Effect of Phospholipid Fractions upon the Coagulation Defects in Patients on Long-Term Dicumarol Therapy

By Paul W. Boyles, Ralph Jones, Jr., and E. Sterling Nichol

The relatively frequent occurrence of bleeding in patients on a long-term anticoagulant regimen emphasizes the need for further study of the effects of such therapy. In this investigation, the coagulation defects following dicumarol administration over prolonged periods were studied. Correction of some of these defects has been effected in vitro and in vivo by phospholipid fractions of human brain or of soy beans.

Materials and Methods

Selection of patients.—The patients under investigation had been on continuous dicumarol therapy (25 mg. to 100 mg. per day) for a minimum period of six months. This group consisted of 30 patients examined weekly in the Out-Patient Clinic of Jackson Memorial Hospital. The severity of cardiac disease and the nature of its management were not taken into consideration in evaluating the data obtained in this study.

Control specimens were obtained from normal individuals. Serum samples from patients with known PTC deficiency and Stuart Factor deficiency were used as indicated under Results.

Coagulation studies.—Venous blood was collected from non-fasting patients with siliconed syringes. Blood allowed to clot in glass test tubes at 25 C. was used to determine the clotting time. Aliquots of the serum obtained from the specimen were incubated at 37 C. for one hour and four hours after clotting for the prothrombin consumption test and the thromboplastin generation test, respectively. Plasma was obtained from whole blood to which 0.1 M potassium oxalate solution had been added. Plasma and serum were separated by centrifugation at 3,000 × g at 25 C. for 15 minutes. Platelet-rich plasma was obtained from whole blood to which 3.8 per cent sodium citrate solution had been added in siliconed test tubes. The RBC were allowed to settle at 25 C. for 15 minutes, and the supernatant plasma was removed for use in the thrombin generation test.

Soy bean phospholipid was prepared by suspending "Lecithin"† in saline (50 mg./ml.) with a Waring Blender and autoclaving the material in 50 ml. vials. This material was stored in the deep freeze (−20 C.) until used. Brain phospholipid (25 mg./ml.) was prepared as described by Bell and Alton* and stored at −20 C. until used.

1. The thromboplastin generation test† was used with brain phospholipid to replace platelet suspensions according to the modification described by Bell† unless otherwise indicated.

From The Coagulation Research Laboratory, Miami Heart Institute, Miami Beach, and the Department of Medicine, University of Miami School of Medicine, Coral Gables, Fla.

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†The Glidden Company, Chicago, Ill.
2. One-stage prothrombin was performed with the use of human brain thromboplastin with 0.025 M calcium chloride.

3. The two-stage prothrombin determination was modified by the use of bovine serum and a preparation of Fraction I* which had been purified by adsorption with barium sulfate and subsequent precipitation with 25 per cent ammonium sulfate.

4. Fibrinogen (fibrin) determinations were carried out by a previously described method.*

5. Accelerator Globulin (AcG) was estimated by the one-stage method of Stefanini.†

6. Antithrombin was determined by the method of Wilson.†

7. Prothrombin consumption was modified by employing bovine Fraction I* to replace deprothrombinized plasma.

8. Thrombin generation was assayed by the method of MacFarlane and Biggs.‡

9. Clotting time was performed by the Lee and White technic.‡

10. Anti-thromboplastin was determined by the procedure of Fantl and Nance.‡

11. Serum prothrombin conversion accelerator (SPCA) was estimated by the one-stage method of Owren.†

The effects of human brain or soy bean phospholipid fractions on the dicumarol-induced coagulation defects were studied in vitro. The thrombin generation test was performed with the use of 0.1 ml. of saline suspension of these phospholipid materials added to plasma. In the determination of the clotting time 0.9 ml. of whole blood was added to 0.1 ml. of phospholipid suspension.

RESULTS

The 30 patients studied had been on long-term dicumarol therapy. Each blood specimen from these patients demonstrated multiple factor deficiencies. Phospholipid fractions corrected the clotting time without alteration in the level of the coagulation factors.

Studies on the Coagulation Defects Induced by Long-Term Dicumarol Administration

In addition to the known effects of dicumarol on the prothrombin conversion factors, long-term anticoagulant therapy was found to cause a defect in thromboplastin and thrombin generation (fig. 1). Ac-globulin level, prothrombin consumption, fibrinogen† and antithrombin† levels were normal in the patients studied.

It was confirmed that prothrombin values were higher than those indicated by the one-stage method (fig. 2).

The thromboplastin generation test was abnormal in patients on long-term dicumarol therapy. This abnormality in thromboplastin generation was not caused by an increase in antithromboplastin (fig. 3). Barium sulfate absorbable components (i.e., serum components) completely corrected the thromboplastin generation test (fig. 4). In 70 per cent of patients PTC-deficient serum partially corrected the serum, and in 30 per cent it completely corrected the defect. Mixtures of dicumarol, PTC-deficient and Stuart Factor-deficient sera completely corrected the abnormal thromboplastin generation, while combinations of any two of these sera resulted in partial correction (fig. 5). The relative independence of the levels of prothrombin and SPCA

*The Armour Company, Chicago, Ill.
†Unpublished data.
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Fig. 1.—Effect of long-term dicumarol on blood coagulation. The dots indicate the actual values obtained in the patients studied. The shaded area represents the normal ranges.

compared to the level of thromboplastin generated from the patient’s serum, illustrated by the serial studies in a typical patient, is shown in figure 6.

Effects of a Soy Bean Phospholipid Fraction on the Hypocoagulable State of Long-Term Dicumarol Administration

In vitro studies.—Phospholipid fractions (of human brain or of soy beans) at concentrations of 2.5 mg. per ml. corrected the abnormal clotting times and increased the prothrombin consumption (fig. 7). Acceleration in the rate of prothrombin consumption by phospholipid suspension is illustrated in figure 8. Thrombin generation of blood from patients on dicumarol therapy was uniformly improved by the addition of phospholipid suspension (fig. 9). The corrections are brought about by the phospholipid fractions without alteration in the levels of the prothrombin, SPCA or the serum thromboplastic factors.

In vivo studies.—In these 30 patients on long-term dicumarol administration, six instances of bleeding were encountered during a 12 month period. Hemostasis was generally established within 24 hours after the intravenous administration of vitamin K. Recurrent thrombotic episodes (i.e., the “rebound” phenomenon), due to rapid changes in the concentration of coagulation factors, were not observed.

One patient developed purpura in addition to the other sequelae of dicumarol therapy (i.e., prolonged clotting and prothrombin times and defective generation of thromboplastin and of thrombin.) After the intravenous administration of 2.5 mg. of vitamin K, the patient’s hemorrhagic status and defective coagulation remained unchanged for 24 hours. Soybean phospho-
lipid, 780 mg. (0.25 per cent suspension in 0.9 percent NaCl), was infused intravenously over a 15 minute period. Immediate correction of the abnormal clotting time and thrombin generation occurred. The prothrombin level and the generation of thromboplastin were changed 24 hours after the administration of the phospholipid suspension. Figure 10 illustrates the data obtained.

One patient with cerebral bleeding from long-term dicumarol therapy was treated with soybean phospholipid, 2,400 mg. (0.25 per cent solution in 0.9 per cent NaCl) intravenously over a two-hour period. The patient's clotting time and thrombin generation improved with the phospholipid therapy but the patient's prothrombin time and thromboplastin generation became progressively more abnormal, and the patient died within four hours after the onset of treatment with phospholipid. Necropsy examination confirmed the presence of massive intracerebral hemorrhage.
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FIG. 3.—Effect of thromboplastin concentration on prothrombin time. The prothrombin times with various dilutions of tissue thromboplastin are plotted for normal plasma and plasma from patients on long-term dicumarol therapy on a logarithmic scale.

DISCUSSION

Our results indicate that prolonged dicumarol therapy depresses the normal plasma prothrombin and SPCA (cf. Owren's report). Recent studies have shown that PTC is reduced with long-term anticoagulant therapy. Mixing of PTC-deficient plasma and blood from patients on anticoagulant therapy for more than 30 days has been shown to correct the abnormal prothrombin consumption in many instances. Our studies are in agreement with these findings. The ability of the blood to generate thromboplastin is completely independent of the coagulation components measured by the prothrombin time determination, which is commonly used as a guide for anticoagulant therapy. The deficiency of thromboplastin formation can be demonstrated
most effectively by the thromboplastin generation test. We have found that dicumarol serum when mixed with the serum of a patient congenitally deficient in PTC partially corrects the thromboplastin generation test. This suggests a deficiency in dicumarol serum which may be caused by PTC.

During the past two years we have observed several patients who were bleeding from short-term dicumarol therapy. These patients had, in general, either an excessively prolonged prothrombin time or some complicating pathologic condition associated with the bleeding. In contrast, we have observed bleeding in patients on long-term dicumarol therapy in whom the bleeding occurred with prothrombin times in the range of 30 to 40 seconds, a prolonged coagulation time and poor thromboplastin formation. In addition, some patients on long-term anticoagulants have developed bleeding in association with prolonged prothrombin times, clotting times and poor throm-
Fig. 5.—Effect of PTC and Stuart Factor-deficient sera on the thromboplastin generation test. The following mixtures of sera were used with normal plasma and suspension of brain phospholipid in the thromboplastin generation test: $\frac{1}{2}$ volume of dicumarol serum and $\frac{1}{2}$ volume Stuart serum; $\frac{1}{2}$ volume Stuart serum and $\frac{1}{4}$ volume PTC serum; $\frac{1}{2}$ volume PTC serum and $\frac{1}{2}$ volume dicumarol serum; and $\frac{1}{3}$ volume PTC serum, $\frac{1}{3}$ volume dicumarol serum and $\frac{1}{3}$ volume Stuart serum (demonstrating complete correction).

These observations taken together with previous reports suggest that the abnormal thromboplastin formation is an important factor in bleeding of patients on long-term anticoagulant therapy. Sise et al.\textsuperscript{21} have recently reported bleeding in 23 patients on long-term anticoagulant therapy in whom 15 of the patients had a Quick prothrombin time between 22 and 39 seconds. These authors consider the level of prothrombin to be the important factor in both recurrent thrombosis or hemorrhage in patients on anticoagulant therapy.

A new clotting factor, the Stuart Factor, was recently reported\textsuperscript{24} to be
Fig. 6.—Coagulation defect from long-term dicumarol therapy.

Fig. 7.—Effects of soybean phospholipid on the clotting time and prothrombin consumption.
Fig. 8.—Effect of soybean phospholipid on prothrombin consumption.

Fig. 9.—Results of thrombin generation test on dicumarol blood.

A = AVERAGE OF MAXIMUM UNITS OF THROMBIN PRODUCED FROM DICUMAROL BLOOD
B = AVERAGE OF MAXIMUM UNITS OF THROMBIN PRODUCED FROM DICUMAROL BLOOD IN PRESENCE OF BRAIN PHOSPHOLIPID
C = AVERAGE OF MAXIMUM UNITS OF THROMBIN PRODUCED FROM NORMAL BLOOD
associated with a serum defect in the thromboplastin generation test which is not PTC. The patient in whom Stuart Factor was described had previously been reported to have congenital hypoproconvertinemia (SPCA deficiency).\textsuperscript{25} Mixtures of blood from patients on anticoagulants revealed a deficiency in Stuart Factor. Our findings support the hypothesis that long-term dicumarol administration produces decreased prothrombin, SPCA, Christmas Factor and Stuart Factor concentrations. The results reported indicate that the combined depression of these various factors to a "critical" level is found in patients bleeding from long-term dicumarol therapy. Phospholipid fractions of brain and of soybean reverse the prolonged coagulation time observed in patients on long-term dicumarol therapy, without interference with the specific anticoagulant effect.

The phospholipid preparation accelerates prothrombin conversion. The mechanism of action is not understood at this time. Phospholipid tested both in vitro and in vivo on PTC-deficient patients shows acceleration of the clotting time, improvement in prothrombin consumption and thrombin generation. The phospholipid preparations, however, did not correct abnormal thromboplastin generation.\textsuperscript{26} These observations suggest that the mechanism of action of these phospholipids is the same in both PTC-deficient and dicumarol bloods.
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SUMMARY

The coagulation defects of the blood of 30 patients after six months of dicumarol therapy have been studied. A preparation of brain or soybean phospholipids corrected the abnormal coagulation time and the thrombin generation test of patients on long-term dicumarol therapy. The deficiency in thromboplastin generation of these patients was not corrected.

SUMMARIO IN INTERLINGUA

Le defectos de coagulation in le sanguine de 30 patientes post 6 menses de therapia a dicumarol esseva studiate. Un preparato de phospholipidos ab cerebro o ab soja corrigeva le anormal tempore de coagulation e le test de generation de thrombina in patientes con therapia a dicumarol a longe vista. Le deficientia in le generation de thromboplastina in iste patientes non esseva corrigite.

REFERENCES

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PAUL W. BOYLES, RALPH JONES, JR. and E. STERLING NICHOL