ANTICOAGULANT THERAPY*

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There is little disagreement about the value of heparin in the prophylaxis and treatment of thrombosis, and considerable quantities of this expensive drug are now used. The control of the dosage, however, often seems to be unsatisfactory, and we feel that more would be gained by giving larger and fully effective doses to a smaller number of carefully selected cases. The aim should be to maintain the clotting time at about 30 minutes instead of the normal 6–10 minutes, and this seems seldom to be achieved by the usual practice of intravenous injections of 50–75 mg. four-hourly. It is probably more efficacious to give a continuous intravenous drip of heparin in saline (say 100 mg. per pint, or 175 mg. per litre) at a rate of 15 to 25 drops a minute, the rate being adjusted at intervals so as to maintain the desired prolongation of coagulation. But until we have available effective preparations of heparin with an action lasting many hours when given intramuscularly it is unlikely that this drug will be employed for more than a few days at a time, and in the great majority of cases of thrombosis effective treatment for much longer than this is desired.

Dicoumarol

This brings us to consider dicoumarol, which is given orally and is thought to prevent the development or extension of thrombosis by lowering the prothrombin and thereby the coagulability of blood and we propose to confine the rest of the paper to a consideration of this drug, which is now widely used in the treatment of many forms of venous and arterial thrombosis.

Dicoumarol has not proved to be one of those drugs which fade into disuse after a short period of popularity. Rather the reverse is true, and as time has passed and more and more reports have appeared its place in the treatment of thrombosis has become more firmly established. It is true, of course, that many of these reports have dealt with such small numbers of cases that their value has been negligible, but several large and well-controlled series have been published, and these are impressive enough to justify its use. A committee of the American Heart Association has recently reported the results in 800 cases of coronary thrombosis (Wright *et al.*, 1948). Of these, 368 patients admitted on even days received conventional treatment without anticoagulants; and 432 admitted on odd days were given the same conventional treatment with the addition of anticoagulants, mainly dicoumarol. While the mortality rate in the control group was 24%, in the treated group it was 14.9%, and this reduction was accounted for mainly by the much smaller number of deaths after one or more thrombo-embolic complications in the treated group.

More than 25% of the control group developed one or more thrombo-embolic complications, as compared with about 12% in the treated group. Moreover, in only about 6% did these complications occur at a time when the anticoagulant therapy could reasonably be expected to be effective.

The incidence of the various thrombo-embolic complications was noted, and it was found that for extensions of the original infarct, for new infarctions, for pulmonary, cerebral, and peripheral emboli, and for venous thromboses the incidence was notably less in the treated group.

These results provide striking confirmation of the effectiveness of dicoumarol as an anticoagulant and are likely to increase its use in coronary thrombosis considerably. The results of its use in venous thrombosis are also striking, although frequently the findings have not been so well controlled

Unfortunately two considerations have to be borne in mind before a decision to use it as a routine can be adopted. First, no uniform scheme of dosage can be prescribed, because of the marked individual variation in response. Secondly, dosage must be carefully controlled, because not infrequently a haemorrhagic tendency, which may be severe, complicates treatment. This is often due to too great lowering of the prothrombin level of the blood. Accordingly, dosage in each case must be controlled by regular estimations of plasma prothrombin—ideally every day, certainly not less often than every second day. In our opinion this consideration should limit the use of dicoumarol to patients in hospitals with adequate facilities for these tests. Even in these circumstances haemorrhage —e.g., severe haematuria—will occasionally occur. Without such careful control one of two things is likely to happen: either small ineffective doses will be used or effective doses will lead to some disastrous haemorrhages.

On the question of what test should be used to control the dosage there has been much difference of opinion and confusion of thought. It is now possible to clear away some of the misconceptions which have arisen in the past few years and therefore to understand better the significance of the different techniques of so-called "prothrombin estimation." Certainly the lack of knowledge concerning these techniques and the resulting confusion have prevented dicoumarol being used on a much wider scale.

The simplest theory of blood coagulation is that of Morawitz, who describes the process as occurring in two steps: (1) thrombokinase + prothrombin + calcium→thrombin; (2) thrombin + fibrinogen → fibrin. It used to be thought that thrombokinase was derived mainly from disintegrating platelets in shed blood and from tissue cells damaged in a wound; now its main source is believed by some to be a precursor in the plasma (Lozner *et al.*, 1942). According to this theory it converts prothrombin to thrombin in the presence of free calcium ions. When enough thrombin has accumulated the soluble protein fibrinogen is converted to strands of fibrin which contain the red cells and serum in their meshes.

Prothrombin Estimation

(*a*) *Quick's Method.*—This is based on the above theory, and is widely used in America and this country in the control of treatment with dicoumarol. In this method the source of thrombokinase is a suspension of acetone-dried rabbit brain. A similar substance can be obtained from most tissues—e.g., several workers have used an extract of lung, and now human brain is widely employed.

The essential steps in the test are: (1) 4.5 ml. of blood is mixed with 0.5 ml. of oxalate solution. The calcium is thereby removed and development of Stage 1 is prevented. (2) To 0.1 ml. of plasma the same volume of brain-powder suspension is added—the tube being held in a water bath at 37° C. (3) Then 0.1 ml. of calcium solution is added

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quickly and the time between this addition and the formation of fibrin is noted, a stopwatch being used.

This is the so-called "prothrombin time." The theoretical justification for regarding this time as an indication of the prothrombin content of the plasma is that when optimal amounts of thrombokinase and calcium are added the only variable in Stage 1 is the amount of prothrombin. Therefore the smaller the amount of prothrombin the longer the time before enough thrombin is formed to bring about Stage 2.

Various workers have found different times with Quick's method in normal plasmas, usually ranging between 15 and 30 seconds. This is because different batches of brain extract vary in potency, and the normal time for each batch must be carefully determined before the test is used in patients. Moreover, the preparation of the powder is tedious.

(*b*) *Russell Viper Venom.*—The foregoing considerations led one of us (H.W.F.) to suggest the use of Russell viper venom as the source of thrombokinase in this test (Fullerton, 1940). Six years earlier Macfarlane and Barnett (1934) had investigated the possibilities of this substance in their search for a local haemostatic effective in haemophilia. They found that the venom was capable of accelerating the clotting of haemophilic blood in dilutions of 1 in 10^{12} or more. It did not clot oxalated or citrated blood unless calcium was added, and it did not clot solutions of fibrinogen. Accordingly, its action was that of a powerful thrombokinase.

As a result of this work Russell viper venom was produced commercially. The best-known preparation is "stypven" (B.W. and Co.): 0.1 mg. of dried venom and 1 ml. of distilled water are supplied in separate ampoules and the solution is made shortly before use. Different batches differ little in potency, and with normal plasmas "prothrombin times" of 18–25 seconds are usually obtained; consequently the careful standardization of each batch is unnecessary. Apart from this difference in the source of thrombokinase the technique is the same as Quick's.

(*c*) *Venom plus Lecithin.*—The venom method was quickly followed by a third. Witts and Hobson (1940) described a series of observations in which they used a mixture of venom solution and ovo-lecithin as the thrombokinase. Normal plasmas gave very short "prothrombin times" of 6–10 seconds, so it is evident that some substance in the ovo-lecithin potentiates the action of the venom. Witts and Hobson, and recently Biggs and Macfarlane (1949), added an alcoholic solution of lecithin to the venom. With this method we have had variable results. We believe that it is much more satisfactory to use water instead of alcohol as the solvent. When ovo-lecithin is dissolved in water containing 0.5% phenol the solution retains its full activity for several months. This solution is added to a freshly opened ampoule of venom in the usual proportion (1 ml. to 0.1 mg. venom). With this modification the method is therefore a very simple one to use. With it we have not observed the marked day-to-day variations recorded by Biggs and Macfarlane during treatment with dicoumarol, nor have we found it insensitive as an indication of the effects of this drug; in fact, definite prolongation of the time has usually been observed on the second or third day of treatment—i.e., at the same time as changes occur when brain powder is used.

The questions then arise: How do these three methods compare? Do they measure the same factor when they are used to control the dosage of dicoumarin, or is one to be preferred to the others when it is used in this way? These questions can be answered satisfactorily if we base our deductions on a theory of coagulation slightly different from that of Morawitz.

Role of the Lipoid Factor

There seems no doubt that a lipoid substance is essential for the coagulation of blood. For example, Macfarlane *et al.* (1941) have shown that oxalated plasma from which the fat has been removed by high-speed centrifuging or by extraction with carbon tetrachloride will not clot after the addition of a fat-free thrombokinase such as viper venom and calcium unless some lipoid material like ovo-lecithin or cream is added as well. We believe that it is not only essential in the first stage of coagulation but that it is a separate and distinct substance—in a way similar to calcium but with the difference that while it is simple to free a plasma of the calcium ions without disturbing the other factors concerned in coagulation it is very difficult to free it entirely of the lipoid substance. Moreover, alterations probably occur in the concentration of this lipoid factor in the plasma of a degree sufficient to alter the coagulability of blood.

If we accept this theory then the following is clear:

(1) That with the venom+lecithin method optimal amounts of thrombokinase and the lipoid activator are added in the test. Therefore this method measures the prothrombin level but will not reveal alterations in coagulability due to variations in the plasma level of the lipoid factor.

(2) Brain extract contains optimal amounts of lipoid factor and variable and suboptimal amounts of thrombokinase. The results of a representative experiment illustrate this point (Table I). All the estimations were made in one sample of plasma. With venom alone an average time of 22.5 seconds was obtained. With the particular batch of brain we were then using the time was 24.1 seconds; with venom+lecithin it was 9.3 seconds. Adding ovo-lecithin to the suspension of the brain powder produced no shortening of the time: this indicates that the brain extract already contained an optimal amount of the lipoid factor. But the addition of venom (0.1 mg. per ml.) to the brain suspension reduced the time to 10.8 seconds, which is not significantly longer than the result with venom+lecithin (9.3 seconds). This indicates that the concentration of thrombokinase in the brain suspension is suboptimal.

TABLE I.—*Prothrombin Time with the Various Methods*

Thrombokinase	"Prothrombin Time"
Venom	22·5 secs.
Brain powder	24·1 ,,
Venom + Lecithin	9·3 ,,
Brain powder + Lecithin	25·4 ,,
Brain powder + Venom	10·8 ,,
Brain powder + Venom + Lecithin	10·2 ,,

We believe, therefore, that Quick's test, like the venom+lecithin method, measures prothrombin but not changes in coagulability due to variations in the lipoid factor. Since optimal concentrations of thrombokinase and the lipoid factor can be assured with the venom+lecithin method it would seem preferable to the brain extract method.

(3) When venom alone is used we have a thrombokinase which is free of lipoid factor. Accordingly, by this method the so-called "prothrombin time"—the "accelerated clotting time" is a better term—is an accurate measure of prothrombin only if the concentration of lipoid factor in the plasma is within the normal range. And, conversely, if the prothrombin is constant this test will measure variations in the lipoid factor in the plasma.

The justification for these statements and their practical importance is based on the following experimental work.

Effect of Alimentary Lipaemia

The effect of lipaemia on the coagulability of blood following a high-fat meal was determined by the "accelerated clotting time," venom being used as the thrombokinase.

When the ordinary ward meals are taken there is no significant variation in the accelerated clotting time throughout the day; this conclusion is based on a study of eight patients. But in a group of six patients given a breakfast containing 85 g. fat (bacon and eggs and bread-and-butter) the results were striking (Table II).

TABLE II

Case	Fasting	During Lipaemia	Difference	Time of Shortest Reading After Meals
	Fat Intake 85 g.			
1	24·5 sec.	10·9 sec.	13·6 sec.	5 hours
2	22·3 ,,	13·4 ,,	8·6 ,,	4¾ ,,
3	23·0 ,,	12·8 ,,	10·2 ,,	3 ,,
4	24·3 ,,	17·5 ,,	6·8 ,,	2½ ,,
5	22·3 ,,	14·8 ,,	8·0 ,,	3¾ ,,
6	24·7 ,,	13·1 ,,	11·6 ,,	3¾ ,,
Average ..	23·5 ,,	13·7 ,,	9·8 ,,	

In the second column is shown the accelerated clotting time in seconds with the patients fasting (average 23.5); in the third the shortest accelerated clotting time during the lipaemia (average 13.7 seconds); next the difference between the fasting and the shortest times (average 9.8 seconds); and lastly the time of the shortest reading in hours after the meal. In each patient a much shorter time was found at the height of the lipaemia.

Chart I shows the details in one case. The curve marked V represents the accelerated clotting times with venom as the thrombokinase falling from about 25 seconds fasting to 10.9 seconds at the height of the lipaemia. The line V+L was obtained by using venom and lecithin as the thrombokinase; it runs in a more or less horizontal line. The line R.B. was obtained by using rabbit brain extract; it too runs horizontally.

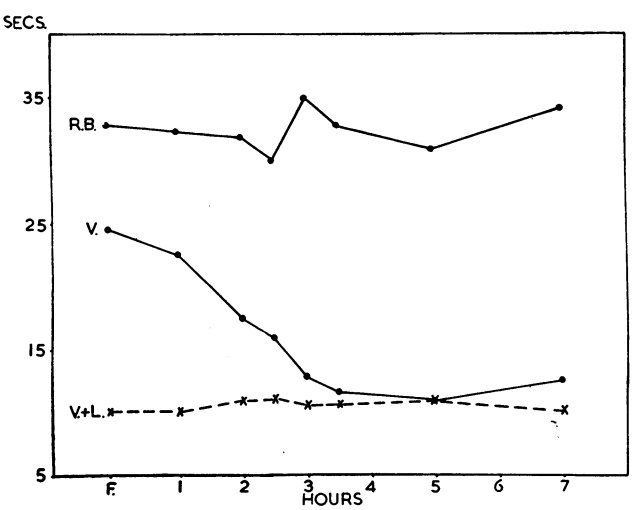

CHART I.—Accelerated clotting times in a patient after a meal containing 85 g. of fat.

Therefore we can conclude that accelerated clotting times determined by the use of venom show marked shortening during lipaemia, a result which is obscured by the use of venom+lecithin and of brain extract, because both these reagents contain an optimal amount of the lipoid factor essential for clotting.

Effect of Alimentary Lipaemia during Treatment with Dicoumarol

The next step was to investigate the effect of alimentary lipaemia in patients in whom dicoumarol treatment had produced a low prothrombin level. This was done in eight patients. The results varied according to the time after the start of dicoumarol treatment when the test was done and also according to the degree to which the prothrombin level had been depressed by the treatment. But it was very clear that the shortening of the accelerated clotting time produced by alimentary lipaemia was much less marked during dicoumarol treatment, and in some cases it was absent altogether.

One example will suffice (Chart II). The patient had received dicoumarol treatment for coronary thrombosis. The accelerated clotting time with venom with the patient fasting was 33.7 seconds. He then took the meal containing 85 g. fat, and at intervals of one hour, two hours, two and a half hours, three hours, and three and three-quarter hours thereafter the times were 32.9, 33.6, 32.6, 31.1, and 33.2 seconds: that is, there was no significant shortening after the meal. The venom+lecithin curve runs horizontally and the rabbit brain extract curve horizontally with some irregularity.

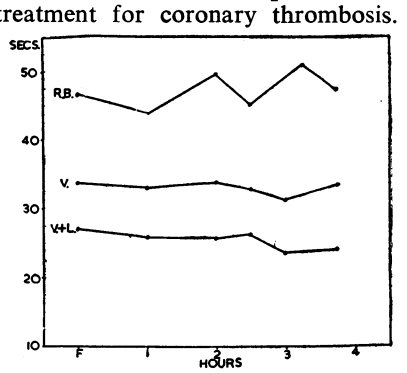

CHART II.—Accelerated clotting times in a patient undergoing dicoumarol treatment and after a meal containing 85 g. of fat.

We believe that the explanation for this striking difference is that when one component in the first stage of coagulation—viz., prothrombin—is depressed by dicoumarol a compensatory increase in another component—the lipoid factor—occurs and so lessens the reduction in coagulability produced by the drug. Alimentary lipaemia produces little or no shortening of the accelerated clotting time when the effects of dicoumarol are marked, because even in the fasting state the plasma level of the lipoid factor may be nearly optimal.

Further evidence in support of this theory is provided by comparing the results obtained by the use of the venom and the venom+lecithin tests throughout the period of dicoumarol treatment. Chart III illustrates the typical findings. Before treatment the accelerated clotting time with venom was 22 seconds; with venom+lecithin 10.5 seconds. Then 1,700 mg. dicoumarol was given over a period of seven days. Increases in the clotting times occurred with both methods, but the difference between the two became less, and it increased again as the effect of the dicoumarol passed off. We believe that this result is due to an increase in the lipoid activator in the plasma, which

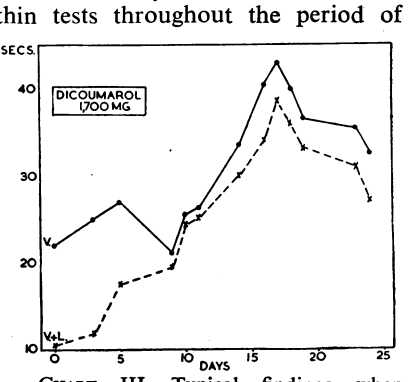

CHART III.—Typical findings when 1,700 mg. of dicoumarol was given for seven days.

after some time reaches nearly an optimal concentration. The effect of this is that the addition of lipoid factor to the venom—as in the venom + lecithin test—produces little or no potentiating action.

On the basis of these results it may be concluded that when dicoumarol is controlled by tests using venom + lecithin or brain extract a reasonably accurate assessment is obtained of the reduction in prothrombin produced by the treatment. But this reduction in prothrombin does not accurately represent the effect of dicoumarol on the coagulability of the blood, since an increase in lipoid factor follows the reduction in prothrombin. The test using venom alone reflects this alteration in coagulability, but does not accurately determine the reduction in prothrombin.

Effect of Haemolysis

One additional point of considerable practical importance is that slight haemolysis produces considerable shortening of the accelerated clotting time when the venom test is used, but not with the venom + lecithin and brain extract tests.

Chart IV illustrates this point. Haemolysed solutions were prepared by adding one volume of packed red cells

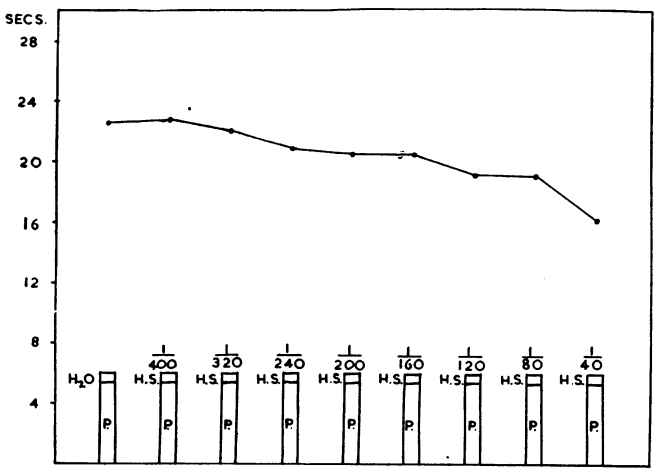

CHART IV.—The effect of haemolysis in shortening the accelerated clotting time with the venom test.

to different volumes of distilled water—one volume of cells in 400 of water, one in 320 of water, one in 240, and so on up to one in 40. One volume of the resulting solutions was then added to nine volumes of plasma, and as a control one volume of water was added to nine volumes of plasma. The accelerated clotting times using venom were then estimated in each, four determinations being made and the average taken. It is clearly seen that as the strength of the haemolysed solution increases the clotting time falls from the control level of 22.6 seconds to 16.1 seconds. We particularly wish to stress that in the range of haemolysed solutions used in this experiment the presence of haemolysis could be recognized macroscopically only at the right-hand end of the scale; in the last plasma slight but definite haemolysis was discernible; in the next a mere trace; in the next it was doubtful; and in the others haemolysis could not be detected.

It is clear, therefore, that when the venom test is used the greatest possible precautions must be taken to avoid haemolysis; the syringe must be dry and must be manipulated gently, and inversion of the tube to ensure mixing of blood and oxalate solution must be done very carefully.

Failure to appreciate the effect of haemolysis has been responsible for some of the confusing results reported. For example, recently there appeared an article by James (1949), who set out to compare the venom and the brain test in patients treated with dicoumarol. He used oxalate crystals instead of solution, and to ensure that they dissolved rapidly in the blood he shook each sample well. Obviously he was producing variable degrees of haemolysis, and accordingly his results with the venom test must be discounted.

The active agent in the red cells which produces this effect is probably the lipoid factor in the cell envelope; hence the effect is not seen with the venom + lecithin and brain extract tests.

Conclusion

Lastly the question arises, Which is the best test to use in the control of dicoumarol treatment? The answer is, I think, that it does not matter greatly which is used: the important points are to understand clearly the significance of the findings and to carry out the test with scrupulous attention to detail.

We use both the venom and the venom + lecithin method in each case. This entails very little extra work and the findings give important information. We aim to maintain the accelerated clotting times at about 25 seconds with the venom + lecithin test and at 35–40 seconds with the venom test—i.e., less prolongation proportionally with the latter. Prolongations of the time with the venom test require a larger dosage of dicoumarol and indicate a lower concentration of prothrombin than comparable prolongations with the venom + lecithin and brain tests. A failure to appreciate this point has caused much confusion and has been responsible for the view that the venom test is not suitable for the control of dicoumarol treatment. We do not express the results as a percentage of normal: this involves making unwarranted assumptions and confuses the picture. Until standard preparations of brain powder are obtainable we can see no theoretical advantage in using them. It would seem that their use is unlikely to give any more valuable information, but a careful comparison of the three tests has not yet been reported.

We give 300 mg. of dicoumarol on the first day and 200 mg. on each of the two succeeding days. Further treatment is not given until the effect of these initial doses is seen, and then the dosage depends not only on the degree of prolongation of the accelerated clotting time but also on the speed with which this develops. No definite rules can be given; each case must be considered individually, and some experience is necessary before the desired prolongation can be attained and maintained. On several occasions haematuria has developed: we have used no special treatment for it apart from stopping dicoumarol, and no harm has resulted.

Summary

The value of heparin and dicoumarol is briefly appraised, and the difficulties of calculating accurate dosage are mentioned. Three methods of determining blood coagulability are described. The role of the lipoid factor in blood clotting is discussed from this standpoint. An account is given of experimental work showing the effect on the coagulability of the blood of lipaemia and haemolysis, and important points of technique in tests of blood coagulability are emphasized.

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