The partial thromboplastin (cephalin) time test

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SYNOPSIS Nine partial thromboplastin (cephalin) reagents have been compared in a parallel investigation of groups of patients on 'long-term' anticoagulants, a group with moderate haemophilia, and patients on heparin infusion.

Results with the seven commercial reagents and a human cephalin extract have been correlated with those of a specially prepared and standardized reference preparation of human brain origin. The comparison was similar in principle to that of the prothrombin time thromboplastin standardization using the British Comparative Thromboplastin (BCT).

Results, which for comparative purposes were expressed as ratio of patients' cephalin times to control cephalin times, varied greatly in all three groups. In the oral anticoagulant group some of the commercial reagents were particularly insensitive to the 'intrinsic' clotting defect. The correlation between the 'standardized preparation' and the other reagents was not good and the use of a reference cephalin material for quality control of cephalin time tests does not appear promising.

In moderate haemophilia the commercial reagents were either relatively poor at picking out the clotting defect compared with the 'standardized preparation' or gave such a bad endpoint that the results were not dependable. The poor endpoint also limited the dependability of the results of oral anticoagulation but the 'standardized preparation' and two of the commercial reagents in controlling heparin administration.

In view of these standardization difficulties, which cannot apparently be resolved by the use of reference material, there is need for bulk, routine supplies of a sensitive, standardized cephalin reagent giving good reproducible endpoints. The method for the provision of such material in a recently introduced national supply scheme is described.

The partial thromboplastin (cephalin) time is employed as an overall measure of 'intrinsic' blood clotting. Its main applications are in the screening for hereditary and acquired 'intrinsic' clotting defects and in assessing the intrinsic defect during oral anticoagulant treatment. The test involves the recalcification of platelet-poor plasma in the presence of a crude phospholipid extract. The origins of the latter vary and different animal tissues are used in their preparation. In addition 'home-made' phospholipid extracts from human brain may be made (Hjort, Rapaport, and Owren, 1955) and used at individual hospitals.

The present report describes the results encountered in clinical practice when a variety of reagents currently available in Britain are used. An attempt has been made to correlate these reagents with a 'standardized cephalin preparation'. The comparison of the cephalin reagents has been on patients on 'long-term' anticoagulants (nicoumalone therapy), a group with moderately severe haemophilia, and patients on heparin therapy.

On the basis of the findings a system for standardizing the cephalin time test has been introduced and involving the supply of a sensitive standardized cephalin preparation.

Part I An assessment of the need for standardization

Method of Study

Nine reagents, used as partial thromboplastins in

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the cephalin time test, have been studied. These comprised seven preparations produced by commercial manufacturers, ie, Platelin, Thrombofax, Thrombofax Activated, Diagen Platelet Substitute

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Diascreen, Hoechst and Hyland, and two of our own extracts of human brain (Hjort *et al*, 1955). Both human brain reagents, designated A and B, were made in lyophilized form at the National Thromboplastin Centre. Batch A was produced in the usual way according to the original technique described by Hjort *et al*. With batch B the technique was modified by the addition of strict methods of quality control introduced to produce a sensitive and reproducible partial thromboplastin reagent. This is described in Part II. Batch human A was used in the coumarin study only.

During the performance of the cephalin time test maximum effort was made to avoid contact activation as follows: clean non-traumatic venepuncture; the use of non-wettable syringes and siliconized containers; storage of specimens in melting ice; centrifugation at 4°C; the performance of the tests in parallel by six technicians and the limitation of the number of patients or controls to six on each day of testing. Two of the technicians who performed tests with more than one reagent tested each plasma in turn with the two cephalins. These stringent precautions were additional to the manufacturers' instructions which otherwise were followed. Results were the means of two duplicate tests on each patient.

With the human cephalin preparations A and B a kaolin-activated partial thromboplastin time was used, modified by Thomson (1970), from the method of Egeberg (1961), using a 10-minute activation period.

A ratio method was used for the expression of results, namely,

$$\frac{\text{patient's cephalin time (sec)}}{\text{mean normal control cephalin time (sec)}}$$

This seemed more valid for assessing the comparative sensitivity of the different reagents than simply recording the results of the individual cephalin times. The same principle is used with the British Comparative Thromboplastin when calibrating Quick prothrombin test reagents by correlation of results on anticoagulated patients (Poller, 1970). The reliability of the ratio system depends on accurate normal values. Twenty fresh normal specimens, taken from healthy adults were therefore tested individually with each reagent to give a good normal mean. The ratios obtained in the coumarin-treated group with each cephalin reagent were plotted against the 'standardized preparation' (human cephalin B). Results were plotted on arithmetic graph paper with the 'standardized' preparation (human B) on the ordinate, as in the prothrombin time thromboplastin correlation.

The first group under study were stabilized 'long-term' patients on oral anticoagulant treatment. These normally show a prolongation of the cephalin time test which reflects depression of factors IX and X. The reliability and sensitivity of the available cephalin reagents were thus compared with the 'standardized preparation'. In the first part of the study, cephalin times were performed with each partial thromboplastin time reagent in duplicate on 26 specimens from patients treated on a 'long-term' basis with nicoumalone, Sinthrome. These patients were adequately anticoagulated having prothrombin ratios greater than 2.0 with the British Comparative Thromboplastin.

The commercial reagent Thrombofax Activated only became available towards the end of the study and had to be compared individually with the 'standardized preparation' (human cephalin B) on the 17 coumarin plasmas.

In order to characterize the sensitivity of the reagents to moderate to severe factor VIII deficiency, seven plasmas from haemophilic patients whose factor VIII lay between 1 and 10% of pooled normal were tested individually with each commercial cephalin reagent and the 'standardized preparation'. The results in this series were also expressed as a cephalin ratio.

A study was also initiated on patients on heparin treatment to assess the sensitivity to the presence of circulating anticoagulant and suitability of the commercial reagents and 'standardized preparation'. The patients were on continuous heparin administration by continuous drip and their plasma recalcification times were approximately twice the upper limit of normality.

Results

ORAL ANTICOAGULANT TREATMENT

The results with the nine partial thromboplastin reagents included in the trial are given in Table I. The figures represent the mean ratio of the 26 coumarin plasmas with the eight cephalin reagents in Table I. Table I also gives the mean cephalin time (sec) of the 20 normal control plasmas tested with each reagent. The least sensitive reagents gave ratios of 1.1 whereas the two most sensitive reagents gave mean ratios of 1.4 on the same group of coumarin plasmas. There was no correlation between the length of the mean normal control time and the sensitivity, ie, no increase in sensitivity to compensate for the increasing tedium of the test arising from the longer clotting times. The results for Thrombofax Activated are given in parallel with the 'standardized reagent'. Individual patient's blood

	Mean Ratio	Mean Control Time (sec)
<i>On 26 plasmas</i>		
Thrombafax	1.1	87.2
Hoechst	1.1	45.8
Human cephalin A	1.2	41.1
Platelin	1.2	95.5
Diagen	1.2	49.4
Diascreen	1.3	56.0
Human cephalin B	1.4	43.0
Hyland	1.4	41.5
<i>On 17 plasmas</i>		
Thrombafax activated	1.2	36.5
Human cephalin B	1.5	43.0

Table I Results on coumarin plasmas.

showed even greater variation than the mean values given in Table I indicated. Examples are given in Table II.

The comparison of the ratios of the different reagents with the standardized preparation, using the method recommended for comparison of Quick test thromboplastins and the BCT proved unworkable. When the results were plotted, in most instances it was impossible to draw a 'best line' by visual assessment with any degree of confidence owing to the degree of scatter of results. When the 'best line' was calculated on the basis of the formula for the regression line the extent of the divergence between the visual line and the calculated line with all of the reagents is seen in the figure. Where the lines coincide a correct discernible correlation was detected by eye. The divergence between the two lines indicates the unreliability of the procedure. There were too many aberrant points in five of the eight correlations. Alderson, Poller, and Thomson (1970) showed that with the British system for anticoagulant control when more than two aberrant points were present out of twelve, the correlation with the BCT was not valid. With prothrombin time ratios an aberrant point was defined as a point more than 0.5 of a ratio from the 'best line' in the vertical plane. With cephalin times the ratios are smaller when patients are at therapeutic levels of anticoagulant treatment but even on the liberal 0.5 ratio definition there were

far too many aberrant points. The degree of scatter of the points was probably due to varying sensitivities of the cephalin reagents to different intrinsic clotting factors and probably also reflects different degrees of contact activation with the various techniques and reagents. The scatter was far greater than previously encountered with the comparison of tissue thromboplastins used in the prothrombin time test either by ourselves or hospitals participating in the national collaborative trials (Poller and Thomson, 1969; Alderson *et al.*, 1970). It is relevant to note that the best correlations were obtained with comparisons of the two different human brain preparations A and B, and of human B with Diagen and Diascreen.

PATIENTS WITH MODERATE HAEMOPHILIA

The mean results of the seven patients, whose factor VIII levels ranged from 1% to 10%, are given in Table IIIa. The mean ratio of the seven patients with each cephalin reagent gives an indication of the sensitivity of the reagent to factor VIII depletion. Comment on the endpoint obtained in the test is also given. With Thrombafax, the mean value could not be calculated because the endpoint was not recordable in four of the seven patients. Table IIIb gives the results with the haemophilic with a 10% factor VIII level. With the exception of Diascreen all the reagents showed reasonable prolongation, ie, were sensitive to the defect at this level. Only the standardized preparation, Diagen, and Diascreen gave good, reproducible endpoints. The ill-defined endpoints and poor reproducibility with the other reagents undermines their clinical value. The standardized preparation was the only sensitive preparation with a good, reproducible endpoint.

PATIENTS ON HEPARIN TREATMENT

Results with the two heparinized patients are given in Table IV. The heparin group was limited to two patients because it was found that the endpoint of the test was poor with all the commercial reagents except Diagen, which was good and Diascreen moderately good, at therapeutic levels of continuous heparin infusion. The best endpoint was with the standardized preparation (human B).

Patient No.	Human Brain A	Human Brain B Standardized Preparation	Diagen	Diascreen	Platelin	Thrombafax	Hoechst	Hyland
1	1.2	1.6	1.2	1.4	1.6	1.3	1.1	1.5
2	1.3	1.6	1.2	1.4	1.1	1.0	1.3	1.4
3	1.4	1.8	1.4	2.0	1.3	1.3	1.5	1.4
4	1.9	2.1	1.8	1.7	1.6	1.5	1.4	2.6
5	1.3	1.7	1.3	1.4	1.3	1.2	1.0	1.5

Table II Examples of different cephalin ratios on five individual patients on nicoumalone (Sinthrome)

Fig. Comparison of cephalin ratios obtained with individual reagents and standardized preparation (human cephalin B) on patients receiving long-term anticoagulant treatment

Reagent	Mean Cephalin Ratio
Human brain B (standardized)	4.6
Diascreen A	2.1
Platelin ¹	2.2
Diagen	2.8
Hoechst ¹	3.2
Hyland ¹	4.1
Thrombofax A ¹	5.1
Thrombofax ¹	Not recordable

Table IIIa Mean cephalin ratios with various reagents in patients with haemophilia

¹Poor endpoints

Reagent	Ratio(patient/mean control)	Endpoint
Human cephalin B (standardized preparation)	1.7	Good
Thrombofax activated	2.0	Ill defined
Thrombofax	1.8	Almost unrecordable
Hyland	1.8	Poor
Hoechst	1.4	Poor
Platelin	1.4	Poor
Diagen	1.2	Good
Diascreen	1.1	Good

Table IIIb Results with haemophilic patient (10% factor VIII level)

	Cephalin Time Ratio	
	Patient 1	Patient 2
Human cephalin B ¹		
'standardized preparation'	2.8	1.2
Hoechst	2.6	1.1
Diascreen	2.2	1.1
Thrombofax	3.7	1.2
Thrombofax A	5.8	1.4
Diagen ¹	4.9	1.2
Platelin	4.0	1.2
Hyland	2.1	1.6

Table IV Results of patients on continuous heparin infusion

¹Good endpoints

QUALITY OF THE ENDPOINT

The endpoint of the cephalin time test is important in ensuring the reliability of the result. A comparison has therefore been made of the quality of the endpoint with the various reagents in the different groups studied. Collective observations in all four groups are given in Table V. Only the human standardized preparation and Diagen gave good results with all four groups. Diascreen gave a good endpoint except for the patients on heparin where the endpoint was not clearly defined. Thrombofax reagent gave a poor endpoint throughout. The new activated Thrombofax reagent gave an improved endpoint with normals and the coumarin group whereas Hoechst and Hyland only gave good endpoints with the normal and coumarins. Platelin did not give good endpoints with any of the four groups, although not as bad as Thrombofax.

Comments

Patients on long-term anticoagulant treatment have a combined defect of factors II, VII, IX, and X and may have an induced anticoagulant, PIVKA (Hemker, Veltkamp, and Loeliger, 1968). Variation in sensitivity to specific clotting factors explains some of the differences between the mean ratios with the various cephalin reagents. Varying degrees of contact activation with the individual cephalin time

techniques may be a contributing factor to the discrepant results. These uncontrolled variables in a group of patients might explain the enormous scatter of results when an attempt was made to correlate the ratios on individual patients with the respective reagents (see figure). Only the three cephalin preparations—human A, Diagen, and Diascreen—gave a correlation with the standardized preparation which was acceptable. In the prothrombin time test good correlations are normally obtained between the Quick test reference reagent (British Comparative Thromboplastin) and other Quick test reagents on coumarin blood samples. The cephalin time correlations are therefore disappointing.

The wide scatter in results indicates that there is considerable variation in the ability of these cephalin reagents to measure the coumarin-induced 'intrinsic' clotting defect. Obviously the larger the ratio with a given cephalin and plasma, the greater the sensitivity and the more useful the reagent. Some of the reagents barely detect any change from normal at therapeutic levels of oral anticoagulant dosage and are unsuitable for use in this context. It might be possible to define a minimum sensitivity for such preparations. It is apparent that even with animal tissue reasonable sensitivity to the coumarin defect can be achieved and good quality endpoints obtained.

The impracticability of the correlation between the standardized cephalin material and the other partial thromboplastin reagents has important implications. It will clearly be impossible to standardize cephalin time results of coumarin-treated patients at different centres by the use of a cephalin reference preparation. If uniform results are to be achieved the same reagent for use with a uniform technique will have to be supplied for routine work to the hospitals.

The value of cephalin time tests as an adjunct to prothrombin times for controlling anticoagulant therapy is well established. We ourselves employ the test in parallel with the Quick test in anticoagulant control of our hospital patients and whenever any of our outpatients on anticoagulants require surgery or dental extraction. The test is also advisable if any patient experiences unexpected bleeding or further thrombosis occurs on anticoagulant treatment. In these circumstances to proceed without using a method sensitive to factor IX would be unwise. The test has also been of value in the authors' experience in deciding whether to give vitamin K₁ in patients with excessively prolonged prothrombin times due to anticoagulant overdosage. Indeed Eastham (1968) has recommended the cephalin time as being more valuable than the Quick test in the regulation of long-term anticoagulant administration.

	Normals	Coumarin	Haemophilia	Heparin
Human cephalin B				
'standardized preparation'	Good	Good	Good	Good
Diagen	Good	Good	Good	Good
Diascreen	Good	Good	Good	Moderate
Platelin	Moderate	Moderate	Poor	Poor
Thrombofax	Poor	Poor	Poor	Poor
Thrombofax A	Good	Good	Poor	Poor
Hoechst	Good	Good	Poor	Poor
Hyland	Good	Good	Poor	Poor

Table V Comparison of quality of endpoints in cephalin time tests

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The value of the cephalin time in the diagnosis of haemophilia has been a matter for dispute. Sawers and Hirsh (1970) state: 'The activated partial thromboplastin time test typically gives a prolonged clotting time in all grades of haemophilia except when the patient has recently received transfusion with fresh blood, or with poorly collected blood samples'. Most workers would accept the view that the partial thromboplastin time would probably give an abnormal result if the factor VIII was below 20% (Ingram, 1969).

In the present investigations the variable sensitivity of the commercial reagents to moderate haemophilia is seen and indicates the great variation in sensitivity of these preparations to low factor VIII levels. The mean cephalin ratios for the seven haemophiliacs varied from 2.1 to 5.1 (see Table III).

Part II The preparation of a 'standardized cephalin' extract

No system of quality control has hitherto been recommended for cephalin reagents and there has been little appreciation of the effect of variation of reagents on the sensitivity of the tests.

The first step in the production of a standardized cephalin preparation was to produce a crude phospholipid suspension which showed good sensitivity to the intrinsic clotting defect induced by oral anticoagulants. By modification of and experimentation with the Hjort method (1955) for the production of cephalin from human brain, more sensitive preparations were made. Initially our preparations were of the order of sensitivity of our human brain A and varied considerably between batches.

The first problem therefore was to develop the maximum sensitivity to the intrinsic clotting defect. Many modifications of the human brain cephalin preparation were compared using results of stabilized patients on oral anticoagulant therapy. The aim was to develop the maximum sensitivity combined with reasonable prolongation of the normal value. It was also considered preferable to activate the plasma, as the unactivated cephalin method, although a good test, is more subject to variability in technique of blood collection and storage *in vitro* than the activated method. A 10-minute kaolin activation was chosen. The kaolin activation time with the different techniques varies from a minute or so up to 20 minutes. Even 10 minutes does not give absolutely complete activation and Hardisty and Hutton (1965) recommended 20 minutes. A 20-minute incubation time makes the test very lengthy, and, as activation is almost complete at 10 minutes, this time was preferred.

A large batch of human brain cephalin B has been

The prolongation of some of the tests was due to the indeterminate nature of the endpoint and not only to sensitivity to factor VIII levels. Only three reagents gave good endpoints, these were: the standardized material (human B), Diagen, and Diascreen, although the two commercial ones were relatively insensitive.

The cephalin time is also used for the clinical regulation of heparin dosage at some centres. The relative value of the different reagents in assessing the response is governed by two processes—the sensitivity to the anticoagulant effect of heparin and the quality of the endpoint. The results varied considerably (Table IV). Only the human standardized preparation, and possibly Diagen and Diascreen could be recommended for heparin control as the endpoint was too poorly defined with the others.

prepared in lyophilized form. It is stored at -70°C , and degradation studies carried out at 50°C , 37°C , room temperature, and -20°C indicate that it should be stable at low temperature for a long time. Subsequent working batches of cephalin reagent have been made to conform with the standardized preparation, in a similar way to the method of preparation of the British Comparative Thromboplastin. Working batches of cephalin reagent have been offered to hospitals in the national system for anticoagulant control for clinical assessment. Over 200 hospitals have requested and received supplies of working cephalin reagent. If the preparation and cephalin time method recommended prove acceptable this could provide a basis for a national system for testing and reporting.

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