 2	29.5	10
18	54	63.5	1.5	23.5	5	14	48	83.1	1.5	18.0	5
						15	50	55.4	1.2	21.6	4
						17	72	56.8	1.8	31.7	5
						19	22	52.2	2.4	45.9	8
						20	82	46.3	1.5	32.3	5

DISCUSSION

Dose: There was no apparent correlation between the incidence of hemorrhagic response and the amount of the drug administered (cf., Table II); moreover, comparable degrees of prothrombin- and coagulation-time prolongation were obtained in both bleeding and non-bleeding patients. As would be expected, the period over which the prothrombin time was prolonged was greater in those patients who received the larger doses. Hemorrhagic complications of equal intensity, equally resistant to therapy were noted in patient No. 5 (Wt. 79.5 Kg.) who had received 36.4 mg./Kg. of the dicoumarin and in patient No. 9 (Wt. 64.5 Kg.) who had received only 18.6 mg./Kg.

In patient No. 5, the bleeding tendency was of five days duration, and responded to two transfusions. In patient No. 9, hemorrhagic signs and symptoms persisted over a ten day period, requiring three transfusions for adequate control.

Link[109] and his associates have observed that response to dicoumarin in animals is enhanced by malnutrition, hepatic disorders, anacidity, dehydration, and abnormally high environmental temperature. Bearing in mind these factors, as possible contributory causes to the development of the hemorrhagic diathesis, a careful analysis of available laboratory and clinical data was made which yielded the following observations:

Temperature: During the course of our investigations the room temperature on the hospital wards was at times uncomfortably high, ranging from 80°-90°, although the average temperature during this period as recorded by the U.S. Weather Bureau was 74.7° F. Whether skin capillary dilatation associated with exposure to high environmental temperature, superadded to the widespread capillary relaxation noted by Bingham,[106] was a factor in the production of hemorrhagic manifestations has not yet been ascertained.

Dehydration:[113-114] No evidence of dehydration was present in any of these patients prior to the onset of hemorrhage. Hematocrit, serum proteins, and plasma specific gravities were within normal range.

Anacidity: Gastric analysis in the patients who manifested the hemorrhagic diathesis revealed normal HCl concentration curves in four; hyperchlorhydria in one; and hypochlorhydria in three. Similar studies in non-bleeding patients showed normal HCl concentration curves in six, hypochlorhydria in three, and hyperchlorhydria in two patients.

Age: The age distribution of those patients, with and without hemorrhagic complications, was essentially the same (cf. Table II). Only three female patients were included in the series, hence no deductions regarding the influence of sex were warranted.

Nutritional status as estimated by weight and height tables was good in all but three patients. None of these showed hemorrhagic tendencies.

Capillary fragility: No apparent relationship between hemorrhagic tendency and capillary permeability could be established on the basis of the test used. There was no increase in capillary permeability over control determinations at the time of hemorrhage. Seven of the twenty patients showed abnormal capillary fragility prior to the administration of the dicoumarin. Of this group only two developed hemorrhagic complications which were not associated with a further increase in capillary fragility.

Sedimentation rate remained at control level throughout the period

of investigation in the non-hemorrhagic group. In those patients showing the hemorrhagic tendency an elevation of sedimentation rate occurred with the onset of hemorrhage, persisting until the hemorrhagic tendency subsided, at which time a slow return to control level was observed.

Renal function: Because of the inability of many of our patients to coöperate, studies of renal function by means of the urea clearance test were incomplete. Mosenthal[117] renal concentration tests showed low fixed specific gravity in four patients of the hemorrhagic group, as contrasted with one patient in the group who showed no complications.

Hepatic function: Two patients showed significant bromsulphalein retention after 1 hour. One of these (patient No. 19) showed hemorrhagic signs; the other (patient No. 11) did not. The cephalin flocculation test was positive in two non-hemorrhagic patients and in one patient who exhibited the bleeding tendency.

From the foregoing it is difficult to attribute the hemorrhagic complications observed in this group of patients to any single factor.

The interval of twenty-four to seventy-two hours which transpires before the initial effect of the dicoumarin is observed would seem to suggest that a comparable interval is required for the dicoumarin to be converted into a prothrombin inhibitor, or to exert a direct effect upon the prothrombin-producing function of the liver. Bingham, Meyer and Pohle[106] reported a similar delayed action with intravenous administration. This demonstrates that the lag is not due to retarded absorption. It cannot be stated at this time whether the site of action of dicoumarin is in the liver or not.

The fact that in vitro studies have shown that the dicoumarin, as such, has no anti-coagulant activity would tend to minimize the importance of renal retention as a probable cause of the hemorrhagic diathesis. Undoubtedly, dose-level influences the tendency to hemorrhagic complications, since other observers who administered smaller or only single doses to patients reported no such manifestations. However, in addition to this, a combination of factors which for want of adequate evidence must be termed individual susceptibility, varying from patient to patient, would seem to be an important determinant in the production of the hemorrhagic tendency.

The conclusions drawn from this study are as follows:

1. In man, the oral administration of dicoumarin will prolong proth-

TABLE III

CONTRASTING CHARACTERISTICS OF DICOUMARIN AND HEPARIN

	Dicoumarin	*Heparin*
Chemical Classification	3, 3'-Methylene-Bis-(4-Hydroxycoumarin)	Mucoitin polysulfuric acid
Preparation	1. Extraction from spoiled sweet clover hay 2. Synthesis	Extraction from blood, liver, lung and other tissues Commercially it is prepared from lung and liver
Effect Manifested by	1. Prolongation of prothrombin and coagulation times	1. Inhibition of platelet agglutination 2. Prolongation of coagulation time
Effect Antagonized by	1. Fresh whole blood	1. Trypsin 2. Thrombokinase 3. Snake venom 4. Salmine sulfuric acid
Dose	Variable (approximately 300 mg. on alternate days in recent cases has proved satisfactory)	Variable (approximately 20 mg./Kg.)
Method of Administration	1. Oral 2. Intravenous	Intravenous {Continuous Drip / Multiple Divided Dose
Initial Response Occurs	24-72 hours	Immediately
Duration of Effect	2-26 days average 11.2 days	1-4 hours
Hemorrhagic Manifestations	Purpura, Ecchymoses, Hematuria, Gingival hemorrhage, Epistaxis, Conjunctival hemorrhage	Hematomata, Hematuria, Cerebral hemorrhage
Hemorrhagic Tendency Controlled by	Transfusions of fresh whole blood or plasma	1. Salmine sulfuric acid 2. Blood transfusion

rombin and coagulation time.

2. Wide variations in degree and extent of response were observed in different patients.

3. Hemorrhagic reactions, resembling sweet clover disease in animals, occurred following the oral administration of dicoumarin in eight patients.

4. Evidence suggests that differences in individual susceptibility may be responsible in part for these hemorrhagic responses.

The contrasting characteristics of these two in vivo anti-coagulants –heparin and dicoumarin–are presented in Table III.

An attempt has been made to summarize the present status of the use of in-vivo anti-coagulants. Of these, heparin and the dicoumarin 3, 3′ methylene-bis-(4-hydroxycoumarin) appear to be the most important and the most promising. They should be considered merely as steps toward our understanding of the factors and mechanisms involved in the important process of the coagulation of the blood. Some therapeutic indications have been outlined.

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