National Survey of Value of Plasma Standards for Anticoagulant Therapy

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The need for standardization of methods for laboratory control of anticoagulant therapy is well established (Poller, 1964, 1967 ; Biggs and Denson, 1967 ; Miale and La Fond, 1967). The emphasis in this country has been on the provision of a thromboplastin standard with lyophilized plasma playing a secondary part (Poller, 1967), whereas Miale and La Fond (1967) used freeze-dried plasma standards in a large prothrombin-time test survey in the United States.

It therefore seemed important to determine the reliability of the use of dried plasma standards at individual hospitals. By the provision, in addition, of standard thromboplastin extract (Manchester Comparative Reagent) it has been possible to observe how plasma results varied even though identical laboratory reagents were used at the different hospitals.

Method of Study

Of the 142 major hospital centres that participated in our previous national survey (Poller and Thomson, 1968) 66 were invited to take part in this additional study. This was not an arbitrary selection, but was guided by our desire to include an equal proportion of hospitals using human brain reagents and commercial animal tissue extracts. Hospitals participating in our routine supply scheme were of course excluded, as they do not use other thromboplastin extracts.

The following were sent to each of the hospitals: (1) a fresh supply of Manchester Comparative Reagent human brain thromboplastin—this is a phenolized liquid preparation ; and (2) two ampoules of lyophilized coumarin plasma—one from a patient with a marked depression of all four clotting factors, and one from a severe pure factor VII deficiency—these samples being from single donors treated with nicoumalone (Sinthrome). The criteria for factor VII deficiency were as follows: (1) gross prolongation of the prothrombin time, (2) normal "intrinsic" system assessed by assay, (3) normal factor X level, and (4) restoration of prothrombin time by dilute stored serum.

The hospitals were asked to give details of their own reagent. They were asked to carry out parallel tests with their own thromboplastin and the Manchester standard on two fresh normal control plasmas. Then they were to perform duplicate tests on the two lyophilized plasmas, using both their own tissue extract and the standard. They were also asked to perform the test within seven days of receipt of the reagents so that their possible deterioration should not influence results.

The plasma specimens were tested by us before dispatch, again on the day of dispatch, and also at the end of the period of study. Plasma ampoules and thromboplastin were left at room temperature for 48 hours to simulate conditions arising during transit.

Results

The results with the lyophilized plasma samples obtained in our laboratory with the Manchester Comparative Reagent did not vary over the period of the study.

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Completed results were returned from 58 of the 66 hospitals circularized. One hospital gave results with two different commercial reagents. All were complete, except for two instances where ampoules were broken in transit (one plasma 1 and one plasma 2). The following types of thromboplastin were used by the hospitals:

(1) "Home-made" human brain extracts	...	...	30
Stayne's	...	...	5
Geigy	...	...	5
Simplastin	...	...	8
(2) Commercial extracts	...	...	5
Difco	...	...	4
"2,7,10"	...	...	1
Ortho	...	...	1

Normal Controls.—The distribution of prothrombin times (in seconds) for the normal controls used in the different hospitals is shown in Fig. 1. The distribution of the prothrombin times with the Manchester standard, the "home-made" human brains, and the commercial reagents is given.

FIG. 1.—Distribution of normal readings.

Coumarin Plasmas.—From the prothrombin-time results and the normal controls at each hospital the prothrombin ratio was calculated by us for each of the plasma samples. It was thought better to do this than merely to give results as prothrombin activity, as by using the term "ratio" some of the individual differences arising from a slightly different technique in the prothrombin-time test might be minimized. Results of

FIG. 2.—Coumarin plasma 1. Distribution of prothrombin ratios.

plasma 1 are plotted as ratios in Fig. 2, which gives the distribution at the various hospitals using the Manchester standard, individual "home-made" human brains, and commercial extracts. Fig. 3 gives the results obtained with the pure factor-VII-deficient specimen (plasma 2) in a similar way. Plasma 2 was particularly of interest because of factor VII insensitivity encountered with some commercial reagents.

FIG. 3.—Coumarin plasma 2 (factor-VII-deficient). Distribution of prothrombin ratios.

Comparison of Plasma Standard with Thromboplastin Standard.—In this investigation we have assessed the reproducibility of the results with dried plasma at the different hospitals. It is our current practice in the Manchester scheme to provide a reference thromboplastin preparation. This enables hospitals to calibrate their own home-made or commercial thromboplastin against the Manchester Comparative Reagent and determine the equivalent therapeutic range, whether in terms of prothrombin activity, ratio, or index. This is done by parallel observation on at least 12 patients within the therapeutic range. Thromboplastin standard is also necessary to determine the "equivalent" prothrombin ratio (Biggs and Denson, 1967), with the use of a similar technique of parallel observation on 20 anticoagulated patients. Our survey on equivalent ratios preceded the present investigation by a few weeks. Data were available from the same hospitals. Only 45 of the 58 hospitals

FIG. 4.—Thromboplastin standard versus single dried plasma standard, using human brain reagents. (Coumarin plasma 1.)

FIG. 5.—As Fig. 4, but using commercial reagents.

which returned results in the present survey could be studied, because in the meantime the others had changed their thromboplastin preparations. We compared the scatter of the equivalent ratios based on a thromboplastin standard with the scatter of ratios at the same hospitals using the single dried coumarin plasma, deficient in all four clotting factors.

In Fig. 4 the distribution of values for the prothrombin ratio equivalent to 1.7 with the standard Manchester reagent is given as well as the distribution of individual plasma ratios at the different hospitals. Fig. 5 gives the similar distributions for the commercial extracts.

Discussion

This study differs in many respects from the survey conducted in the United States by Miale and La Fond (1967). Though far fewer hospitals have been included in our investigation more data are available from each centre, as in addition to the provision of standard plasma we have provided standard thromboplastin reagent together with a recommended uniform technique for parallel studies with their own reagents at the different hospitals.

The distribution of results obtained from the fresh normal control plasma by the use of the Manchester standard thromboplastin is a reasonable one. Only a small number of results fell outside the normal range we have established at our own laboratory. The normal results with the other human brain preparations and commercial animal reagents were not so close. The variation in the normal values was taken into account, as the results of dried plasma 1 and 2 were expressed as a ratio of the normal result in seconds at each hospital.

There was more reproducibility with the Manchester standard than with the individual home-made human brain extracts and also than with the commercial extracts with both the dried plasma specimens. The Manchester reagent and the home-made human brain reagents were also more sensitive to the coumarin defect than the commercial extracts with both dried plasma specimens. This parallels the result in the previous survey, where none of the commercial reagents gave as good prothrombin ratios as the human brain preparations—that is, animal preparations were less sensitive to the coumarin defect (Poller and Thomson, 1968).

The ability of a single dried plasma standard to characterize an unknown tissue thromboplastin is seen to be very poor when contrasted with the provision of a standard sensitive thromboplastin—for example, human brain—for parallel studies. Figs. 4 and 5 show the very good agreement at different centres where the equivalent prothrombin ratio was determined by parallel observation on groups of 20 fresh coumarin plasma samples. They also show the wide distribution and lack of agreement between the prothrombin ratios obtained from dried coumarin plasma at the same hospitals. Apart from assessing the sensitivity of a thromboplastin to the depression of individual coagulation factors by using deficient plasma, results obtained from a single dried plasma standard appear relatively valueless owing to uncontrolled variations in laboratory technique.

With use of the same reagent based on the technique with the Manchester standard prothrombin ratios on the same plasma varied between 2.7 and 7.6 with plasma 1 and 2.7 and 11.5 with plasma 2. With different home-made thromboplastin the distribution was even greater. The scatter was not so great with commercial extracts, but these were uniformly insensitive to the coumarin defect (see Fig. 5).

All the many variables which affect prothrombin time results in seconds affect the readings with dried plasma, and these are uncontrolled. With standard thromboplastin and the Manchester method for determination of the equivalent therapeutic range, at least 12 therapeutic coumarin plasmas are tested in parallel with both thromboplastin reagents. The best line is

drawn to determine the equivalent prothrombin activity, ratio, or index. With the Biggs and Denson (1967) method 10 patients are tested on two consecutive days in parallel, the prothrombin ratios are plotted, and the equivalent to a fixed point on the standard (1.7 with Manchester reagent) is determined by parallel observation and drawing the "best line." With both these methods the system of parallel observation on fresh plasma specimens means that differences in techniques and handling at the individual hospitals are minimized. In the case of dried plasma standards the absence of a standard thromboplastin means that these variables cannot be controlled in this way. In addition, the multiplicity of plasma specimens used with standard thromboplastin means that the technical errors are minimized. The lyophilized plasma standard is also subject to many difficulties not encountered with thromboplastin. The handling of dried plasma in the laboratory introduced additional variables—for example, contact activation, deterioration on storage, and inactivation in reconstitution and subsequent handling, which are not encountered with standard thromboplastin, particularly if prepared in liquid form as is our phenolized extract.

It is almost impossible to obtain human dried plasma specimens which correspond to a predetermined prothrombin percentage activity or ratio without artificial manipulation. This is because of the contact activation and/or inactivation of

From this survey it appears that the place of a dried plasma standard should be limited to that of a useful adjunct to standard thromboplastin in the provision of a uniform service for laboratory control of anticoagulants (see Table). Where a suitable sensitive human brain standard is available it is doubtful if standard dried plasma provides much additional information. It might be possible at some future date to improve the poor performance of plasma standards by greater attention to the many variables, but a lot more work is required before this becomes a practical proposition.

Summary

A study has been carried out to determine the reliability of the use of dried plasma standards at individual hospitals. By the provision, in addition, of standard thromboplastin extract (Manchester Comparative Reagent) it has been possible to observe how plasma results varied even though identical laboratory reagents were used at the different hospitals.

The following were sent: (1) a fresh supply of Manchester Comparative Reagent human brain thromboplastin, and (2) two ampoules of lyophilized coumarin plasma—one from a patient with a marked depression of all four clotting factors, and one from a severe pure factor VII deficiency. Results were returned from 58 hospital centres. The ability of a single dried plasma standard to characterize an unknown tissue thromboplastin was seen to be very poor when contrasted with the provision of a standard sensitive thromboplastin. It appears that the place of a dried plasma standard should be limited to that of a useful adjunct to standard thromboplastin in the provision of a uniform service for laboratory control of anticoagulants. Where a suitable sensitive human brain standard is available it is doubtful if standard dried plasma provides much additional information. It might be possible at some future date to improve the poor performance of plasma standards, but a lot more work is required.

Comparison of Thromboplastin Standard with Plasma Standard

Human Brain Thromboplastin Standard (Manchester Comparative Reagent)	Dried Plasma Standard
1. Parallel observation controls variables	1. Variables largely uncontrolled
2. Use of many coumarin plasma specimens to obtain equivalent values minimizes technical errors	2. Isolated plasma specimens only
3. Large bulk available as standard. Many years' supply prepared	3. Relatively small volumes available, particularly of factor-VII-deficient
4. Every batch identical to previous batch	4. Not easy to make identical preparations
5. Easy to handle	5. Difficult to handle—e.g., contact activation and factor V lability
6. Very reliable and good reproducibility between hospitals (Poller and Thomson, 1968)	6. Poor agreement at different hospitals

clotting factors, particularly of factor V, which occurs in lyophilization. It is also difficult to get a sufficiently large pool of human plasma depleted of the three relevant clotting factors—that is, prothrombin, factor VII, and factor X—without artificial adsorption. Factor-VII-deficient plasma is probably a valuable reagent and might be used for screening animal products against factor VII insensitivity in the absence of a human brain standard. This can be obtained, in small volumes, only from human donors, and cannot be prepared satisfactorily by artificial means.

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