A Micro-Method for the Determination of the Combined Activity of Factor II and VII (Prothrombin and Proconvertin)

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This method is intended for use on patients receiving long term anticoagulant therapy, whose veins are either deep seated or covered with haematoma as a result of previous venous punctures. Often with such patients, difficulty arises, when venous blood is required at close intervals. Either the patients show considerable objection to having their blood taken, or it is impossible to obtain the blood without much pain and anxiety to them. Such incidents are not uncommon in older patients, and those confined to bed most of the time.

Technically, this micro-method requires the utmost care and accuracy in collecting the blood and performing the test, if reproducible results are to be obtained. It is therefore not a test ideally suited for use all the time in a busy routine laboratory when there are many specimens to be tested. However, as the results are parallel with those obtained by using the techniques of Owren or Koller et al. using venous blood plasma, it can be adopted whenever venous blood is difficult to obtain, with no fear of disturbing a series of results due to changing a method.

During the period of investigation, over 300 specimens from in-patients undergoing long term anticoagulant therapy were tested at least in duplicate on the same specimen of venous blood, using the new micro-method and Owren's technique. The same amount of diluted plasmas of 1 : 10 concentration and reagents were used in both techniques. Diluting fluid used was buffered saline, a modification of Owren's diluting fluid II (Toohey [1958]). The results obtained by both methods were closely identical with each other in all cases.

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Three hours after these original venous blood samples were taken, the authors personally collected capillary blood from the patients, by the method later described. It was not possible to take venous and capillary blood at the same time, the venous blood being taken on the wards before normal working hours. The results obtained from the capillary blood specimens varied only very slightly from those of venous blood. The differences can be attributed to the fact that the "P & P"*) clotting time of these patients was constantly varying. For the comparison of a sample of the results obtained, see table 1.

Tab. 1

Venous blood plasma				Capillary blood plasma	
Owren		Micro		Micro	
Secs.	%	Secs.	%	Secs.	%
32	59	32	59	32	59
44	35	44	35	45	34
45	34	45	34	43	37
52	27	51	28	52	27
55	25	55	25	55	25
60	22	60	22	62	21
65	19	65	19	72	16
74	16	74	16	74	16
80	14	80	14	83	13
85	13	85	13	85	13
89	12	90	11 3/4	94	11
90	11 3/4	90	11 3/4	90	11 3/4
94	11	93	11	97	10 1/2
100	10	99	10	99	10
110	8 1/2	110	8 1/2	110	8 1/2
120	7 1/2	120	7 1/2	120	7 1/2
130	6 1/2	130	6 1/2	110	8 1/2
240	2 1/2	240	2 1/2	240	2 1/2

Method

Wassermann test tubes are convenient for performing the tests. 0.05 ml of test plasma is diluted in 0.45 ml of buffered saline, giving a 1 : 10 dilution. 0.2 ml of this diluted plasma is accurately pipetted into another tube containing 0.2 ml of $BaSO_4$ adsorbed ox plasma, and 0.2 ml of saline-extract human brain thromboplastin is then added. The mixture in the tube should now be gently

*) = Prothrombin-Proconvertin.

rocked to mix, and is incubated in the water-bath at 37° C for three minutes. After the incubation, 0.2 ml M/30 $CaCl_2$ is added and a stop-watch started simultaneously. After adding the $CaCl_2$ the tube is again gently rocked, and left in the bath standing for 20 seconds before it is examined for clot. All tests should be done in duplicate. It is advisable that the duplicate is carried out at an interval of 10 seconds after starting the first incubation of the first tube. The first serves as a guide, while the second is less disturbed and gives more accurate clotting time. By observing these points mentioned, the authors were able to obtain very close duplicate results of within one second on all specimens tested. It is important that the thromboplastin and $CaCl_2$ should be warmed to 37° C for 15 minutes before the test. The actual clotting time in seconds is converted to a percentage from the standard dilution curve plotted on double log. paper.

Normal control. It is important that all the reagents used are standardised. To do this, a normal control test is carried out preferably before each day's tests. Normal pooled human plasma is used, which has been collected nine parts of blood to one part of citrate-heparin anticoagulant solution. The pooled plasma is kept deep-frozen at — 20° C in tightly stoppered siliconised tubes and in convenient amount enough for each day's test. To 0.05 ml normal pooled plasma, add 0.15 ml Owren's diluting fluid I and mix well. This gives a primary dilution of 25%. From this dilution, 0.05 ml is accurately pipetted into another tube containing 0.45 ml of buffered saline. The actual test is carried out as above. The clotting time should be within about 2 seconds of the time shown as 25% concentration of plasma on the standard dilution curve. In our experience it is usually in the 53—57 seconds range, when the Quick one-stage prothrombin-complex clotting time of the pooled plasma is 12—13 seconds.

Collection of capillary blood. Capillary blood is obtained from either the ear lobe or the finger tip. The site chosen should be rubbed vigorously to stimulate good blood circulation, and warmed if necessary. The area is sterilised preferably with ether, and a quick one-action deep puncture is made by either using a sterile Hagerdorn surgical needle or a sterile „blood-gun". The first drop of blood is wiped away. A micro pipette gratuated to 0.2 ml in 0.01 ml divisions is attached to a polythene sucking tube, and citrate-heparin anticoagulant is sucked up to the 0.02 ml mark. Using this pipette the free flowing blood is then sucked up until the fluid column reaches the 0.2 ml mark. The mixture is then rapidly blown out into a Dreyer precipitin test tube and mixed by tapping the bottom of the tube, but avoiding bubbles while doing so. The specimen is then centrifuged and the supernatant layer is examined for fibrin clots before the test is commenced. In none of the 300 or more specimens taken by the authors, was there any clotting.

Reagents

Buffer solution. Sodium-diethyl-barbiturate 11.75 g, sodium chloride 14.67 g, and N/10 HCl 430 ml. This mixture is diluted to 2000 ml with distilled water. The final pH of the solution should be 7.35.

Diluting fluid I. 100 ml citrate-heparin anticoagulant mixed with 500 ml of physiological normal saline.

Buffered 1/5 normal saline (Toohey [1958]). 200 ml buffered solution is added to 800 ml of 1/5 normal physiological saline.

Anticoagulant. To 250 ml of 3.13% trisodium-citrate solution, 25 mg standard heparin (25 000 IU in 5 ml ampoules) and 25 mg "Merthiolate" are added and mixed well.

When not in use, all the above mentioned solutions should be stored at 4° C to prevent mold contamination.

Preparation of standard dilution curve, saline extract of human brain thromboplastin, and the prothrombin-proconvertin-free ox plasma have been described by Toohey (1958).

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(I) Grade "A" shellback pipettes with capacities of 0.05 ml and 0.2 ml with a single mark are obtainable from Messrs, C. E. Payne & Sons Ltd., 131 Clapham High St., London S.W. 14.

(II) Grade "A" pipettes graduated to 0.2 ml in 0.01 ml divisions are obtainable from, Messrs, H. J. Elliot Ltd., Treforest Indust. Est., Glamorgan, England.

(III) "Blood-gun" with adjustable depth control is obtainable from Messrs, Baird and Tatlock, Freshwater Road, Chadwell Heath, Essex, England.

Summary

A Micro-method for the determination of the combined activity of factors II and VII (prothrombin-proconvertin) based on the techniques of Owren and Koller et al. using capillary blood plasma, is being described. 0.18 ml of the free flowing blood from either the ear lobe or the finger tip is sucked into a micro-pipette previously containing 0.02 ml of anticoagulant. 0.05 ml of the centrifuged plasma is used to make a 1 : 10 dilution with buffered saline for the test. The clotting times in seconds are converted to percentages from the standard dilution curve plotted on double log. paper. Results obtained on over 300 capillary blood samples from in-patients receiving long term anticoagulant therapy were compared with those done by both Owren's technique and the described micro-method on venous blood plasmas. These results were closely identical with each other.

Résumé

Les auteurs décrivent une microméthode pour mesurer l'activité combinée des facteurs II et VII (prothrombine-proconvertine) d'après les techniques d'Owren et Koller et al. en utilisant du sang capillaire. On prend 0.18 ml de sang capillaire d'un lobe de l'oreille ou d'un doigt avec une micropipette qui contient 0.02 ml d'anticoagulant. Pour le test on emploie 0.05 ml de plasma et on le dilue à raison de 1 : 10 avec de la solution de chlorure de sodium tamponnée. Les temps de coagulation sont calculés en pourcentages à l'aide d'une courbe standard sur papier à double échelle logarithmique. La méthode d'Owren et la microméthode décrite ci-dessus ont donné des resultats identiques pour plus de 300 cas testés en parallèle.

Zusammenfassung

Es wird eine Mikro-Methode beschrieben, die es — basierend auf den Methoden von Owren und Koller et al. — gestattet, die gemeinsame Aktivität der Faktoren II und VII (Prothrombin-Proconvertin) im Kapillarblut zu bestimmen. Mit einer 0.2 ml Mikropipette wird zuerst 0.02 ml Antikoagulans und dann bis zur Marke 0.2 ml Blut aus dem Ohrläppchen oder der Fingerbeere aufgezogen. Für den Test macht man mit 0.05 ml Plasma eine 1 : 10 Verdünnung. Mit Hilfe einer Eichkurve auf doppelt log. Papier werden die Gerinnungszeiten in Sekunden in Prozente der Norm umgerechnet. Über 300 Bestimmungen mit der Originalmethode nach Owren und der hier beschriebenen Mikromethode zeigten eine strenge Parallelität der Resultate.

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