J. clin. Path., 1971, **24**, 460-463

International and national standardization of control of anticoagulant therapy in patients receiving coumarin and indanedione drugs using calibrated thromboplastin preparations[1]

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In most parts of the world anticoagulant therapy with coumarin drugs is controlled by the one-stage prothrombin time test, and because of different sensitivities of the methods to the coumarin defect and different methods of reporting the results, there is difficulty in comparing the level of anticoagulation at one centre with that at another. The provision internationally of a reference preparation of thromboplastin is an attempt to provide a means whereby the degree of anticoagulation determined at different centres may be compared and therapy more uniformly controlled.

A research standard of thromboplastin, human (67/40) is obtainable, with information about it, on application to the Division of Biological Standards, National Institute for Medical Research, Mill Hill, London. (This is a freeze dried preparation which is stable and which has been characterized in an international collaborative study by Bangham, Briggs, Brozovic, and Denson, 1970.) This could be used by laboratories to calibrate their own thromboplastin preparations. It is hoped further that national reference thromboplastin preparations will be made and calibrated with this research standard[2] and that commercial preparations should be similarly calibrated against the national reference preparations. This would enable better control of therapy in individual patients, particularly for patients travelling from one hospital to another or travelling abroad. In addition, information obtained from patients included in a clinical trial at different centres should be comparable if the clotting time ratios are expressed in terms of the national or international reference preparations.

[1]This memorandum is published on behalf of the International Committee for Haemostasis and Thrombosis.

[2]The use of this reference preparation was recommended by/at the Meeting of the International Committee for Thrombosis and Haemostasis at Bath in October 1969.

Received for publication 29 October 1970.

Clotting Time Ratios as an Index of Measurement

The comparative procedure involves the use of the clotting time ratio derived by dividing the clotting time obtained with the patient's plasma by the clotting time obtained with normal plasma. For example, if the patient's plasma has a clotting time of 24 sec and the normal of 12 sec, the clotting time ratio would be 2·0.

Collection of Fresh Normal Plasma and Plasma Samples from Patients Stabilized on Anticoagulant Therapy

Blood is collected into one-tenth volume of 3·13% trisodium citrate, the plasma is separated by centrifugation and tested the same day. The research standard (67/40) is normally reconstituted with 3·2 mM $CaCl_2$. If it is the practice to collect blood into one-tenth volume of 3·8% trisodium citrate for the method under comparison, the same plasma samples may be used for the research standard 67/40 but in this case the reagent should be reconstituted with 4 mM $CaCl_2$.

Test Procedure

Of the thromboplastin reference preparation, 0·4 ml is pipetted into a glass tube and warmed to 37°C. Then 0·05 ml of citrated plasma is added and at the same time a stopwatch is started and the clotting time recorded.

Experimental Comparison of Test and Normal Plasma Samples

The test procedure is carried out in duplicate on four fresh normal plasma samples and preferably not less than 20 different samples of fresh plasma from

patients stabilized on anticoagulant therapy. The testing may be carried out on two separate days if this is convenient, a pool of two normal plasma samples and 10 abnormal samples being tested on each day. The same plasma samples are tested on the same day by the method for the thromboplastin preparation to be compared. An example of such an experiment is shown in Table I.

Calculation

The clotting time ratios for all the samples of test plasma are calculated by dividing the clotting time of each test sample by the mean clotting time for the normal plasmas used in each day's work. The clotting time ratios with the research standard (67/40) are then plotted against the clotting time ratios obtained with the preparation being calibrated for each plasma sample. This has been done for the results of Table I in Figure 1. The best straight line is drawn through the points. It is then possible to calculate the ratios, using the reference thromboplastin preparation, that are equivalent to those using the test thromboplastin preparation. In the example given in Fig. 1 a ratio of 2·0, which has been assigned to the research standard thromboplastin, is equivalent to one of 1·5 with the test thromboplastin. The ratio read off from Fig. 1 equivalent to the ratio of 2·0 obtained with thromboplastin human research standard (67/40) is called the 'thromboplastin sensitivity ratio' of the thromboplastin preparation being calibrated. Similar equivalence of ratios may be obtained for any other values and these are recorded in Table II. A preparation having any other thromboplastin sensitivity ratio could be used as a calibrated reference preparation.

In England the national thromboplastin comparative reagent is a phenolized saline extract of human brain preparation made in Manchester and used in many laboratories. Successive batches have almost the same sensitivity ratio (2·0), determined by extensive tests such as those of Table I (Fig. 1), as the research standard (67/40).

The use of 20 samples of plasma from patients stabilized on anticoagulant therapy is probably adequate for calibration of local or home-made preparations. For the accurate calibration of national reference preparations against the research standard 67/40 it is desirable to test at least 100 samples from patients stabilized on anticoagulant therapy. The international preparations which have been calibrated to date have been tested extensively using many hundreds of different plasma samples.

It may be found when drawing the best line through the points that several obviously outlying points appear. These points probably represent the summation of experimental error and real differences in the sensitivity of the thromboplastins under com-

Sample		*Test Thromboplastin*		*Research Standard 67/40*	
		Mean Clotting Time[1]	*Ratio*	*Mean Clotting Time*[1]	*Ratio*
Average clotting time of 4 samples of plasmas		13·1	1·0	17·0	1·0
Dindevan plasma samples	1	31·0	2·35	61·9	3·64
	2	26·8	2·05	57·8	3·05
	3	27·1	2·07	56·8	3·34
	4	21·4	1·64	40·8	2·4
	5	22·5	1·72	41·6	2·45
	6	22·3	1·71	41·7	2·45
	7	27·9	2·12	47·5	2·8
	8	33·7	2·1	63·8	3·75
	9	24·4	1·86	46·7	2·75
	10	20·8	1·58	35·2	2·07
	11	18·1	1·38	29·8	1·75
	12	22·7	1·74	40·0	2·35
	13	15·3	1·16	22·6	1·33
	14	19·7	1·50	36·6	2·15
	15	20·7	1·59	35·7	2·10
	16	21·3	1·63	34·5	2·03
	17	29·4	2·24	57·8	3·40
	18	17·8	1·36	28·7	1·69
	19	16·9	1·29	23·8	1·4
	20	21·0	1·60	35·0	2·06

Table I *An experiment on the standardization of thromboplastin for the one-stage prothrombin time used for the control of anticoagulant therapy*

[1]Mean of two readings.

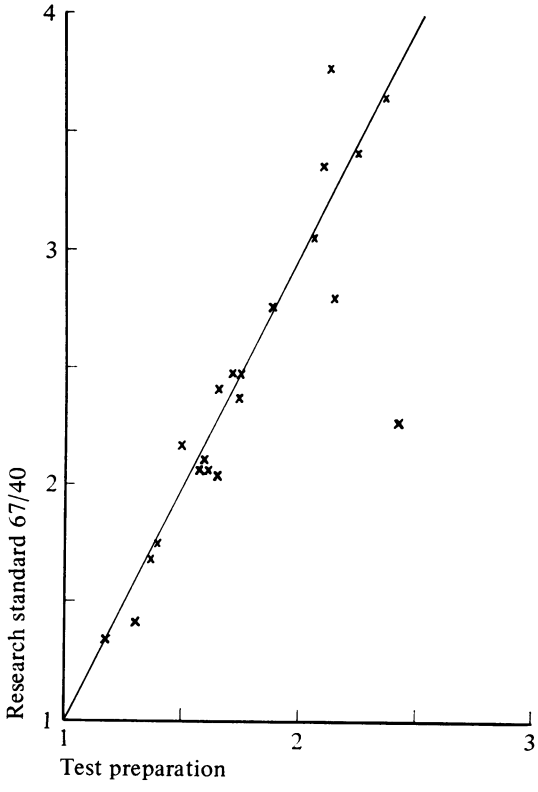

Fig. 1 *The clotting time ratios of 20 samples from patients on anticoagulant therapy for the research standard 67/40 plotted against the clotting time ratios for the same samples using a test preparation.*

parison with individual plasma samples. Whilst it is reasonable to ignore one or two such points among 20 samples, if more than two such outlying points are obtained then this is probably largely the result of experimental error and the comparison should be repeated with a further 20 samples and the line plotted using points from 40 samples.

Practical Application of a Calibrated Thromboplastin Reference Preparation

TRANSFER OF A PATIENT FROM ONE HOSPITAL TO ANOTHER INSIDE ONE COUNTRY

For example, a patient in England might transfer from a hospital using a reagent of thromboplastin sensitivity ratio of 1·5 to a hospital using a reagent whose thromboplastin sensitivity ratio has been determined as 2·2. If it was desired to maintain the patient within a range of ratios from 2·0 to 2·5 (by the test using a thromboplastin whose sensitivity ratio is 1·5) then reference to Table II shows that an equivalent level of anticoagulation would correspond to a ratio range of 3·4 to 4·5 with the thromboplastin at the second hospital.

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	3·74	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	2·98
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

Table II *Clotting time ratios for a test preparation equivalent to the clotting time ratios for the research standard 67/40 when the sensitivity ratio of the preparation has been determined using research standard 67/40 or some other characterized preparation*

TRANSFER OF A PATIENT FROM ONE HOSPITAL TO ANOTHER IN A DIFFERENT COUNTRY

This would offer no additional problem if the thromboplastin sensitivity ratio of the local reagent has been determined relative to the national and international reference preparations.

COMMERCIAL PREPARATIONS

Successive batches should be calibrated in a similar manner by means of a calibrated reference preparation. The thromboplastin sensitivity ratio should be determined and stated in the accompanying literature. A calibration table giving a set of ratios and the equivalent ratios using the national and international preparations, should be supplied with each batch. For example, a thromboplastin calibrated as having a sensitivity ratio of 1·5 should quote the column headed 1·5 in Table II.

THERAPEUTIC RANGE

The therapeutic range at different centres may differ according to the lesion treated and according to a particular physician's preference. The usual range for patients with cardiac infarction or venous thrombosis corresponds to a ratio range of 1·8 to 3·0, using research standard (67/40) or the British comparative reagent, or an equivalent ratio using another calibrated reference preparation.

CLINICAL TRIALS

It should be possible to compare results from patients included in clinical trials at different centres if the clotting time ratios are expressed in terms of the international reference preparations or of calibrated national preparations.

Reference

Bangham, D. R., Biggs, R., Brozovic, M., and Denson, K. W. E. (1970). Collaborative study of two thromboplastins. In *Proceedings of the 1969 Meeting of the International Committee on Haemostasis and Thrombosis*, p. 341. Schattauer Verlag, Stuttgart.