Standardisation of the Laboratory Control of Anticoagulant Therapy

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SUMMARY

The sensitivity of human and rabbit brain thromboplastin preparations to the coumarin-induced plasma defect was compared with the British comparative thromboplastin (BCT). The human thromboplastin yielded prothrombin ratios that compared favourably with those obtained with the BCT. Furthermore, the dose of anticoagulant required to maintain a prothrombin ratio in the therapeutic range was approximately similar whether the local human brain thromboplastin or the BCT was used.

Rabbit brain preparations showed considerably less sensitivity to the coumarin defect.

A stable suspension of human brain thromboplastin for use as a local standard was prepared and is available to laboratories in South Africa.

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Anticoagulant therapy with the coumarin group of drugs has been used in clinical practice for more than a quarter of a century. The most widely used form of laboratory control of the treatment is the Quick one-stage prothrombin time.[1] This simple test proved to be satisfactory in most cases, but discrepant results can and do occur, often due to inadequate standardisation of the reagents. Different batches of thromboplastin may produce very different results, so that the anticoagulant dosage level required to achieve the therapeutic range of prothrombin time, as reported by different laboratories, may vary very widely. Poller[2] and others[3] have investigated the value of a standardised brain thromboplastin for use in this test. Their aim was to produce a standardised preparation against which any laboratory could compare its own products and, if necessary, correct the results to agree with those obtained with the standard preparation. In this way, prothrombin assays would be comparable from laboratory to laboratory and within the same laboratory at different times.

In 1969 the British Anticoagulant Panel[4] introduced a national system for anticoagulant control in the United Kingdom. This involved the use of a phenolised standard human brain thromboplastin designated as the British comparative thromboplastin (BCT). BCT was made available to us in 1971 and has been used to standardise human brain thromboplastins.

To facilitate comparison, Denson[5] introduced a modification, the thromboplastin sensitivity ratio (TSR), to tabulate the varying sensitivities of different thromboplastin reagents to the coumarin-induced effect. This ratio has made it possible to convert results obtained with any thromboplastin of known TSR to the results that would be obtained had the BCT been used. All preparations of brain thromboplastin we prepared were therefore assigned a TSR value as described below.

At present there is also variation in the method of expressing the results of the prothrombin time tests. In South Africa many laboratories use the prothrombin index. However, Poller[6] has recommended the use of the prothrombin ratio which is calculated by dividing the clotting time of the patient's plasma by the clotting time of pooled normal plasma. It is then possible to convert the prothrombin ratio obtained with any thromboplastin to the ratio that would be obtained if the BCT had been used. This corrected ratio is known as the British corrected ratio (BCR).[6] Theoretically, the BCR obtained by all laboratories using thromboplastins of known TSR, as calculated from a comparison with the BCT, should be identical.

In the present study the TSR of the human brain thromboplastins we prepared were determined. A number of commercial rabbit brain preparations were also tested. From these results an attempt was made to define limits of acceptability for thromboplastin preparations.

In addition, 89 patients on anticoagulant therapy were studied. Their prothrombin times were converted to the BCR and the relationship between the BCR, the therapeutic range advised by Poller[7] and the dosage of Warfarin used, was analysed. This information, it was hoped, would clarify the value of the prothrombin time, as determined locally, in regulating the dosage of coumarin therapy and therefore the degree of anticoagulant effect produced.

MATERIAL AND METHODS

British comparative thromboplastin was obtained (by courtesy of Dr L. Poller) as a liquid phenolised suspension, delivered every 4 weeks by air freight from the UK, and stored at 4°C.

Human brain thromboplastin was prepared by standard procedure.[8] An acetone-extracted powder was prepared

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and then used as a 3% suspension in saline, to which 0,2% phenol (w/v) was added. Fresh suspension was prepared each day.

Twenty-three brain thromboplastin preparations from separate brains were studied over a period of approximately one year. Normal plasmas yielded prothrombin times of between 10 and 13 seconds with these preparations.

Rabbit brain preparations were reconstituted according to the manufacturer's directions.

Fresh normal plasma was prepared from a pool of 5 normal donors and stored at −20°C in small aliquots. This plasma was collected with one-tenth volume of 3,8% sodium citrate as anticoagulant. There was no significant difference between the prothrombin times of citrated and oxalated control plasmas.

Coumarin plasmas were obtained from patients on Warfarin or other coumarin-type anticoagulant therapy. Blood was collected from the patients with 2 mg/ml of a mixture of ammonium oxalate (6 parts) and potassium oxalate (4 parts) as anticoagulant. The plasma was separated after centrifuging at 3 000 *g* for 15 minutes and tested within 4 hours of collection. For the purpose of determining the TSR, patients with varying degrees of Warfarin-induced coagulation defects, ranging from mild to severe, were selected.

Prothrombin times were determined by adding 0,1 ml thromboplastin suspension to 0,1 ml plasma, followed by recalcification with 0,1 ml 0,025M $CaCl_2$ at 37°C. The clotting times were determined manually in duplicate. The results were expressed either as (*a*) prothrombin index, viz.

$$\frac{\text{prothrombin time of control}}{\text{prothrombin time of patient}} \times 100$$

or as (*b*) prothrombin ratio, viz.

$$\frac{\text{prothrombin time of patient}}{\text{prothrombin time of control}}$$

Method of Calculating the Thromboplastin Sensitivity Ratio (TSR)

Prothrombin times were determined for the control and coumarin plasmas using the BCT and the test thromboplastin. The prothrombin ratio was then calculated for both preparations. Between 10 and 20 coumarin plasmas were tested with each thromboplastin preparation used. The ratios obtained with the two thromboplastin preparations were plotted against each other on arithmetic graph paper. The line of regression was fitted by the method of least squares. The point at which the curve intercepted the abscissa was noted (see results below). The ratio obtained with the test preparation that corresponded to a ratio of 2,0 with the BCT represented the TSR for that preparation (Fig. 1).

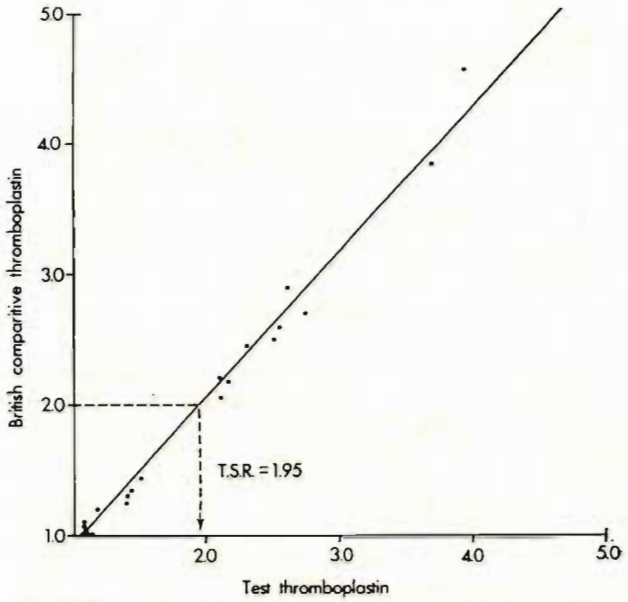

Fig. 1. Determination of thromboplastin sensitivity ratio. The prothrombin ratios of a series of coumarin plasmas tested with the BCT (on the ordinate), and the locally prepared human brain thromboplastin (on the abscissa) are plotted as shown. In this experiment a ratio of 2,0 using the BCT is the equivalent of 1,95 using the local thromboplastin. The TSR of the latter is therefore 1,95.

Method of Converting Prothrombin Ratios to the British Corrected Ratio (BCR)

The ratio of the patient to control prothrombin times was determined using a preparation of known TSR. Reference was then made to the table devised by Denson[2] (see Table I). The column headed by the TSR of the thromboplastin used was located. The ratio of the specimen tested was then found in this column. This ratio corresponded to the ratios shown on the same horizontal line for preparations with the TSR values shown at the top of each column. Thus the BCR (by definition) would be the ratio on the same horizontal line in the column headed by a TSR of 2,0.

For example: if a ratio of 1,87 were obtained on a patient using a thromboplastin preparation having a TSR of 1,7, then the BCR for that patient would be 2,23.

Stability of Phenolised Suspensions of Human Brain Thromboplastin

The acetone-dried brain thromboplastin from one human brain was divided into two aliquots. One aliquot was suspended in 0,2% and the other in 0,5% phenol saline. These in turn were divided into two aliquots. One aliquot was refrigerated at 4°C and the other was kept at room temperature. During the experimental period the room temperature fluctuated between 15° and 28°C. The thromboplastin sensitivity ratio of the 0,2% and 0,5% phenolised suspensions was determined on the day of preparation and at weekly intervals for the following 12 weeks.

TABLE I. CLOTTING TIME RATIOS FOR A TEST PREPARATION EQUIVALENT TO THE CLOTTING TIME RATIOS FOR THE RESEARCH STANDARD (from Denson[5])

Thromboplastin sensitivity ratio (research standard = 2,0)												
1,3	1,4	1,5	1,6	1,7	1,8	1,9	2,0	2,1	2,2	2,3	2,4	2,5
2,4	2,87	3,35	3,84	4,33	4,79	5,27	5,72	6,23	6,68	7,15	7,63	8,1
2,16	2,55	2,94	3,34	3,37	4,1	4,5	4,93	5,28	5,67	6,06	6,45	6,84
2,0	2,32	2,66	2,99	3,34	3,65	3,98	4,34	4,66	4,98	5,33	5,64	5,98
1,85	2,14	2,43	2,73	3,03	3,29	3,57	3,88	4,17	4,44	4,73	5,03	5,32
1,77	2,0	2,25	2,52	2,77	3,0	3,26	3,54	3,78	4,03	4,28	4,53	4,8
1,68	1,88	2,12	2,34	2,57	2,78	3,02	3,25	3,47	3,69	3,92	4,14	4,37
1,6	1,8	2,0	2,23	2,42	2,61	2,83	3,02	3,23	3,43	3,63	3,82	4,04
1,55	1,73	1,92	2,09	2,28	2,45	2,64	2,82	3,0	3,18	3,33	3,55	3,73
1,5	1,67	1,83	2,0	2,18	2,33	2,5	2,67	2,84	3,0	3,18	3,34	3,52
1,47	1,62	1,77	1,93	2,09	2,24	2,39	2,54	2,7	2,85	3,0	3,15	3,32
1,43	1,57	1,71	1,85	2,0	2,14	2,28	2,44	2,57	2,7	2,85	2,99	3,14
1,39	1,52	1,65	1,78	1,93	2,05	2,18	2,32	2,44	2,56	2,7	2,84	2,08
1,37	1,48	1,62	1,74	1,87	1,98	2,1	2,23	2,35	2,47	2,59	2,72	2,85
1,34	1,45	1,57	1,68	1,8	1,92	2,02	2,14	2,25	2,37	2,48	2,59	2,72
1,32	1,42	1,53	1,63	1,74	1,85	1,95	2,08	2,17	2,28	2,38	2,48	2,58
1,3	1,4	1,5	1,6	1,7	1,8	1,9	2,0	2,1	2,2	2,3	2,4	2,5
1,23	1,3	1,37	1,45	1,54	1,6	1,69	1,76	1,83	1,9	1,98	2,05	2,14
1,17	1,23	1,29	1,34	1,41	1,47	1,53	1,59	1,64	1,69	1,75	1,83	1,87
1,12	1,15	1,18	1,23	1,27	1,3	1,34	1,37	1,42	1,45	1,49	1,53	1,57
1,07	1,1	1,12	1,15	1,18	1,2	1,23	1,25	1,28	1,3	1,33	1,35	1,37
1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0

Patients on Long-Term Anticoagulant Therapy

Eighty-nine patients on long-term anticoagulant therapy were studied. The patients were on treatment with Warfarin for periods of between 1 and 12 months (mean ± SD = 6,7 ± 2,8 months). The treatment was instituted for myocardial infarction, deep vein thrombosis of the legs or pulmonary embolism. The dosage of Warfarin was prescribed by one of the authors (B.A.B.). All patients had prothrombin times tested at regular intervals of between 1 and 4 weeks, depending upon the stability of their prothrombin times. The mean of the prothrombin ratios determined during the period of study was calculated for each patient. The mean weekly dose of Warfarin was also calculated for each patient. The mean and standard deviation for the prothrombin ratios and weekly dose of Warfarin of the group of 89 patients were calculated from the individual mean values.

Two brain preparations were used during the study period. Both had a TSR of 1,6. All results were converted to the BCR as described above.

RESULTS

Human Brain Thromboplastin

Thromboplastin sensitivity ratios were determined for 23 human brain thromboplastins. The results are shown in Table II and Fig. 2. The mean TSR was 1,73 (standard

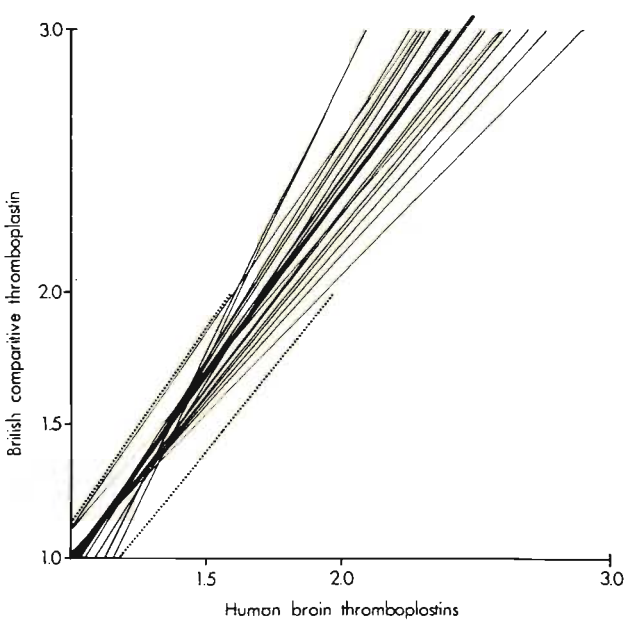

Fig. 2. Thromboplastin sensitivity ratios of 23 human brain preparations. The thin lines indicate individual curves for each brain; the heavy line is the mean curve for all 23 brains, and the dotted lines indicate the limits of TSR and point of interception with the abscissa.

TABLE II. THROMBOPLASTIN SENSITIVITY RATIO OF 23 HUMAN BRAIN PREPARATIONS

	TSR	Deviation from origin
Mean	1,73	0,03
SD	0,14	—
Range	1,6 to 1,95	−0,15 to +0,17 (NB: 14 of 23 curves passed through the origin)

deviation 0,14; range 1,6 - 1,9). Most of the experiments yielded a straight line passing through the origin. In 9 experiments the line missed the origin. These lines all intercepted the abscissa within 0,17 units of the origin. The mean deviation was 0,03 units from the origin. Eight of the lines intercepted the positive and one the negative side of the abscissa.

Fig. 2 shows all 23 curves obtained. The limits between which these curves fell could be defined by the range of TSRs obtained *and* the range of points of interception from the origin. These limits are shown by the dotted lines in Fig. 2.

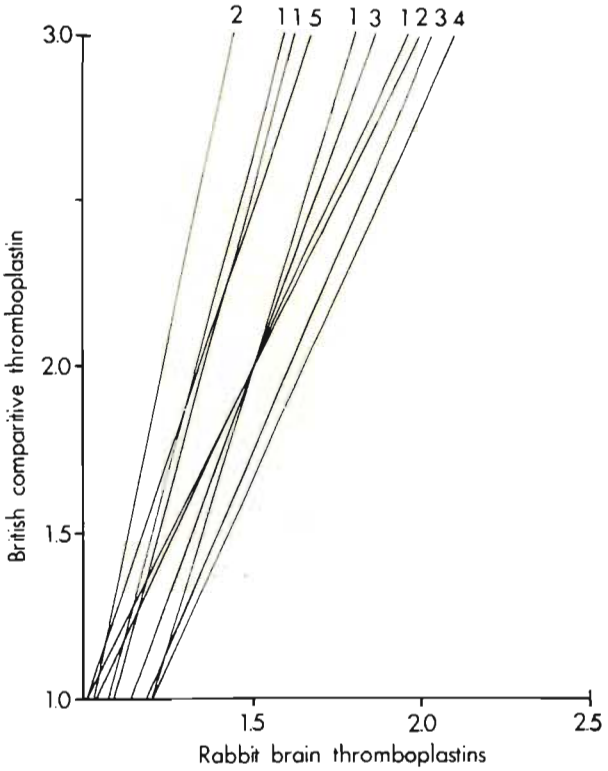

Fig. 3. Thromboplastin sensitivity ratios of 5 rabbit brain preparations. (Note: some preparations were tested more than once). 1 = Ortho; 2 = Hyland; 3 = Dade; 4 = Simplastin, and 5 = Boehringer.

Rabbit Brain Preparations

Five commercial rabbit brain preparations were tested. The results are shown in Table III and Fig. 3. The mean TSR was 1,48 (range 1,24 - 1,65). In all cases the curve intercepted the abscissa away from the point of origin.

TABLE III. THROMBOPLASTIN SENSITIVITY RATIOS OF 5 COMMERCIAL RABBIT BRAIN PREPARATIONS

Preparation		TSR	Deviation from origin
Ortho	1	1,5	+0,20
	2	1,35	+0,10
	3	1,32	+0,07
Dade	1	1,6	+0,18
	2	1,5	+0,15
Simplastin		1,65	+0,20
Hyland (dried)		1,24	+0,02
Hyland (liquid)		1,52	+0,02
Boehringer		1,57	+0,02
Mean		1,48	+0,11
Range		1,24 to 1,65	−0,02 to +0,20

Stability of Human Brain Thromboplastin Preparation

The results of storage of 0,2% and 0,5% phenolised human brain thromboplastin preparation are shown in Table IV.

The 0,2% phenolised suspension showed a marked change after one week at room temperature, in that the prothrombin time of normal plasma was markedly prolonged and the TSR showed a significant increase. At 4°C the suspension was remarkably stable for approximately 7 weeks.

The 0,5% phenolised suspension was stable at room temperature for approximately 5 weeks. Further storage resulted in a progressive lengthening of the prothrombin time of normal plasma and a decrease in the TSR. Since at 8 weeks the prothrombin time of normal plasma had lengthened to 19,0 seconds, this part of the experiment was discontinued. When stored at 4°C, however, the 0,5% phenolised suspension was completely stable for at least 7 weeks. After further storage the TSR showed some irregular fluctuation, but there was no definite upward or downward trend. The prothrombin time of normal plasma showed no significant change even after 12 weeks' storage.

Patients on Long-Term Anticoagulant Therapy

Table V shows that the mean prothrombin ratio for the group of 89 patients was 1,65 (standard deviation 0,15). The corresponding values for prothrombin index are given for convenience since this method of expressing the results is widely used in South Africa. The mean prothrombin index was 60,4%. The thromboplastin preparations used in this study had TSR values of 1,6. Reference

TABLE IV. STABILITY OF 3% HUMAN BRAIN THROMBOPLASTIN SUSPENSIONS PRESERVED WITH 0,2% AND 0,5% PHENOL RESPECTIVELY, AT ROOM TEMPERATURE (18 - 28°C) AND AT 4°C

	0,2% phenol				0,5% phenol			
Period of storage in weeks	Prothrombin time of normal plasma (seconds)		TSR		Prothrombin time of normal plasma (seconds)		TSR	
	Room temp.	4°C	Room temp.	4°C	Room temp.	4°C	Room temp.	4°C
0	12,8	13,1	1,85	1,85	16,1	16,1	1,85	1,85
1	24,5	12,8	2,25	1,86	15,8	15,0	1,85	1,95
2	67,9	13,7	—	1,81	16,2	16,0	1,91	1,86
3	—	12,6	—	1,86	16,0	14,6	1,86	1,93
4	—	11,5	—	1,92	15,6	14,4	1,87	1,87
5	—	12,6	—	1,85	15,0	14,0	1,95	1,95
6	—	12,0	—	1,86	17,0	14,0	1,77	1,94
7	—	12,0	—	1,85	17,2	14,0	1,74	1,91
8	—	13,4	—	1,64	19,0	15,2	1,62	1,75
9	—	—	—	—	—	—	—	—
10	—	13,7	—	1,83	—	14,6	—	2,14
11	—	—	—	—	—	—	—	—
12	—	13,6	—	1,85	—	16,3	—	2,00

to Table I shows that a ratio of 1,65 obtained with these thromboplastins corresponds to a BCR of 2,09. The mean weekly dose of Warfarin administered was 34,0 mg, or 4,86 mg daily.

TABLE V. PROTHROMBIN TIME TEST IN 89 PATIENTS RECEIVING WARFARIN THERAPY (All PATIENTS TESTED WITH HUMAN BRAIN THROMBOPLASTIN OF TSR 1,6)

	Prothrombin index (%)	Prothrombin ratio	British corrected ratio	Daily Warfarin dosage (mg)
Mean	**60,46**	**1,65**	**2,09**	**4,86**
SD	**6,6**	**0,15**	**0,36**	**2,87**

DISCUSSION

Two objectives were defined in this study. One was to determine the sensitivity of the thromboplastin preparations to the coumarin-induced coagulation defect, and the other was to determine the clinical suitability of the preparations as a means of regulating coumarin dosage. Sensitivity to the coumarin defect was quantitated by determining the TSR in relation to the BCT as described above. A preparation with a TSR of 2,0 was defined as being identical with the BCT in its sensitivity. A lower TSR indicated less, and a higher TSR indicated more sensitivity than the BCT. Clinical suitability was assessed in the case of the human thromboplastin preparations from the study of 89 patients on long-term coumarin therapy.

Human Thromboplastin Preparations

In the present study all 23 preparations made from separate human brains showed TSRs of less than 2,0, indicating less sensitivity than the BCT. The variation in sensitivity among the 23 preparations was, however, relatively small. The range of TSR was from 1,6 to 1,9. This indicated comparatively little batch-to-batch variation in the preparation of the reagent.

A difficulty could arise in calculating the TSR of a thromboplastin if the 'best fit' line through the points obtained by experiment missed the origin. Poller[6] stated that such a line may still be valid. This may be so within acceptable limits of error, provided that the divergence of the line from the origin was not too great. If the divergence exceeded defined limits, the use of Denson's table[5] in order to derive the BCR would result in unacceptable errors.

Of the 23 preparations studied, 9 produced lines that missed the origin. The mean deviation from the origin was expressed in terms of the units plotted on the abscissa of Fig. 1 (i.e. units of the ratio of patient to control prothrombin times). The thromboplastins we prepared can therefore be described as having TSRs within the range of 1,6 to 1,9, and having curves that intercept the abscissa less than 0,17 units from the origin.

The study of 89 patients on long-term Warfarin therapy provides information on the clinical suitability of these preparations. In this study the two thromboplastin preparations used had TSRs of 1,6. The mean BCR of the 89 patients studied was 2,09. This result falls within the therapeutic range of 1,8 to 3,0 recommended by Poller[7] (corresponding to a prothrombin index of 54 - 33%). However, the mean BCR of a large group of well-controlled patients should be close to the midpoint of

this range, i.e. 2,4. The present group of patients therefore appear to be underdosed. This conclusion is supported by the fact that the mean daily dose of Warfarin used was 4,86 mg, which is less than the usually recommended average maintenance dose of 8 mg daily.[9]

It is therefore reasonable to infer that the BCR obtained with our human brain preparations is close to the BCR that would have been derived if the BCT had been used.

The study of these 89 patients on anticoagulant therapy provides evidence to validate the concept of the BCR and the TSR as defined by Poller[7] and Denson,[5] by demonstrating that patients whose prothrombin ratios were insufficiently prolonged (in terms of the recommended therapeutic range), were in fact receiving comparatively small doses of Warfarin.

Rabbit Thromboplastin Preparations

The preparations tested showed TSRs of 1,24 to 1,65, indicating less sensitivity to the coumarin defect than the human preparations. In addition, all of these preparations produced curves that intercepted the abscissa away from the origin. If the description of the human brain preparations in terms of their TSR *and* point of interception on the abscissa are accepted as criteria of acceptability, then none of the rabbit brain preparations tested can be regarded as acceptable. That is, none of the rabbit thromboplastins showed a TSR of 1,6 or more and intercepted the abscissa within 0,17 units of the origin.

The lack of sensitivity of rabbit brain thromboplastin to the coumarin defect has been documented by Poller.[3] The present results confirm his findings. In addition, the consistent deviation of the TSR curves from the origin renders conversion of the prothrombin ratio to the BCR unreliable in the case of the rabbit preparations.

Recommendations

The determination of the thromboplastin sensitivity ratio of a thromboplastin preparation is a procedure within the scope of any routine laboratory, provided the BCT or another stable preparation of known TSR is available. Supplies of the BCT are limited, but preparations of known TSR as determined locally by reference to the BCT, are available in the Republic. Such preparations are supplied in the form of a 0,5% phenolised liquid extract of human brain, which is stable at 4°C for 7 weeks and at room temperature for 5 weeks. Any laboratory can use this preparation for the determination of the TSR of the thromboplastin normally used.

In addition, all preparations of thromboplastin should have the TSR value stated on the label. In the case of dried thromboplastin in powdered form, this TSR will only be valid if the instructions for preparation of a phenolised saline suspension of the powder are followed precisely. It is recommended that the TSR of any 'home-made' thromboplastin should be checked periodically against the phenolised liquid standard available locally.

The results obtained with any thromboplastin preparation of known TSR can be converted to the BCR by reference to Denson's table as described above. Both the BCR and whatever method of expressing results (e.g. prothrombin index) was previously in use, should be stated on the laboratory report.

If this method were adopted throughout the Republic of South Africa it would provide considerable benefit to patients on long-term anticoagulant therapy. Many such patients require tests when travelling, and errors of dosage would be avoided if comparable results could be assured at any laboratory in the country. Furthermore, the results obtained in the Republic would be comparable with those obtained at many centres in the UK, Europe, Australia and other parts of the world. Perhaps of greater importance to the individual patient would be the fact that batch-to-batch variation of thromboplastin used within a single laboratory would also be controlled. This would ensure greater stability of results at various times in the same laboratory.

In view of their better sensitivity to the coumarin defect and less divergence from the origin in the TSR plot, human brain thromboplastins are preferred to those prepared from rabbit brain.

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