

Partial thromboplastin time test with kaolin

Normal range and modifications for the diagnosis of haemophilia and Christmas disease

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SYNOPSIS The partial thromboplastin time test provides a convenient and sensitive screening procedure for deficiencies of thromboplastic factors, especially factors VIII and IX. The test is carried out after preincubating the plasma for 10 minutes with kaolin, and Inosithin is used as a platelet substitute.

The 'normal range' of the test has been estimated in terms of the differences encountered between random normal plasmas tested in pairs, because individual patients are usually tested against single control subjects. A patient's partial thromboplastin time should be regarded as abnormal if it is more than six seconds longer than the control time. In the diagnosis of haemophilia, patients' plasmas with concentrations of factor VIII as low as about 20% might be regarded as being within the range of normal, if the selected control subject's factor VIII happened to lie near the lower end of the normal range.

When mild haemophilia is suspected, discrimination may be improved by diluting both the patient's and the control plasmas 1 in 20 in haemophilic plasma. With the test modified in this way the clotting time is prolonged, though the range of differences among normal subjects is unaltered, and plasmas with factor VIII concentrations below about 30%, *i.e.*, in undiluted plasma, would be unlikely to be regarded as normal.

The partial thromboplastin time may be similarly modified as a screening test for factor IX deficiency.

Some clinical examples are reported.

The partial thromboplastin time test of Langdell, Wagner, and Brinkhous (1953) is a simple, one-stage procedure of similar design to the Quick prothrombin time, but sensitive to deficiencies of clotting factors required for the formation of blood thromboplastin. It has been in regular use for a number of years as a screening test (Nye, Graham, and Brinkhous, 1962), especially for the lifelong, hereditary bleeding disorders, of which haemophilia (factor VIII deficiency) and Christmas disease (factor IX deficiency) are, in that order, the commonest.

In the partial thromboplastin time test as described by Rodman, Barrow, and Graham (1958), the clotting time was recorded after mixing 0.1 ml. volumes of citrated or oxalated plasma, a 0.3% saline suspension of ethereal brain extract (supplying

phospholipid), and 0.02 M calcium chloride solution. Tests on 206 normal controls gave a mean clotting time of 76 sec. with a standard deviation of ± 13 sec. corresponding to a coefficient of variation of 17%.

Following the work of Margolis (1957, 1958) and others, it has been appreciated that a large part of the clotting time of blood is taken up in activating the contact system (factors XII and XI), and also that uncontrolled conditions during this stage account for much experimental variability (Hardisty and Macpherson, 1962). A preliminary incubation of the plasma with kaolin conveniently shortens the partial thromboplastin time, and the variability is more than correspondingly reduced. Proctor and Rapaport (1961) obtained a mean partial thromboplastin time from 40 normal persons of 45 sec. after preincubation for three minutes, with a standard

deviation of ± 3.0 sec., giving a coefficient of variation of 7%.

It was not clear whether the smaller range of normal variation observed by Proctor and Rapaport was mainly due to a greater homogeneity of their tested population or to a lower experimental error of their kaolin test (since the effects of subject and experimental variation would be additive). It was therefore interesting to compare experimental and normal subject variation with a kaolin-modified test. We also wished to establish a working limit for the normal variation appropriate to the difference between test times obtained from a patient and a single control subject, on the lines previously suggested for the prothrombin time (Ingram and Armitage, 1959). Particular attention was given to the problem of the diagnosis of 'mild haemophilia'.

METHODS

Blood was taken into one ninth of its volume of 3.2% sodium citrate solution in polystyrene containers, and plasma obtained by centrifugation.

THE TEST For the test, 0.1 ml. of plasma is mixed with an equal volume of a suspension of light kaolin, 1 mg./ml., in aminotris(hydroxymethyl)methane buffer, 0.05 M, pH 7.2 at 37°C. (Gomori, 1946) and incubated for 10 min. at 37°C. in a glass or polystyrene clotting tube. The reaction mixture is then completed by the addition of 0.1 ml. volumes of a suspension of Inosithin¹ (a soy-bean phospholipid preparation) about 1 mg./ml. and 0.025 M calcium chloride solution, and the clotting time is determined. Other commercial phospholipid sources were investigated by Quick and Geppert (1963) and Fenichel, Baker, and Rose (1964), and may be used instead of Inosithin, and preliminary tests have suggested that slightly more reproducible results are obtained by adding the phospholipid preparation after the kaolin treatment rather than with the kaolin, as has been previously suggested. Tests can be set up at one-minute or two-minute intervals (depending upon the clotting time expected) so that numerous samples may be tested concurrently.

Two replicate readings are obtained from each plasma sample. With the object of eliminating systematic trends related to the passage of time, one replicate reading is obtained in order from each plasma sample, including the control, and then the second replicates are obtained serially in the opposite order (Cox, 1951). The means of the duplicate readings are taken as the partial thromboplastin times. The differences between duplicate readings provided the measure of experimental error used in the present investigation.

Like any other clotting test, the partial thromboplastin time may be prolonged either because a necessary clotting factor is missing or because the plasma contains an inhibiting substance. While examples of the second situa-

tion are rarer than the first, it is not difficult to set up the test so as to detect a prolongation by an inhibitor. When investigating a single patient, it is therefore usually convenient to employ the 10 min. preincubation period in setting up six tests at 60- or 90-sec. intervals in the following order: control, control and patient 50:50, patient, patient, control and patient 50:50, control. If the patient's result is normal, the tests on the mixtures are uninformative; but if the patient's time is prolonged, a near-normal clotting time of the mixture suggests a deficiency in the patient's plasma (made good by the equal volume of normal plasma) whereas a long clotting time of the mixture suggests an inhibitor.

An inactivator of a clotting factor, *e.g.*, an anti-factor VIII, may be demonstrated by progressive prolongation of the partial thromboplastin time when the 50:50 mixture is incubated. In this case the relevant control is the partial thromboplastin time of a freshly made 50:50 mixture of the patient's and the control plasmas which have been incubated separately alongside the incubated mixture.

RESULTS

SENSITIVITY OF THE PARTIAL THROMBOPLASTIN TIME TO FACTOR-VIII DEFICIENCY Plasmas from six ostensibly normal adults were separately diluted in plasma from a severely affected haemophiliac, to give 80, 60, 50, 40 and 30 % plasma from each subject, and

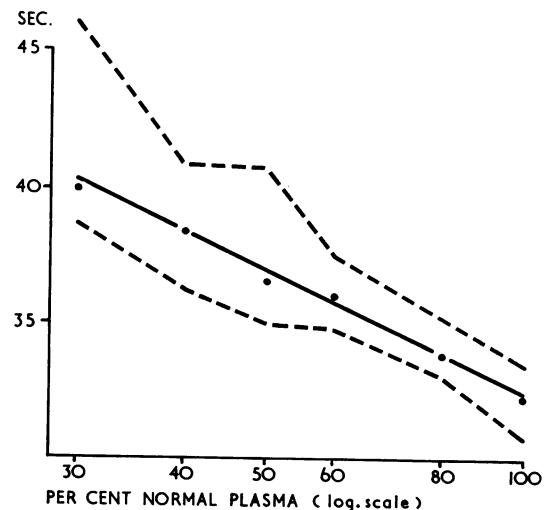


FIG. 1. Kaolin partial thromboplastin times were obtained on dilutions of six normal plasmas in the plasma of one severely affected haemophiliac (kaolin partial thromboplastin time about 105 sec.). Two readings were obtained at each dilution of each plasma, and all the tests were made on the same day. The broken lines show the range of the means of the duplicate readings of the six normal plasmas at each dilution. The solid line shows the regression of the means of the means (solid circles) on the concentration of normal plasma plotted logarithmically.

¹Associated Concentrates, Inc., N.Y.; obtainable in the U.K. through A. V. Howe and Co. Ltd., 46 Pembridge Road, London, W.11.

each normal plasma and each of the dilutions made from it were separately tested as described. All the tests were made on the same day by one observer. The grand mean clotting time, and the range of individual plasma means are shown for each dilution in Figure 1. It is apparent that when individual variation is eliminated by taking grand means, the test responds sensitively to small degrees of factor-VIII deficiency, but that it would not be possible to exploit this fully when testing individual subjects. The next step was therefore to assess the practical limits of discrimination when using the test under ordinary clinical conditions. It was also important to verify that differences between persons tested would be large compared with differences introduced by experimental error, before it could be assumed that observer differences might justifiably be ignored.

EXPERIMENTAL ERROR The differences between the replicate clotting times in the above experiment were examined, and the standard error for a single clotting time was found to be ± 1.07 sec. (Appendix, formula 1). Similar calculations were made on replicate pairs of clotting times from 77 normal plasmas, used as controls in routine clinical tests carried out by various other members of this laboratory; these data yielded a corresponding value of ± 1.31 sec. The two estimates did not differ significantly and were therefore pooled to give a value of ± 1.23 sec., corresponding to a coefficient of variation of 3.5% for experimental error. From this it could be calculated that the contribution of experimental error to the coefficient for the difference between the means of two clotting times from each of two persons would be 4.9% (Appendix, formula 4).

RANGE OF DIFFERENCES BETWEEN PARTIAL THROMBOPLASTIN TIMES OF RANDOMLY PAIRED NORMAL SUBJECTS The commercial phospholipids are reasonably stable preparations, but other factors besides phospholipid activity may possibly influence actual partial thromboplastin times obtained on different occasions. It therefore seems wise to base clinical tests on paired patient-control comparisons unless the test is in constant use and regular control is separately undertaken. In practice, therefore, the significant question will usually be whether the mean clotting time from a patient exceeds that from a given random normal person more than would be reasonably expected if normal subjects were tested together in random pairs.

Plasmas from 42 ostensibly healthy adult subjects of both sexes were therefore tested in groups of two to five. In this way they provided 70 different pairs of plasmas where both members of the pair had been tested in the same experiment as described.

The means of the replicate readings from each of the 42 subjects were obtained and ranged from 29.2 to 38.0 sec., about a grand mean of 33.7 sec. The 70 differences between the means of the paired subjects were then calculated, and these yielded a standard error for a single subject of ± 3.01 sec. (Appendix, formula 2), corresponding to a coefficient of variation of 8.9%. From this, the standard error for the difference between mean clotting times from two random subjects (Appendix, formula 3) was found to be ± 4.25 sec., corresponding to a coefficient of variation of 12.6%, of which it has been shown above that experimental error would contribute less than half (4.9%).

It was thus clear that experimental error was small compared to differences between subjects, so that variations in the experimental error among different observers could reasonably be ignored, and the estimate of subject variation accepted for all observers. It was also found that the difference between subjects did not tend to increase in proportion to their mean partial thromboplastin time over the range encountered with normal persons. The results from patients and controls could therefore be compared simply by taking the differences between them, and it was unnecessary to calculate a series of critical differences appropriate to different control results, as had been required for the prothrombin time (Ingram and Armitage, 1959). It was also found that the results showed no long-term fluctuations over a period of eight months, which suggested that the reagents remained stable.

In the diagnosis of the bleeding disorders, the question asked is whether the patient's partial thromboplastin time is significantly longer than that of the control subject; shorter times than the control are of no consequence in this context. A 'one-sided' significance test is therefore appropriate, and the patient's partial thromboplastin time would significantly exceed the control value at the 5% probability level if it were longer by $4.25 \times 1.65 = 7.0$ sec. or more (where 1.65 is the *t*-table entry for the 10% level on ∞ d.f. for the ordinary two-sided test). The corresponding limit at the 1% level would be $4.25 \times 2.33 = 9.9$ sec. It is suggested that 7 sec. be taken as the critical difference for ordinary purposes, a difference of 10 sec. or more being clearly abnormal.

Now, reference to Fig. 1 will show that a difference of 7 sec. represents a 2.8-fold difference in factor-VIII concentration over the range illustrated. Thus, if the chosen control subject happened to have a factor-VIII concentration of 100%, a patient whose partial thromboplastin time were 7 sec. longer would (other things being equal) have a factor-VIII concentration of $100/2.8 = 36\%$. If, however, the control subject lay near the lower end of the normal

range of factor-VIII levels, with a concentration of, say, 60% of average normal (Preston and Barr, 1964) the suggested difference of 7 sec. would be exceeded only if the patient's factor-VIII level were below $60/2.8 = 22\%$. If other factors tended to make the patient's plasma clot more rapidly than the control plasma in this test, an even lower factor-VIII level in the patient might pass for 'normal'. In fact, this is somewhat unfair, because below 30% the dose-response curve begins to rise more steeply, so that a 7 sec. difference in clotting time would represent less than a 2.8-fold difference in factor-VIII concentrations at the lower normal limit; but nevertheless it is apparent that a certain number of mild cases of haemophilia might be missed by adopting the suggested criterion of normality when using a randomly-chosen normal person as control.

A modification of the test was therefore investigated in an attempt to improve discrimination for haemophilia.

SCREENING TEST FOR FACTOR-VIII DEFICIENCY If the test and control plasmas are diluted in haemophilic plasma, the effects of factors other than VIII will be largely eliminated, and also the test will operate on a steeper part of the dose-response curve where discrimination is inherently greater.

Modified test for factor-VIII deficiency The patient's and the control plasmas are each diluted 1 in 20 in plasma from a severely affected haemophilic. (This material can be stored for some months at -20°C . in small lots, which should be discarded when the prothrombin time begins to rise, suggesting that factor V is becoming reduced.) The test is then performed on the dilutions, as described.

This test was investigated in a similar manner to that employed above. Plasmas from 21 normal adults provided 34 pairs of readings, each 'reading' being the mean of two replicate clotting times. The mean values ranged from 54.9 to 69.1 sec., about a grand mean of 63.6 sec. Again it was found that experimental error (standard error for a single clotting time, ± 1.84 sec.) was small by comparison with differences between persons (standard error for a single subject, ± 3.24 sec.; coefficient of variation, 5.1%). The standard error of the difference between mean clotting times from two random subjects was ± 4.57 sec., corresponding to a coefficient of variation of 7.2%.

Taking the corresponding criteria to those previously adopted, the patient's mean would significantly exceed the control mean in the modified test at the 5% probability level if it were longer by $4.57 \times 1.65 = 7.6$ sec. or more. The corresponding limit at the 1% level would be $4.57 \times 2.33 = 10.7$ sec. It is suggested that for simplicity 7 sec. is taken as

the working critical difference, which is conveniently the same value as that suggested for the unmodified test.

Next it was necessary to consider the difference in factor-VIII concentration represented by a difference in clotting time of 7 sec. at a 1 in 20 dilution of test and control plasmas in haemophilic plasma. Blood was therefore taken from two normal persons known to differ widely in their factor-VIII levels: each plasma was diluted 1 in 10 and 1 in 40 in the same haemophilic plasma, and the means of four replicate clotting times obtained on each plasma at each dilution. From each plasma the difference between mean clotting times at the two dilutions was taken to represent the slope of the dose-response curve at a dilution of 1 in 20 (since the regression of response on log. dose was known to be approximately linear in this region). Despite a 2.4-fold difference in factor-VIII concentration between the two subjects' plasmas, the slopes were almost identical and a common slope was taken. On this basis it was found that a difference in 7 sec. represented a 1.92-fold difference in factor-VIII concentration over this region.

Thus, if the factor-VIII level of a random control subject happened to be 100%, a patient's plasma would be regarded as normal by the modified test if his factor-VIII concentration did not lie below 52%. If, on the other hand, the control plasma level were only 60%, the 'normal range' would extend down to 31%.

While this 'range' is still wide, it appears unlikely that a significant degree of factor-VIII deficiency would be missed by the modified test, for 25 to 30% has been regarded as a sufficient haemostatic level for surgical traumata (Macfarlane, Mallam, Witts, Bidwell, Biggs, Fraenkel, Honey, and Taylor, 1957; Maycock, Evans, Vallet, Combridge, Wolf, McGibbon, French, Wallett, Dacie, Biggs, Handley, and Macfarlane, 1963), which probably form the major risk for mild haemophiliacs. Thus the test would be unlikely to miss those haemophiliacs who might have serious post-operative bleeding.

SCREENING TEST FOR FACTOR-IX DEFICIENCY A similar screening test can be set up for factor-IX deficiency, by diluting the patient's and control plasmas 1 in 20 in plasma from a severely affected case of Christmas disease. If stored Christmas disease plasma is to be used, it is important that it should not have become deficient in factor VIII, or the discrimination of the test will suffer. The factor-VIII content may conveniently be supplemented by first diluting the Christmas-disease plasma with an equal part of fresh, normal, barium-sulphate treated, oxalated plasma, and then making the 1 in 20 dilu-

tions in this mixture. We think that barium-sulphate adsorbed oxalated plasma is more reliably deficient in factor IX than alumina-adsorbed citrated plasma. A convenient quantity is prepared by treating 2.0 ml. of plasma with 200 mg. of barium sulphate at room temperature for 20 minutes.

We have not investigated the normal range of this modification in the same detail with which we studied the factor-VIII screening test, because we are less certain of the validity of assay procedures for factor IX, so that a critical haemostatic level cannot be well defined. In the following section we therefore present clinical material part of which suggests that the two screening tests have an approximately similar discriminatory ability.

CLINICAL EXPERIENCE WITH THE TESTS DESCRIBED
Table I presents the findings on two men referred on account of bleeding symptoms, in both of whom the

TABLE I
'FOR DIAGNOSIS'

Age (yr.)	Clinical Features	Differences between Patient's and Control Clotting Times (sec.)	
		Partial Thromboplastin Time Test	Screening Tests For Factor VIII Deficiency IX Deficiency
18	Life-long bleeding symptoms, including haemarthrosis	33	9 74
29	Several episodes of prolonged dental bleeding	24	38 2

partial thromboplastin time tests gave a clearly abnormal result. It is immediately apparent from the findings in the screening tests that one patient is factor-VIII deficient and the other IX-deficient, and

TABLE II

MILD FACTOR-VIII DEFICIENCY

Age (yr.)	Clinical Features	Differences between Patient's and Control Clotting Times (sec.)		Factor VIII Assay
		Partial Thromboplastin Time Test	Factor VIII Screening Test	
10	Life-long bleeding symptoms, including haemarthrosis	88	65	2%
52	Bled for 24 hr. after dental extractions. Two weeks' oozing from wounds following traffic accident, with immediate response to fresh-frozen plasma infusion	20	20	14%
74	Bled for 3 days after dental extractions	22	20	17%

differences of this order have been our general experience.

Table II shows the results in three patients with varying degrees of factor-VIII deficiency, two being rather mildly affected. With factor-VIII levels around 15% these two men gave clearly abnormal tests, although obviously less so than the more severely affected boy.

Table III gives the range of clotting times observed in various members of a large kindred transmitting Christmas disease. Part of this kindred has already been described (family B6) by Davies, Gavin, Goldsmith, Graham, Hamper, Hardisty, Harris, Holman, Ingram, Jones, McAfee, McKusick, O'Brien, Race, Sanger, and Tippet (1963), including information supplied by Dr. P. Flute of King's College Hospital Medical School. It will be seen that 15 persons who gave no history of abnormal bleeding yielded clotting times up to 6.5 sec. longer than the control in the partial thromboplastin time test and up to 10.5 sec. above control in the IX-screening test. Two persons whose symptoms were difficult to assess gave results within this range whereas four whose symptoms were clearly abnormal gave obviously abnormal test results. Thus the factor-IX screening test allowed the members of the kindred to be clearly divided into affected and unaffected persons.

TABLE III

MEMBERS OF A KINDRED TRANSMITTING CHRISTMAS DISEASE

Clinical Classification	No. of Persons	Range of Age (yr.)	Range of Differences between Patient's and Control Clotting Times (sec.)	
			Partial Thromboplastin Time Test ¹	Factor IX Screening Test
Normal	15	6 to 63	-3.0 to 6.5 ²	-7.0 to 10.5
Doubtful (numerous epistaxes only; easy bruising)	2	1, 28	0.5, 3.0	-1.5, 2.0
Abnormal (easy bruising, prolonged post-traumatic bleeding; response to infusion of fresh-frozen plasma)	4	6 to 65	13.0 to 21.5	29.0 to 61.0

¹A minus difference indicates that the patient's time was shorter than the control time.

²Only 9 persons tested.

DISCUSSION

The partial thromboplastin time test, modified by preincubating the plasma for 10 min. with kaolin, provides a simple and sensitive one-stage clotting test for deficiencies of thromboplastic factors, particularly factors VIII and IX.

The reagents appear to be stable, but for routine

clinical use it is probably wise to test patients' plasmas in parallel with normal controls. Investigation of the discriminating ability of the test with respect to haemophilia showed that the variation in normal factor-VIII levels might lead to patients being regarded as normal down to a factor-VIII concentration of about 20%. However, if the patient's and the control plasmas are first diluted 1 in 20 in haemophilic plasma, an abnormal result may be expected if the patient's factor-VIII level is below about 30%. It is considered that this level of discrimination is sufficient for clinical purposes.

If the partial thromboplastin time test is in frequent use, with intervals of not less than, say, a week between tests, a valid alternative approach, which could be expected to improve discrimination, would be to maintain a 'control chart' of the normal values plotted against date of testing, so that a range of normal variation could be established for a particular laboratory and its reagents, and temporal trends could be seen. Patients' values could then be compared visually with the charted normal range.

SCREENING TESTS FOR FACTOR-VIII AND FACTOR-IX DEFICIENCY If a long partial thromboplastin time is found in a patient who might be suffering from a life-long bleeding tendency, the two most likely deficiencies would be of factors VIII or IX. On demonstrating a prolonged partial thromboplastin time in such a patient, our practice is to repeat the test with the patient's and the control plasmas diluted 1 in 20 in both haemophilic and Christmas-disease plasmas. Our experience suggests that the modified tests possess the same order of discrimination for Christmas disease as for haemophilia. On comparing the results of the two tests carried out together, there is seldom any doubt which defect is present (Table I).

CLINICAL CONSIDERATIONS These tests provide no exception to the rule that laboratory results should not be considered in isolation from their clinical background.

If on clinical grounds it is likely that a patient suffers from either haemophilia or Christmas disease, both modified tests should be done even though the kaolin partial thromboplastin time test itself appears to be normal. This is especially important when the patient has no affected relatives or is related to mildly affected cases: the diagnosis presents much less difficulty in the relatives of severely affected persons, who, if they are affected will also have a severe abnormality which will easily be detected.

In possible cases of the defibrination syndrome, a normal kaolin partial thromboplastin time test is

good evidence that significant depletion of the haemostatic reserve has not occurred, especially if the fibrinogen level and reactivity and the platelet count are also normal. On the other hand, an abnormal partial thromboplastin time in this syndrome does not necessarily imply factor-VIII depletion, because the test would clearly be affected by serious fibrinogen depletion.

RELATION TO OTHER TESTS We find the kaolin partial thromboplastin time test no less informative than the thromboplastin screening test of Hicks and Pitney (1957), and, with the suggested modifications, more convenient for the diagnosis of haemophilia and Christmas disease than the thromboplastin generation test of Biggs and Douglas (1953). However, the modifications which we have discussed require plasmas from known, severe cases of haemophilia and Christmas disease, whereas the thromboplastin generation test does not. If the kaolin partial thromboplastin time test is markedly prolonged, a presumptive diagnosis of haemophilia or of Christmas disease may be made with the same degree of confidence as with the thromboplastin generation test if the patient's partial thromboplastin time is much shortened by the addition of one-fifth of a volume of adsorbed normal plasma or of normal serum respectively. (Alumina-treated, citrated plasma or barium-sulphate treated, oxalated plasma may be used.) However, these manoeuvres only give a clear-cut answer if the patient's plasma is severely deficient, so that the patient's partial thromboplastin time is considerably longer than that of the control.

It is particularly informative to compare the results of the partial thromboplastin and prothrombin times, for if either is normal it may be inferred that an abnormality in the other is unlikely to be due to a defect in the conversion of prothrombin or in the formation of fibrin, and also that the thromboplastic factors required by both tests (factors V and X) are normal. If the partial thromboplastin time alone is abnormal, factors VIII or IX (or possibly XI or XII) are probably reduced, whereas an abnormality of the prothrombin time alone points to a reduction in factor VII.

We would like to thank Professor P. Armitage, of the London School of Hygiene and Tropical Medicine, for his advice with the statistical analysis. Professor G. Wetherley-Mein kindly read the manuscript.

ADDENDUM

Since the above was written, Mr. R. A. Hutton, has kindly pointed out to us that clotting times some

3 to 5 sec. shorter may be obtained by increasing the kaolin concentration to 5 mg./ml. in the reagent suspension. Since, in the above experiments, the coefficient of variation between persons was not increased by the dilution in haemophilic plasma which prolonged the mean clotting time from 34 to 64 sec., it is unlikely that the coefficient would be much reduced by shortening the mean normal clotting time to about 30 sec. It seems wise to apply a maximal contact stimulus, and if the higher concentration of kaolin is therefore used, it is probable that our values for the 'normal range' of the test can still be used.

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APPENDIX

FORMULAE EMPLOYED IN CALCULATING STANDARD ERRORS

1 STANDARD ERROR FOR A SINGLE CLOTTING TIME ('EXPERIMENTAL ERROR')

$$S.E. = \sqrt{\frac{\sum d^2}{2n}}$$

where d is the difference between paired replicate clotting times from n subjects.

2 STANDARD ERROR FOR A SINGLE SUBJECT (SUBJECT VARIATION)

$$S.E. = \sqrt{\frac{\sum D^2}{2N}}$$

where D is the difference between the means of two replicate clotting times from each of N pairs of subjects.

3 STANDARD ERROR FOR THE DIFFERENCE BETWEEN THE MEAN CLOTTING TIMES FROM TWO SUBJECTS

$$S.E. = \sqrt{\frac{\sum D^2}{N}}$$

4 CONTRIBUTION TO (3) FROM EXPERIMENTAL ERROR

$$S.E. = \sqrt{\frac{\sum d^2}{n}}$$