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Plasma Thromboplastin Component: Influence of Coumarin Compounds and Vitamin K on Its Activity in Serum. (22180)

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A new blood clotting factor, termed plasma thromboplastin component (PTC), was recently described by Aggeler, *et al.*(1) and subsequently called Christmas factor by Biggs, *et al.*(2). Little is known about the formation of PTC or about its disappearance from the blood stream. Early workers demonstrated that the level of prothrombin in the blood stream is influenced by vit. K and the coumarin compounds. Since these compounds have been shown to influence "stable factor" as well(3,4), it is of interest to determine whether PTC is similarly affected. The present study demonstrates that patients deficient in vit. K develop a depression of PTC, and confirms the observation of Sise, *et al.*(5) that following the administration of the coumarin compounds there is also a depression of PTC. In both groups we find that PTC activity is restored, in part at least, by the administration of vit. K analogues.

Methods. One-Stage Prothrombin Time was determined by the Link-Shapiro(6,7) modification of Quick's method(8), using commercial rabbit brain thromboplastin* (normal: 14 seconds). *One-Stage Prothrombin Activity* ("per cent" of normal). Normal oxalated plasma was serially diluted with saline (90%, 80%, etc.), and the one-stage prothrombin times of various dilutions determined. Observed times were then plotted against dilution, to provide a standardization curve for comparison of unknown plasmas with normal ones. *Assay of Hemophilioid Factors; Principles Involved*: Thromboplastin is essential for normal conversion of prothrombin into thrombin. Thromboplastin obtained from rabbit brain will react with normal recalcified plasma to form a clot in 14 seconds. This is the basis of the one-stage prothrombin time test. Washed human platelets can form an equally potent thromboplastin, but unlike rabbit brain, must first be incubated with the hemophilioid factors and calcium(9). Hemophilioid factors include: plasma thromboplastin component (PTC), plasma thromboplastin antecedent (PTA) (10) and anti-hemophilic factor (AHF). The ability of these factors, in combination, to transform platelets into a potent thromboplastin, the equivalent of that obtained from rabbit brain, is the basis for the thromboplastin generation test, Biggs, *et al.*(9,11). In the present experiments the thromboplastin generation test is modified so that it becomes a quantitative rather than merely a qualitative assay. Thus the relative levels of any one of the hemophilioid factors can be determined. In the quantitative procedure a reaction mixture containing platelets and calcium is supplied with two of the hemophilioid factors, each in high titer. The third factor—the one to be assayed—is added as an unknown or test preparation. A deficiency of this third factor will be demonstrated by faulty "activation" of thromboplastin. Of the three possible assay procedures, only one was used in the present study—the assay for PTC. *Details of the PTC Assay*: A standard platelet suspension is obtained by the differential centrifugation of citrated normal

*Prepared by Warner-Chilcott Laboratories.

human plasma(9). Adsorbed plasma is prepared by adding 0.2 cc aluminum hydroxide suspension(12) to 2.0 cc of citrated human plasma, agitating for 5 minutes at 37°C, and centrifuging at 2500 rpm for 15 minutes. The supernatant plasma is then removed and diluted 1:5 with 0.85% saline. Serum is prepared by incubating clotted human blood at 37°C for 90 minutes. To insure uniformity, reagents are then frozen and stored at –40°C until used. Sera provide the PTC and PTA needed to activate the platelet suspension; adsorbed plasma provides the AHF. A test serum under investigation, or a normal control serum, is the source of PTC. The assay is based upon variations in PTC activity supplied by such sera. These sera also contain PTA, but unknown sera cannot be used as a reliable source of this factor, since PTA levels in them may vary. We therefore make it a practice to add a stabilizing surplus of PTA, in the form of serum from a patient rich in PTA but congenitally deficient in PTC. Finally, a stabilizing amount of AHF is likewise supplied by adding normal plasma, adsorbed with $Al(OH)_3$; by virtue of the adsorption, this plasma is almost entirely free of PTC and prothrombin.

The reagents are then mixed. An appropriate amount of saline is included in Sol. A to increase sensitivity of the assay.

Sol. A: .10 cc test serum (unknown or normal control serum); 8.20 cc .85% saline; .46 cc serum from patient congenitally deficient in PTC but rich in PTA; 1.24 cc 15% calcium-free acacia in .85% saline to maintain colloidality.†

Sol. B: .20 cc normal $Al(OH)_3$ adsorbed plasma, diluted 1:5 with .85% saline; .20 cc human platelet suspension; .20 cc .02 M $CaCl_2$. Incubate 0.20 cc of Solution A with .60 cc of Solution B in a test tube at 37°C. In this mixture, the activity of thromboplastin reaches a peak after 3-6 minutes of incubation; it then diminishes. Since the amount of PTC in the incubation mixture limits the level reached by this peak, the peak level was chosen as an appropriate measure of PTC. To detect the topmost level with certainty, determinations of thromboplastic activity are made periodically in the region of the peak. At one minute intervals, 0.10 cc aliquots of the incubating thromboplastin are added, simultaneously with 0.10 cc of 0.02 M $CaCl_2$, to 0.10 cc of normal citrated plasma and the clotting times recorded. The citrated normal plasma provides labile and stable factors, prothrombin and fibrinogen. The shortest clotting time of the mixture—recorded at the moment of maximal activation—provides a measure of peak thromboplastic activity. It is recorded as the measure of PTC.

† Acacia was later found to be unnecessary and was replaced by saline.

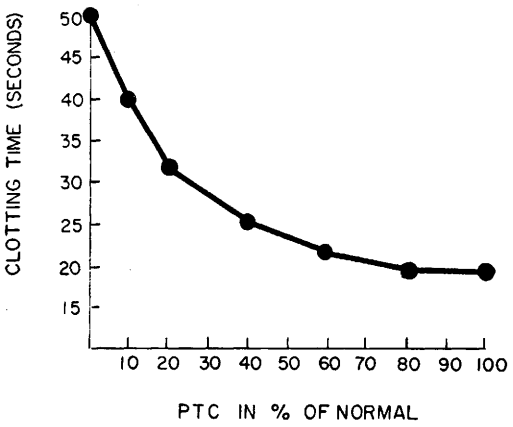

FIG. 1. Standardization curve of PTC assay. The assay is standardized by supplying PTC in graded amounts in the form of serially diluted normal serum.

A PTC standardization curve was prepared for the comparison of PTC content of unknown and normal sera. This was accomplished by supplying PTC to the assay in graded amounts in the form of normal serum, serially diluted. The clotting time at each dilution was plotted in per cent of normal (Fig. 1). Such a curve must be prepared for each new set of reagents. By means of the curve, the clotting times of unknown sera can be interpreted in per cent of normal PTC activity.

Results. The activity of PTC in coumarin-treated and vit. K-deficient patients was studied by means of the PTC assay. The well known deficiency of prothrombin and stable factor which these patients develop was measured by the one-stage prothrombin

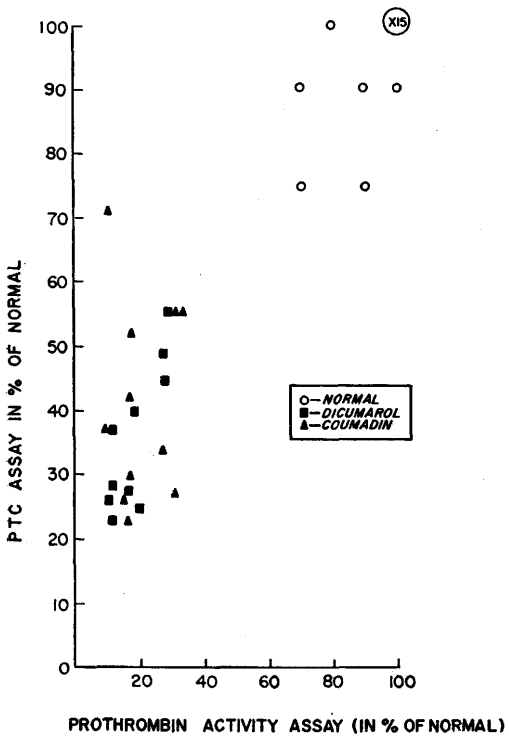

FIG. 2. Relationship between coumarin anticoagulation and PTC activity in 42 treated and untreated patients.

time test and the prothrombin time activity test. These latter assays are not influenced by variations in PTC, since the thromboplastin used in them is fully "activated" already.

Effect of 4-Hydroxy Coumarin Compounds on PTC Levels: Blood specimens were collected from 20 normal persons. A one-stage prothrombin activity test and PTC assay were done on each sample. These same studies were undertaken on 21 patients to whom either 3-3′-methylenebis (4-hydroxy coumarin), known as Dicumarol, or 3 (α-phenyl-β-acetylethyl)-4-hydroxy coumarin, known as Coumadin, had been administered for 4 or more days. These latter patients were suffering from either thrombophlebitis or myocardial infarctions. In Fig. 2 are recorded the test results obtained in both normal and treated patients. PTC levels are plotted against one-stage prothrombin activities. The Dicumarol-treated patients demonstrated significant deficiencies of PTC as well as a depression of the prothrombin activity test. Only one Coumadin-treated patient failed to demonstrate a severe deficiency of PTC. In a second group of patients there was an opportunity to study clotting factor levels before as well as during Dicumarol administration. Representative data, collected from a patient with thrombophlebitis, are shown in Fig. 3. A progressive depression of one-stage prothrombin activity, reflecting drug effect, was accompanied by a similar depression of PTC activity.

Effect of Vit. K_1 on a Depression of PTC Induced by Dicumarol: In patients treated with Dicumarol an attempt is made to maintain the one-stage prothrombin activity between 18% and 30% of normal (27-20 sec.) In one such patient, the drug dosage was inadvertently increased over a 3-day period so that the prothrombin activity decreased to 9% of normal and PTC activity to 34% of normal. At this time, 50 mg of vit. K_1 was given intravenously as a corrective measure. Six hours later, the prothrombin activity had risen to 28% of normal and the PTC activity

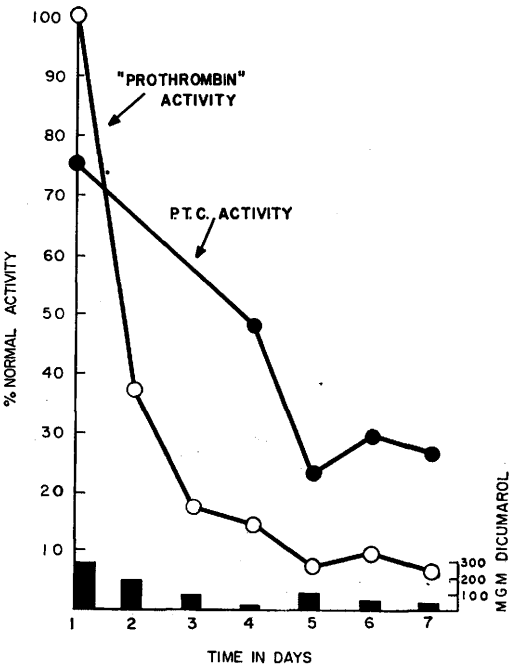

FIG. 3. Effect of Dicumarol on PTC levels and on the one-stage prothrombin activity test in a single patient.

to 43% of normal. After 18 hours, the results were 45% and 57% respectively.

Fat Maladsorption Syndrome: A child, aged 2 years, was found to be unable to adsorb fat-soluble vitamin K. This patient had cystic fibrosis of the pancreas. When tested she demonstrated a marked prolongation of the one-stage prothrombin time (58 sec., or 7% of normal) and a marked deficiency of PTC (8% of normal). Then 15 mg of menadione sodium bisulfite were administered intramuscularly. One hour later, the clotting factor activities had not changed. Eighteen hours after the vitamin administration, the prothrombin time was 18 seconds (57% of normal) and the PTC activity was 65% of normal.

Discussion. Deficiencies of prothrombin, fibrinogen, labile factor and stable factor can be congenital in origin; they can also reflect a vitamin deficiency or be manifestations of hepatic injury. In this regard, plasma thromboplastin component(PTC) is not different. Both vit. K and its coumarin antagonists exert a measurable influence on the activity of PTC, comparable to their effect on prothrombin and stable factor. This influence has been previously investigated, but with the exception of Sise *et al.*(5) the technics employed were too insensitive to demonstrate the moderate alterations that occur(1,13,14).

It is clear that stable factor and PTC have many common properties and characteristics. The one-stage prothrombin time test seems to present the strongest evidence for regarding these two factors as distinct. This test detects deficiencies in prothrombin, stable factor, labile factor and fibrinogen but not in PTC. The rabbit brain used as thromboplastin supplies a PTC-like activity artificially. However, human platelets when used as thromboplastin do not supply PTC-like activity. The necessary "activation" of human platelets by PTC and the other hemophilioid factors makes assay of these factors possible. With further investigation, PTC may prove to be as biologically important as are prothrombin and stable factor.

Summary. Deficiencies of plasma thromboplastin component (PTC) are not necessarily congenital in origin. Serum levels of PTC were depressed following the administration of two 4-hydroxy coumarin compounds to 24 patients. PTC activity was also reduced in a patient who developed a deficiency of vitamin K subsequent to an inability to adsorb fats (cystic fibrosis of the pancreas). In both groups of patients, PTC activity rapidly increased towards normal following the administration of vit. K analogues.

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