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Dicumarol : its physiological effects

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DICUMAROL,
ITS PHYSIOLOGICAL EFFECTS

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INTRODUCTION

The recognition, isolation, and finally synthesis of the substance causing hemorrhagic sweet clover disease in cattle, dicoumarin 3,3'-methylene-bis-(4-hydroxycoumarin), have been responsible for one of the most interesting and spectacular pharmacological developments in the field of medicine in recent years.

Although, from 1922 until 1941, dicumarin was studied strictly from the standpoint of veterinary medicine, it became obvious to workers in the field of peripheral vascular disease that the drug, which is easily prepared and administered, might prove to have a number of therapeutic qualities.

By emphasizing the physiological effects of dicumarin in both laboratory animals and human beings, it is hoped that this will enable the reader to evaluate accurately the merits and untoward results of the use of this drug and to judge accordingly about its application in the field of internal medicine.

At this point it should be mentioned that the term "Dicumarol" is the registered collective trademark adopted by the Wisconsin Alumni Research Foundation for the synthetic preparation 3,3'-methylenebis (4-hydroxycoumarin). Another way of spelling dicumarin is "dicoumarin."

HISTORY

The history of the drug, 3,3'-methylenebis (4-hydroxycoumarin), now known as dicumarin, actually had its beginning in 1922, when Schofield (1), at the Ontario Department of Agriculture, published a remarkably accurate description of a new hemorrhagic disease in cattle. He mentioned that the disease was quite similar to hemorrhagic septicemia, but that cultures of the blood failed to reveal bacteria. Of greatest significance was his keen observation that all cases of the disease reported were in cattle which had recently eaten sweet clover, and that the sweet clover invariably showed traces of mould! One series of cases in particular was of unusual value in the discovery of the etiology of the strange disease. Six animals, all members of a herd which had recently eaten sweet clover, were dehorned in the usual manner. Within six hours, all were dead for reasons undetermined. Shortly thereafter Schofield concluded that the cause lay in the eating of the sweet clover and that the disease occurred in two forms: a hemorrhagic type with the presence of subcutaneous swellings, and an anemic type which followed an operation such as dehorning or castration. Little did he realize, however, that some 18 years were to elapse before the hemorrhagic constituent

of the mouldy sweet clover hay would be isolated and synthesized.

During the winter of 1922, a number of reports of this mysterious disease of cattle were received by the Ontario Veterinary College. The disease resembled blackleg, but it was again noted that in every case sweet clover had been eaten by the animals just prior to their death. Schofield (2), at that time a pathologist at Ontario, conducted a microscopic examination of the tissues obtained from six acute cases of the disease and reported the following: necrosis of the liver cells; marked nephrosis; large amounts of pigment in the spleen; hemorrhages of small and large size in the wall of the intestine; degeneration of the heart muscle; tremendous destruction of erythrocytes. He concluded that the disease was caused by a toxic substance which damages the cells of important tissues and vital organs.

Further studies of "Sweet Clover Disease" and its pathology were conducted by Roderick (3), at the North Dakota Experiment Station. Although his work was simultaneous with that of Schofield, and of a similar nature, his publications did not appear until 1929.

The decade following the work of Schofield and Roderick was most uneventful in the history of

dicumarin, and it was not until 1940 that Link (4) finally isolated the hemorrhagic agent in spoiled sweet clover hay. Working with Campbell and other associates at the University of Wisconsin, he was able to chemically identify and synthesize the substance within the following year.

Shortly after this, two groups of clinicians, headed by Meyer (5) at the University of Wisconsin and by Butt, Allen, and Bollman at the Mayo Clinic, began clinical studies on dicumarin as a means of retarding intravascular clotting in human beings. Since 1942, many aspects of the drug have been tested and tried in innumerable hospitals throughout the United States.

CHEMISTRY

In 1931, Roderick and Schalk (3), after several years of research and investigation by the Veterinary Department of the North Dakota Agricultural Experiment Station, published a lengthy and detailed account of the so-called "Sweet Clover Disease." They described cumarin as being "the characteristic sweet-smelling constituent of new-mown sweet clover, the lactone ester of o-hydroxy-cinnamic acid." They found it to be non-toxic to rabbits and concluded that it must not be the cause of the disease--unless the disease results from a decomposition product of cumarin.

From 1931 to 1940 interest was lost in this mysterious substance of spoiled sweet clover which, when eaten by cattle, produced fatal hemorrhage. There was nothing of importance, chemically or pharmacologically, reported. Apparently the veterinarians, having discovered the manner by which the cattle contracted the disease, were content to advise simply prophylaxis.

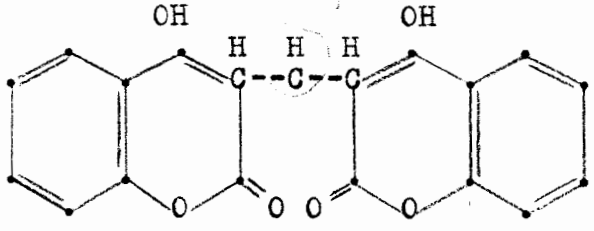
In 1940, however, Campbell, Roberts, Smith, and Link (4) were the first actually to isolate the hemorrhagic agent in spoiled sweet clover hay (melilotus alba). They prepared a concentrate which, when fed to rabbits, reduced the plasma prothrombin time to 10 per cent of the normal in 40 to 48 hours. Although unable,

at the time, to determine exactly the chemical nature of the agent, they found it to be free from the following classes of substances: fats, waxes, sugars, glycosides, water-soluble polysaccharides, amines, alkaloids, water-soluble proteins, and water-soluble decomposition products of chlorophyll. Just a few months following, Link (6), along with Campbell, accomplished that which was to revive interest in the subject and transform it into a source of pharmacological research: By means of fractionating processes, which represent a masterpiece in the field of chemistry and which are too detailed and intricate to be readily understood by those outside this field, these workers were able to extract a hemorrhage-producing concentrate with a prothrombin-reducing activity 200 times greater than that of spoiled hay. This has been isolated from both experimentally produced spoiled hays and hays which have been known to kill cattle, the process of extraction being accomplished in 16 steps, with an efficiency of about 66 per cent.

Having never before been found in nature, this hemorrhagic agent has the empirical formula $C_{19}H_{24}O_4$, is optically inactive, insoluble in acid media and of low solubility in ordinary organic solvents, readily soluble in basic solvents, white and crystalline, and

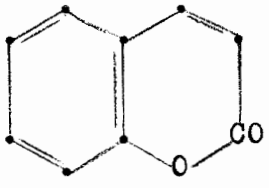
entirely inconsistent in its behavior towards usual identification reagents. One and one-half milligrams of this crystalline substance are equivalent to about 50 gm. of an usual spoiled hay sample in reduction of prothrombin activity.

Through a series of degradation reactions and by synthesis, it was proved by Stahmann and Huebner (7) that the hemorrhagic agent isolated by Campbell and Link, also of the Wisconsin Agricultural Experiment Station, and found in spoiled sweet clover hay is dicumarin, 3,3'-methylenebis (4-hydroxycoumarin), a substance with physical properties identical in natural and synthetic forms but quite different from any of the 60 or more naturally occurring coumarins previously reported. A derivative of coumarin, this product has a melting point of 288 to 289 degrees and a structural chemical formula:

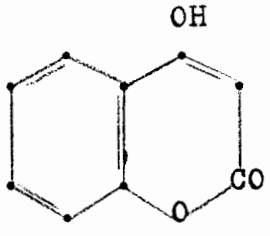


It was concluded from these experiments that the natural bitterness of sweet clover and its tendency to become a hemorrhagic agent when improperly cured have

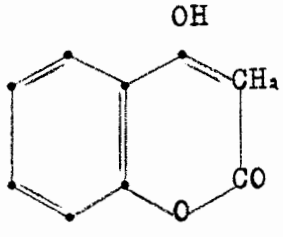
a common basis in the coumarin molecule; and, that when spoilage occurs, this coumarin is oxidized to 4-hydroxycoumarin which is in turn coupled with formaldehyde. The following formulas will aid not only in following the steps involved in the formation of dicumarin from coumarin, but also in the study of the definite antihemorrhagic fraction appearing in this series of compounds:



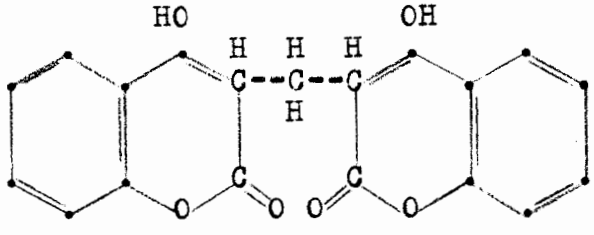
Coumarin



4-hydroxycoumarin



3-methyl-4-hydroxycoumarin



3,3'-methylene-bis (4-hydroxycoumarin)

With further degradation of the hemorrhagic agent, Huebner and Link (8) produced a diketone which, when combined with 10 per cent sodium hydroxide, is 1,3-disalicylylpropane.

Lehmann (9), doing clinical work in Sweden similar to that of Allen and Barker at the Mayo Clinic,

made a series of experimental studies of the effects of coumarin, dicoumarin, and some of their derivatives, on the prothrombin levels of rabbits. He found no compound which showed any advantage over the original 3,3'-methylene-bis (4-hydroxycoumarin); nor was he able to find an active stable water-soluble substance, a derivative of the dicoumarin, for intravenous injection, as had been hoped. By finding a drug for intravenous use, he had hoped to eliminate the 12 to 48 hour latent period which exists when dicumarin is given orally. Contrary to the findings of Roderick and Schalk (although Lehmann admits some irregularity in his results), he found coumarin and its derivatives to be toxic to rabbits even though the prothrombin time was not increased markedly. Introduction of a hydroxy group in position 4 of the coumarin produced 4-hydroxycoumarin, a compound four times as active in decreasing prothrombin activity.

Supplementing the work of Lehmann, Overman and others (10) tested the hypoprothrombinemia-inducing capacity of more than 150 compounds closely related to 3,3'-methylenebis (4-hydroxycoumarin). All of their assays were made on 12.5 per cent plasma of standardized susceptible rabbits which had been fed various quantities of these drugs in gelatine capsules.

Whereas the dicumarin was found to be the most potent of the series, all compounds having an intact residue of the 4-hydroxycoumarin, with the 3 position substituted by a hydrogen atom or a carbon residue, showed definite anticoagulating activity. Higher potency was found to be due to the 4-hydroxycoumarin with a 3-substituent containing a keto group in the 1,5 position with respect to the 4-hydroxyl group. The above structural formulas are helpful.

PHYSIOLOGICAL EFFECTS

That dicumarin is a strange drug having many curious effects on various laboratory animals and on human beings can not be disputed. It is unfortunate, indeed, that its manner of action, correct dosage, excretion by the body, and contraindications are not so well-defined as are those of the majority of the popular drugs in the internist's repertoire.

Schofield (2), as early as 1923, recognized an altered coagulation of the blood in animals with spoiled sweet clover disease, but was unable to tell definitely how this was brought about. His tests, however, did reveal that the inability of the blood to clot was not due to a deficiency in calcium salts or fibrinogen, but due to an absence of thrombin or an inhibition of thrombin.

After it became established that the damaged sweet clover was responsible for the death of cattle from fatal hemorrhage, Roderick (11) conducted a series of studies on the pathologic and functional features of this unusual hemorrhagic complex. That hemorrhage, preceded by a delay in the coagulability of the blood, is the fundamental lesion and cause of death was quickly realized. Hemorrhages were found

in all parts of the body; the lateral ventricles of the brain, the wall of the bladder, the medullary cavity of long bones, and in subcutaneous and intermuscular fascia. External evidence was most apparent in the regions of the neck and shoulders and the wall of the thorax. This was usually in the form of a large hematoma, either superficial or interposed as a sheet between the muscles. The hemorrhages, although common on serous surfaces, were rarer in the mucosa, although epistaxis was not uncommon. A most interesting feature was the inability, upon dissection and microscopical examination, to find any injury or visible openings in the vessel walls, despite the fact that the extent of the hemorrhage would indicate that the mechanism was not by diapedesis. Roderick was of the opinion that the bleeding occurred in the arteriolar bed rather than from the capillaries, veins, or larger vessels.

Further work on the blood composition, urine, and internal organs was also undertaken. Blood non-protein nitrogen, sugar, icterus index, and acid-base balance were found undisturbed, indicating the absence of a hemolytic phenomenon. This, along with the absence of albuminuria and cholesterolemia, together with normal gross and microscopic appearance of the

kidneys, tended to eliminate toxicity as a factor. He saw no evidence of liver function impairment.

After conducting analytical work on a series of animals fed on damaged sweet clover, Roderick (12) published a paper dealing with alterations of the coagulation process of the blood. Of greatest significance, of course, was the delayed coagulability, involving a reduction in prothrombin. There was no evidence of calcium deficiency, increase of an inhibitory substance such as heparin or antithrombin, or of alteration in the number of blood platelets present.

Quick (13) soon realized that the hemorrhagic tendency seen in sweet clover disease of rabbits was accompanied by a low plasma level and a reduction of prothrombin. He also noted that transfusion with citrated whole blood elevated the prothrombin for a short time and that feeding alfalfa had a decidedly beneficial effect in retarding the disease and in improving the condition once it had developed. Being especially interested in the prothrombin aspect, he concluded that the toxic principle either destroys prothrombin or prevents its formation. The alfalfa, he believed, exerted its effect by furnishing some accessory food factor necessary for the organism to produce

prothrombin. No vitamin which would prevent the disease could be found at this time.

In 1940, interest in dicumarin was revived by Campbell (14) and a number of his associates. These workers studied the syndrome produced by feeding spoiled sweet clover hay or hemorrhagic-producing concentrates prepared from it to rabbits. Instead of employing the method of prothrombin determination as outlined by Quick, they used a somewhat modified procedure in order to facilitate greater accuracy. Other precautionary measures such as selection only of susceptible animals, use of thromboplastin specially prepared from dry rabbit brain tissue, uniform dilution of the plasma to be tested, and standardization of the animals through preliminary feeding were taken.

It was found that the rabbits suffered no permanent injury as long as excessive doses of the hemorrhagic substance were not given. The animals developed no acquired immunity or increased susceptibility to the agent. Although there was invariably a latent period of from three to eight hours before decreased prothrombin activity and increased clotting time became manifest, the maximum depletion of the prothrombin level, regardless of the dosage and sensitivity of the rabbit, was usually 38 to 48 hours.

Prothrombin restoration always followed cessation of the drug, reaching a normal level more quickly when smaller doses were given. This varied from 4 to 6 days after the plateau of maximum depletion, but the feeding of stock grain mixture and alfalfa immediately after reaching this plateau caused the normal prothrombin level to be attained more rapidly.

Their results in regard to actual effect of the hemorrhagic substance on the blood clotting mechanism were less impressive, it being concluded that there is either a disturbance of continuity of prothrombin formation by the liver or a qualitative alteration in the prothrombin already formed. This was essentially the same explanation as that advanced by Quick. In contrast to other natural and synthetic anticoagulants, this hemorrhagic agent does not appear to affect the clotting power of normal blood or plasma in vitro. Its unique physiological activity becomes manifest only when the drug is fed by mouth or injected intravenously.

As soon as the isolation and synthesis of this new anticoagulant by Campbell and Link was revealed, numerous men interested in peripheral vascular disease began experimenting with the drug, attempting to employ it as a therapeutic agent. In June of 1942,

Richards and Cortell (15), in an attempt to show the efficacy of the drug in preventing thrombosis, injected segments of the saphenous veins of dogs with ethanolamine oleate to promote thrombosis; then observed the effects of dicumarin given by mouth. The result was a definite reduction in the incidence and degree of thrombus formation. Just a short time later, Lehmann (16) treated seventeen cases of thrombosis of the extremeties by oral administration of the drug. These were human subjects. In all cases the course of the disease was shortened, and no further thrombosis occurred after the fall in prothrombin index. Such success was not typical of the experimentation of many other medical men, however, as will be seen.

Prandoni and Wright (17), treating a series of patients by oral administration of dicumarin in varied doses (from 7.8 to 45.9 mg.;Kg.) over varying lengths of time, observed the following characteristics:

> "Responses in the form of prolongation of the prothrombin and coagulation time varying widely in degree and duration--even in patients receiving the same relative doses.
>
> "The initial effect manifested itself as a significant prolongation of prothrombin time, which occurred in from one to five days, average 3.2 days.
>
> "The coagulation time at the period of maximal prolongation varied from eight to thirty-three minutes, averaging thirteen minutes.

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

"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 was two to twenty-six days, averaging 11.2 days.

"The duration of effect, after discontinuing the administration of dicumarin, varied between one and twenty-three days, averaging 11.2 days."

With but few exceptions, these findings typify the results gathered by the majority of clinicians.

Butt, Allen, and Bollman (18), at the Mayo Clinic, were other early experimenters whose results compared quite favorably with those of Dr. Prandoni. They were favorably impressed by the therapeutic qualities of the drug as well as its economy, ease of administration, and prolonged action, making it superior to heparin in many respects. It might be well, at this point, to state that these men have made a major contribution to the treatment of thrombophlebitis and pulmonary embolism by their extensive work along this line.

Wasserman and Stats (19) gave dicumarin in repeated doses to a group of 71 adult patients, and their results and conclusions furnish a great deal of valuable information regarding the physiological effects of the drug. Gelatin capsules containing from 200 to

500 mg. of the substance were given orally at intervals varying from 1 to 7 days. Each patient received an average of eight doses. From this it was learned that oral administration of dicumarin produces a marked fall in the prothrombin content, after a 24 to 72 hour latent period, along with a prolongation of the coagulation time. However, there is great variability in the degree of response to this drug, making a definite fixed dosage impossible. Moreover, fear of hemorrhage prevents the use of this agent during or shortly after operative procedures or in patients with lesions from which bleeding might occur; yet this fear is not always warranted. It should be remembered, though, that once hemorrhage occurs, due to dicumarin, transfusions with fresh blood might not arrest the condition.

Instances have been observed in which embolism and thrombosis have occurred despite a low blood prothrombin induced by dicumarin. Furthermore, it is known that thrombosis or embolism which has already occurred is affected but little, if any. This was first revealed by Barker (20) at the University of Minnesota. Davis and Porter (21) treated 43 cases of varying degrees of postpartum thrombosis and are of the belief that dicumarin is not only effective in

preventing postoperative thrombophlebitis and pulmonary embolism in man, but that it also limits the extention of an already established thrombosis.

Some question has arisen concerning the effect of dicumarin on the sedimentation rate of the red blood cells. The importance of this is obvious since many patients receiving the hemorrhagic agent have diseases in which this laboratory test may be of importance. Barker and his associates (20) believe the sedimentation rate to be elevated in the great majority of cases; although other writers neglect to comment on this phase of the subject.

Quite recently, Dr. G. Alexander Young (22) has successfully used dicumarin in cerebrovascular diseases. While it is, of course, difficult to determine definitely the extent to which the drug retards or improves cerebral thrombosis, Dr. Young is of the opinion that improvement is more rapid with its use. In fact, the Douglas County Hospital Psychiatric Department, treating cases of cerebral arteriosclerosis associated with psychotic symptoms, is using dicumarin with apparent clearing of the mental symptoms. It is hoped that the drug might be used in combating sinus thrombosis associated with mastoid infections or orbital cellulitis.

For the benefit of those interested in comparing the important advantages and disadvantages of dicumarin and heparin, the following information is presented: Dicumarin, first of all, has two important advantages over heparin. It can be given orally, and it is much less expensive. These factors alone are responsible for the greater part of its recent popularity. Conversely, heparin has an advantage in that the delay in coagulation time appears within a few minutes after its administration and ceases rapidly when it is withdrawn. This makes toxic effects less likely, even though frequent coagulation time determinations are necessary--as is also true with dicumarin. In the event of hemorrhage due to overdosage, the effect of heparin can be remedied by a simple blood transfusion. This is not true of dicumarin, as will be shown later.

Both heparin and dicumarin produce clinical effects which are quite similar. Neither can dissolve a thrombus either in vivo or in vitro. Ottenheimer (23) believes that their value lies in the prevention rather than the treatment of thrombophlebitis.

In all probability these drugs affect the blood clotting mechanism differently. Heparin inhibits platelet agglutination, while dicumarin exerts its

effect directly on prothrombin, either actually destroying it or inhibiting its formation.

FACTORS INFLUENCING THE EFFECT OF DICUMARIN

Since dicumarin has come to be used therapeutically, it has been revealed that there are a number of various important agents which alter, or threaten to alter, the physiological effects of the drug. Undoubtedly more and more of these will be discovered as time passes. The subsequent presentation includes factors very likely to exist in patients upon whom anticoagulant therapy is frequently of great practical importance.

Pregnancy:

This has not given rise to any serious problem inasmuch as treatment of human beings is concerned. However, it is well known that during pregnancy a great number of women are susceptible to thrombophlebitis, for which dicumarin might be indicated. This has led to experimental investigation by certain workers. Field, Overman, and Baumann (24), studying the effect of dicumarin on rats during and immediately after pregnancy, found the drug to have a less pronounced ability to produce hypoprothrombinemia in pregnant than in normal animals; whereas, in lactating rats the anticoagulant was only 18 per cent as effective as in non-lactating controls. Even since these experiments in 1942, no satisfactory explanation

for this has been found. It is now a well-known fact that lactating animals have a much greater capacity to recover from such induced hypoprothrombinemia. Perhaps Lehmann (9), who also published an article on this subject in 1942, had the correct answer to this when he discovered that lactating women excrete the drug in their milk.

Vitamin C:

Further experiments on rats were conducted by Baumann and others (25) with hopes of finding some substance which could conveniently be used to combat untoward effects of dicumarin. By feeding adult rats a basal diet and then allowing a fasting period of 12 hours before feeding 2.5 mg. of dicumarin, these men made some interesting observations in regard to its effect on the prothrombin time of blood which was taken by heart puncture 24 hours later. Emphasis was placed on the effects of l-ascorbic acid on these animals; and, in accordance with the belief that vitamin C is not closely linked with blood coagulation, the results showed that the latter failed to alter the hypoprothrombinemia induced. It also failed to prolong the lives of rats receiving the anticoagulant. Peculiarly, however, the substances, chloretone and carvone, which stimulate synthesis and excretion of

vitamin C in the rat, lower the degree of hypoprothrombinemia and markedly prolongs the lives. This would suggest that substances which stimulate the synthesis of vitamin C also affect the mechanism counteracting hypoprothrombinemia. But there is, as yet, much to be explained in regard to this mechanism. Lehmann, on the contrary, believes that ascorbic acid is definitely indicated along with dicumarin; especially in cases of actual or suspected vitamin C deficiency.

Fever:

Since it is often necessary to administer dicumarin to patients who have fever and since increased temperatures are known to accelerate biochemical reactions, Richards (26) conducted a series of experiments with rats in which fever was induced by typhoid vaccine. The results indicate clearly that a much higher prothrombin time is produced by giving the drug to animals with elevated temperatures. As is the case in normal patients, the degree of this increase varies considerably and shows no definite relationship to the number of degrees the temperature is increased. In general, however, the response is more pronounced in patients with higher degrees of fever. Doctors using dicumarin therapeutically are little concerned about

the effect of fever on the action of the drug because coagulation and prothrombin times must be taken frequently in all instances and the dosage judged accordingly.

Trauma and Gangrene:

Brambel and Loker (27) have recently directed their efforts to the study of trauma and gangrene, relative to the war with its consequent trauma, and to industry with its accelerated production and use of dangerous machinery by workers whose experience is limited. Gangrene caused by diabetes, arteriosclerosis, and frostbite has also been studied in this connection.

Patients suffering from the above conditions have an enhanced coagulation mechanism and show definite resistance to the effects of dicumarin when compared with normal persons. Larger doses of the hemorrhagic agent are necessary to produce the same effect, and the period of duration is shorter. Contrary to expectations, administration of the hemorrhagic compound produces no bleeding from the wounds.

Brambel and Loker, incidentally, believe dicumarin to be highly efficacious not only in preventing gangrene, but also in eliminating the necessity of amputation following crushing injuries in which

gangrene would be likely to develop.

Anesthetics:

It has been known for some time that chloroform, used as an anesthetic, produces a reduction in prothrombin levels of dogs. Steggerda and Richards (28), therefore, decided to test the effect of dicumarin on a series of rats receiving nembutal, pentothal, and chloroform. From their experience it is concluded that these anesthetics do not alter the susceptibility of rats to oral doses of dicumarin; nor do they change the prothrombin time of the blood. Although this is indicative of what might be expected in the human subject, it should be remembered that the liver of the rat may be more resistant to chloroform insofar as prothrombin formation in this organ is concerned.

Heparin and Thyroid:

Because each of the two important anticoagulants, dicumarin and heparin, has significant advantages and disadvantages when used in the human subject, Walker and Rhoads (29) studied the synergistic action of these drugs in a series of three groups of patients. Depending on the quantity of either drug given, a much smaller amount of the other was required to produce the same effect as was achieved in controls. The

results of this indicate clearly that heparin and dicumarin definitely enhance one another. Fortunately, these drugs can be used simultaneously in cases requiring the advantages of both.

In answer to the question concerning the effect of the various thyroid compounds on the prothrombinopenic action of dicumarin, it may be stated that no evidence has been presented to show that patients receiving thyroid are any more susceptible to the action of the hemorrhagic agent. Wakim, Chen, and Gatch (30) showed that rats and dogs receiving desicated thyroid are not in any way altered in susceptibility to the prothrombinopenic effect of dicumarol.

DOSAGE

The problem of finding a standardized dose of dicumarin has not been solved. To accomplish this promises to be an impossibility because of the varied degrees of response to the drug by different individuals. However, during the last three years, a more or less systematized method of administering this hemorrhagic compound has been found.

The first to use dicumarin on human subjects were Meyer, Bingham, and Pohle (5). This was early in 1942. They found 4 to 5 mg. per kilogram, orally or intravenously, to be within safe limits and used oral administration of daily doses of 1 to 1.5 mg. per kilogram without deleterious effects. Of course, at this experimental stage, the necessity of determining the prothrombin and coagulation times at frequent intervals and judging the dosage accordingly is quite apparent.

Evans (31), working at the Lahey Clinic, was in agreement with Bingham, Meyer, and Pohle as to dosage; namely, an initial dose of 5 mg. per kilogram, followed by a daily dose of 1.5 to 3 mg. per kilogram. He was the first to combine dicumarin and heparin in the treatment of thrombophlebitis and pulmonary embolism.

His procedure was as follows: As soon as the diagnosis of phlebothrombosis, thrombophlebitis, or pulmonary embolism was made, heparin was immediately given intravenously until the clotting time was raised to from twenty to twenty-five minutes; then an initial dose of 5 mg. per kilogram of dicumarin was given orally. Thirty-six hours after this initial dose of dicumarin, the heparin was discontinued; but a daily maintenance dose of 1.5 to 3 mg. per kilogram of dicumarin was given for two to three weeks if necessary. Such a procedure was found to be convenient, inexpensive, and as successful therapeutically as the use of either drug by itself. In fact, Evans' method of combined heparin and dicumarin therapy has not been altered substantially since its introduction in 1942.

Le Fevre (32), another early experimenter with dicumarin in human subjects, used 5 mg. per kilogram on the first three successive days, but eventually reduced the third quantity to 2.5 mg. per kilogram. Meyer, Bingham, and Axelrod (33) adhere to the plan whereby an initial dose of 5 mg. per kilo is followed by daily doses of 1.5 mg. per Kg.

There is no doubting that calculating the accurate dose of dicumarin for each individual is difficult. And yet, the importance of this can not be

over emphasized. Shapiro and Sherwin (34) successfully treated five cases of embolism without toxic results by using dicumarin. They attribute this success to their method of judging an accurate dosage level for each individual patient: this level was kept at the point where plasma diluted to 12.5 per cent with normal saline showed a prothrombin time of two or three times normal, while the prothrombin time of whole plasma was only moderately increased. By this time, methods fundamentally similar are used by all workers.

Late in 1942, a different system of administering dicumarin was begun by Butsch and Stewart (35). Instead of using the patient's weight as an index of the dose to be given, they simply gave 300 mg. the first day and followed this by 200 mg. the second and 300 mg. the third, fourth, and fifth. In children, 200 mg. on two successive days proved to be a satisfactory dose. Such is the plan followed by the majority for the last couple of years, discounting, of course, minor variations in number of milligrams and methods of prothrombin determination.

By the middle of 1944 large numbers of patients at the Mayo Clinic were receiving dicumarin. The procedure here is to administer to patients with

pulmonary embolism or thrombophlebitis, or patients having a history of these, 300 mg. of the drug on the first day of treatment and 200 mg. on the second day. Thereafter, 200 mg. is given on each day that the prothrombin time is below 35 seconds. (It should be mentioned that they use a modified Quick test of prothrombin in which the normal is 17 to 19 seconds.) Using this standard, it is found that thrombosis is definitely inhibited above 27 seconds and that bleeding will not occur below 60 seconds. The necessity of daily accurate tests of prothrombin time is obvious. Zucker (36) uses an initial dose of 300 mg. orally, with an additional 200 mg. the following day. Subsequent doses are given in 200 mg. quantities with the aim of maintaining the plasma prothrombin time level between 30 and 60 per cent of his normal. This is identical to the procedure of Walker and Rhoads (29), only they use a prothrombin concentration between 20 and 30 per cent of normal. Eckstam (37), at the University of Minnesota, also employs this dose, with prothrombin time being 25 to 50 per cent of normal.

Since, after operations and for other reasons, administration of dicumarin by the oral or intravenous routes is not always satisfactory, Meyer and

Spooner (38), at the University of Wisconsin Medical School, tried giving the drug rectally in aqueous suspension or in suppositories. Four patients responded in a manner similar to that seen after oral administration while thirty-four others showed no significant decrease in prothrombin or coagulability of the blood. This would indicate that such a method of giving dicumarin is not at all reliable.

Recently, Hurn and others (39) at the Mayo Clinic have devised a standardized method of determining prothrombin time, thereby eliminating the necessity of establishing new criteria for administration of dicumarin each time a new supply of thromboplastin is used. This also enables various laboratories to compare and correlate results of their experiments.

As late as February, 1945, Barker and his associates (40) at Mayos' still had found no satisfactory preparation of dicumarin for parenteral administration, but were continuing to give single oral doses; 300 mg. the first day, 200 mg. the second, and 200 mg. on succeeding days that the prothrombin time is greater than 20 per cent of their standard normal.

TOXICOLOGY

The facts which are known concerning the toxic effects of dicumarin have been gathered from two experimental sources--from laboratory animals and from human beings. The manifestations of these toxic effects may well be divided into three groups: pathological findings in laboratory animals, pathological findings in man, and symptomatic manifestations in man. Because of the fact that considerable difference of opinion exists in regard to toxicology, this arrangement of presentation is adopted.

Pathological Findings in Laboratory Animals:

Rose, Harris, and Chen (41), working in the Lilly Research Laboratories in 1942, estimated the median lethal doses of dicumarin in a series of different animals. Death was found to occur uniformly in rabbits with intravenous injection of daily doses of 1-2 mg. per Kg.; in dogs with oral administration of 5-50 mg. per Kg. daily; and in mice and rats with 0.01-1% dicumarin in food. When daily intravenous doses of 0.1-0.5 mg. per Kg. were given to rabbits, the majority could tolerate this for about 6 weeks. With the occurrence of death, most animals were found to have hemorrhage into various tissues and organs, plus

pulmonary edema. Central liver necrosis was found in about half of the rats, but only occasionally in rabbits, mice, and dogs. These findings compare quite favorably with those of Richards and Cortell (15), who found a considerable difference in tolerance between various species of animals--also among individual animals of the same species. Although necrosis of the liver was found in many animals, the significance of this was questioned because of the severe anemia produced by the drug. The level of vitamin C in guinea pigs appeared to exert definite influence on the extent of liver necrosis and damage.

To study the intoxicating effects of dicumarin prior to the onset of hypoprothrombinemia, Wakim, Chen, and Gatch (42) gave lethal doses of the drug to anesthetized dogs, rabbits, and rats. The intravenous route was used. After a series of control tests in which other ingredients of the solution administered were tested and found to produce no effects, dicumarin was added, making a 0.5 per cent solution. It was noted that when the total quantity given reached between 40 and 60 milligrams per kilogram of body weight, a number of decidedly significant physiological changes occurred in the animals. These were as follows: rapid irregular respiration followed by

severe dyspnea; generalized vasodilatation; darkening of the blood (in spite of rapid and deep respiration), extra systoles and other cardiac irregularities; hyperglycemia; hyperpyrexia; then simultaneous cessation of heart action and respiration. Autopsy showed a dark and congested liver, a contracted spleen, and rigor mortis, the onset of which was almost immediate. And all of these findings had occurred before any change in coagulation and prothrombin times! In nearly all instances death supervened within an hour after the original doses were given. It should again be emphasized that these results were obtained with lethal doses.

In 1943, Richards and Steggerda (43), studying the effects of dicumarin in rats with impaired liver or kidney function, made some practical observations of interest. They produced liver damage by means of subcutaneous injection of 0.2 cc/Kg. of carbon tetrachloride and kidney damage by unilateral or bilateral nephrectomy. Neither of these procedures in itself was responsible for a change in prothrombin level without the use of dicumarin, showing that the experimental results were accurate. It was found that injury to the liver, which in itself does not influence the prothrombin level, definitely sensitizes

rats to the action of dicumarin, the extent of the sensitivity being quite parallel to the liver damage. In regard to the kidneys, it was found that only bilateral nephrectomy influenced the effect of the drug, apparently because of its retention in the blood due to lack of elimination. This was indicated further by the fact that, in bilaterally nephrectomized animals, the prothrombin level was depressed for a continued and increased length of time. This work is the basis for the present belief that dicumarin is contraindicated in patients suffering from impairment of kidney function or having even a mild degree of liver damage.

Pathological Findings in Man:

The effect of the administration of dicumarin upon blood cytology, liver function, plasma proteins (especially fibrinogen), and the kidneys, revealed nothing of significance as to abnormality, according to Davidson and Mac Donald (44). However, they qualify this by stating that the drug does have variable effects upon blood coagulability, prolonged action after discontinuation, and difficulty in control, all of which render it a poor heparin substitute. They advise extreme caution in its use!

In prolonged use of dicumarin, Bingham, Meyer, and Howard (45) report that blood in the urine, as

demonstrated by a positive benzidine test is not uncommon. They also believe its use to be hazardous without close supervision, but state that it may be given for periods as long as 90 days without producing undesirable objective findings.

In June of 1943, Dr. Cahan (46), of New York City, reported a case in which the patient received 2800 mg. of dicumarin over a thirty-two day period and developed hemorrhagic and purpuric manifestations along with a prothrombin deficiency. There was, however, no platelet reduction, capillary fragility, or abnormality in clot retraction. His belief is that the drug induces an increased sensitivity of the vascular bed to trauma, along with a hypoprothrombinemia. Wright and Prandoni (47) are in agreement with Cahan, as regards capillary fragility. They found none, even in cases which were hemorrhagic.

The question as to whether liver damage acts as the mechanism by which the prothrombin is inactivated is still undecided. Icteric index, bromsulphalein, cholesterol, and total protein studies do not seem to demonstrate liver damage as a result of the giving of dicumarin. With but few deaths from excessive amounts of dicumarin, autopsy findings are rare; this accounts for the scarcity of pathological material.

Symptomatic Manifestations in Man:

In 1942, when dicumarin was first being tried in human subjects, Lehmann (16) observed a few cases of mild toxic symptoms in patients when initial doses of the drug were given. These were vomiting and diarrhea chiefly. But with continued treatment, nothing further happened. Liver and kidney function tests were normal. Nevertheless, Lehmann believes that kidney, heart, and liver diseases contraindicate the use of the product. Likewise, Zucker (36) reports cases in which nausea appeared when the drug was first taken, only to disappear shortly thereafter. He mentions also that danger of hemorrhage following venipuncture has been mentioned by some, but that he has seen no instances of this complication.

Apparently, a single large dose of dicumarin produces less physiologic and toxic effects than total equivalent doses administered in smaller daily portions. Other early toxic signs, in addition to nausea and vomiting, are lassitude and general malaise with aching in the costovertebral angles. Occasionally, hemorrhage occurs in patients whose prothrombin times are much lower than those of others having no such tendency. Coagulation times, while they should be taken frequently, are not as reliable a guide to

overdosage as prothrombin time determinations. Wright and Prandoni (47) find that the incidence of toxic reactions is not significantly influenced by age, sex, state of nutrition, or hematocrit studies.

One of the most complete reports of the appearance of manifestations of a hemorrhagic tendency is presented by Wright and Prandoni from their study of eight toxic patients. These manifestations were as follows:

Weakness and lassitude	7
Purpura	4
Gingival hemorrhage	4
Hematemesis	4
Sublingual ecchymosis	3
Conjunctival hemorrhage	3
Hematuria	3
Bleeding at wound site	3
Epistaxis	2
Vertigo	2

Most authors agree that there are certain contraindications to the use of dicumarin. These are as follows: hemorrhagic tendencies and existing low prothrombin times, liver and kidney damage, and subacute bacterial endocarditis. Many feel that the drug should not be used routinely as a method of prophylaxis because the incidence of hemorrhagic manifestations due to its use is greater than the incidence of complications for which its therapeutic administration is indicated.

ANTIDOTES

From the time that dicumarin was first considered for use in human beings, interested experimenters have worked feverishly in an attempt to find something to combat the toxic effects from overdosage of the drug. Thus far, however, the only successful measures have centered around the use of vitamin K or transfusions with blood or plasma.

Use of Transfusions:

Evans (31), using both heparin and dicumarin therapeutically, finds transfusion, repeated if necessary, to offer almost immediate safety from dicumarin bleeding by replacing absent prothrombin. But all others do not agree. Richards and Cortell (15) found only temporary benefit from single blood transfusions in dogs which were extremely depressed by the drug, and Davidson and Mac Donald (44) observed whole blood transfusions to have only a transitory effect, or no effect, upon the abnormal clotting mechanism of patients receiving dicumarin. Young (22) finds transfusions with fresh whole citrated blood extremely efficacious, which agrees with the opinions of Ottenheimer, Prandoni, and Meyer, and the majority of others. But it should be emphasized that the blood

must be whole and fresh and the transfusions multiple, in order that the prothrombin time and coagulation time be shortened.

The Use of Vitamin K:

Concerning the value of vitamin K as a method of combating hemorrhage caused by excessive amounts of dicumarin, there is more unanimity of opinion than is showed in relation to transfusions. Nevertheless, certain experimenters with laboratory animals have found results contrary to the majority.

Overman, Field, Baumann, and Link (48), early in 1942, tested 260 adult rats of both sexes by feeding either a stock ration or an artificial ration low in vitamin K; then incorporating a desired dose of dicumarin in 2 gm. of corn starch and oil after a fasting period of 12 hours. They not only found the effect of the drug to be greater upon the group receiving low vitamin K rations, but also discovered that all forms of this vitamin, whether given before, with, or after the anticoagulant, counteracted the hypoprothrombinemia induced. With supplements such as ascorbic acid, wheat germ, hydrogenated fat, and choline, however, no benefits were derived.

With the ability of Link and Baumann and their associates to show that vitamin K counteracts the

hypoprothrombinemic effect of dicumarin in rats and with the failure of other workers to demonstrate a similar effect in man, Shapiro, Redish, and Campbell (49), conducted a further series of experiments with human subjects, using larger doses of vitamin K and smaller doses of the anticoagulant. The results, in accord with those of Link, were the first to reveal that the vitamin neutralizes the hypoprothrombinemia induced by dicumarin in man as well as in various laboratory animals. But extensive experience in human beings does not bear this out, and the work of these two groups furnishes the extent of the support to the usefulness of vitamin K.

While Townsend (51) thinks the vitamin might be of some value, Wasserman, Ottenheimer, Prandoni & Wright, and Meyer all denounce it entirely.

By the middle of 1944, large numbers of patients at the Mayo Clinic were receiving dicumarin. When it was learned that some 27 per cent of these patients were hypersensitive to the drug and showed excessive elevation of the prothrombin time after receiving only the initial dose of 300 mg., Cromer and Barker (50), in an effort to find some means of quickly restoring a safe level, began working with synthetic vitamin K. In view of the fact that previous experimenters had

observed that the vitamin was ineffective, these men decided to give it in much larger doses. Single doses of menadione bisulfite were given intravenously to thirty-seven patients who had developed an excessive deficiency of prothrombin after receiving dicumarin, and in all but two cases the prothrombin time fell to within safe limits within eighteen hours. There were no toxic or untoward reactions. By February, 1945, Barker (40) had given dicumarin to 1000 patients with postoperative venous thrombosis, pulmonary embolism, or thrombophlebitis, with definite success. The extremely few cases which showed a tendency to bleed were immediately controlled by intravenous administration of 60 to 64 mg. of menadione bisulfite (synthetic vitamin K) or by transfusions with whole fresh citrated blood with large doses of menadione bisulfite. To date, this is the latest and most effective method of controlling the hemorrhagic effects of too much dicumarin.

Nobody has found any other vitamin of value as an antidote for dicumarin.

CONCLUSIONS

Despite the fact that there is yet much to be learned about dicumarin and that it is still in the experimental stages, there are a number of characteristics of the drug which are known with certainty:

1. Dicumarin is a white crystalline substance which is active when given orally and may also be given intravenously.
2. Dicumarin is active only in vivo.
3. "There is a latent period of twenty-four to seventy-two hours before the prothrombin time is prolonged following administration of dicumarin. Dicumarin also prolongs clotting time, clot retraction time, and the red blood cell sedimentation rate. It dilates the capillaries, small arterioles, and venules. The bleeding time is not consistently affected because of the tissue factor present when the skin is injured. Dicumarin does not dissolve the clot that has already formed, but it prevents that clot from extending and allows it to become organized." Such is the epitome as given by Eugene E. Eckstam (37).
4. Some persons are highly resistant to dicumarin and others are remarkably susceptible. A definite

fixed dosage schedule cannot be made.

5. The exact mechanism by which dicumarin reduces the prothrombin content of the blood is still unknown.

6. When giving the drug, it is advisable to carry out daily prothrombin and coagulation determinations.

7. Absolute contraindications to the use of dicumarin are: subacute bacterial endocarditis, renal insufficiency from any cause, liver damage, existing prothrombin deficiency, blood dyscrasia with tendency to bleeding.

8. The only methods of combating an excessive dose are repeated transfusions with fresh whole blood and/or administration of large intravenous doses of menadione bisulfite (synthetic vitamin K).

9. The effectiveness of oral administration, the cheapness with which it may be administered, and its prolonged action make dicumarin preferable to heparin in many instances.

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