Simple screening tests for the diagnosis of isolated clotting factor defects

With special reference to 'contact factor' defects

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SYNOPSIS Reagents may be prepared from normal plasma and used with the prothrombin time and partial thromboplastin time tests to distinguish isolated defects of factors I, II, VII, VIII, IX, X, XI, or XII.

The prothrombin time (PT) and kaolin partial thromboplastin time (KPTT; also called kaolin cephalin clotting time) tests are widely used as screening procedures for clotting defects. In the diagnosis of isolated, life-long clotting disorders, it is well recognized that both tests will be prolonged by defects of fibrinogen, prothrombin, factor V, and factor X; that a defect of factor VII prolongs only the PT; and that defects of factors VIII, IX, XI or XII prolong only the KPTT.

The most satisfactory discrimination between the defects is obtained by testing the ability of the patient's plasma to correct the prolonged clotting times of known, naturally occurring defective plasmas obtained from reference patients, either as qualitative tests or as assays. However, where such plasmas are not available, reagents may be prepared instead from normal plasma. Two alternative principles may be involved: either a concentrate of one or more factors may be added to the patient's plasma in an attempt to correct the defect and thus identify the factor *missing* from the patient's plasma; or the patient's plasma may be added to reagents with known defects (as in using natural plasmas) to see which are corrected, and thus to identify the components *present* in the patient's plasma.

The commonest life-long clotting disorders are haemophilia, Christmas disease, and von Willebrand's disease, involving defects of factors VIII or IX. In either defect the PT will be normal but the PTT prolonged; and it has been pointed out that correction of the PTT by the addition of 1 in 5

Al(OH)₃-adsorbed fresh normal plasma suggests a factor-VIII defect whereas correction by an eluate from the Al(OH)₃ suggested a factor-IX defect (Knights and Ingram, 1967). This technical approach has now been extended to cover other clotting factor defects.

Experiments undertaken in developing the method are described, the findings in reference patients are given, and the suggested diagnostic screening procedure is set out as a dichotomous key.

Materials

Phospholipid: Thrombafax (Ortho) was diluted 1 in 2 for use. Thromboplastin: Manchester Comparative Reagent was kindly supplied by Dr L. Poller, National Reference Laboratory for Anti-coagulant Control Reagents, Withington Hospital, Manchester M20 8LR.

BLOOD PRODUCTS

Citrated plasma was obtained by centrifuging a mixture of 9 vol whole blood and 1 vol 110 mM trisodium citrate solution in plastic tubes. Plasma was adsorbed by incubating 9 vol with 1 vol of a 1 in 20 (v/v) suspension of Al(OH)₃, BDH moist gel, in 50 mM tris buffer, pH 7.4, at 37°C for 3 min and centrifuging to remove the alumina. Eluate was obtained by washing the deposited alumina × 3 in cold distilled water and then incubating with 0.1 × the original plasma volume of phosphate buffer pH 8.0, at 37°C for 3 min, centrifuging to remove the alumina and finally diluting the supernatant 1 in 8 in tris buffer. The phosphate buffer is prepared by dissolving (a) 1.118 g Na₂HPO₄·2H₂O,

and (b) 0.91 g KH_2PO_4 , each in 100 ml water; take 9.7 vol of (a) with 0.3 vol of (b). More detail is given by Knights and Ingram (1967).

Aged normal serum was made by allowing normal blood to clot in glass tubes at 37°C , centrifuging after 1 hr and removing the serum which was incubated for a further 6 hr; it may be stored frozen, and diluted 1 in 5 in tris buffer for use. A more standardized product was made by adding an equal volume of 25 mM CaCl_2 solution to citrated plasma with 1% (v/v) brain thromboplastin, mixing, and incubating for 6 hr: the separated serum is diluted 1 in 2 in tris buffer for use.

Celite-adsorbed normal plasma, with factors V and VIII added, was made by the method of Giddings (1971) as an artificial XI-deficient substrate.

Contact product was made as described by Wilson, Ingram, and Hills (1971) and latterly by the modifications given by Pérez-Requejo and Ingram (1975).

Methods

The PT was performed as described by Hardisty and Ingram (1965). The KPTT was performed as described by Matchett and Ingram (1965), following Hardisty and MacPherson (1962), pre-incubating equal volumes of citrated plasma and a suspension of light kaolin 5 g/l in 50 mM tris buffer for 10 min at 37°C before completing the reaction with equal volumes of Thromboplastin and 25 mM- CaCl_2 . Tests involving additional reagents (0.1 ml) were performed as follows:

TRIS BUFFER CONTROL

The initial test was re-run, tris buffer having been added to both patient's and control tubes.

TEST ADDITIONS

In the PT, the specified reagents were added to the patient's and control tubes before the thromboplastin and CaCl_2 solution. In the KPTT, reagents were added either before or after the 10-min pre-incubation with kaolin, as specified. The performance of the test may be illustrated from the following modification of the procedure of Knights and Ingram (1967) for adding adsorbed plasma and eluate to the KPTT before incubation with kaolin (table I).

Into each of six clotting tubes in the water bath at 37°C was placed 0.1 ml kaolin suspension. To the first tube was added 0.1 ml tris buffer and 0.1 ml control plasma; the contents were mixed and a watch was started. One minute later 0.1 ml tris buffer and 0.1 ml patient's plasma were added to the second

<i>Tube</i>	<i>Time Set Up (min)</i>	<i>Reagent Added</i>	<i>Plasma Tested</i>
1	0	Tris	Control
2	1	Tris	Patient
3	2	Adsorbed plasma 1 in 5	Control
4	3	Adsorbed plasma 1 in 5	Patient
5	4	Eluate	Control
6	5	Eluate	Patient

Table I Arrangement of partial thromboplastin time screening tests

Each tube receives 0.1 ml kaolin suspension 5 g/l, and is warmed; then, at intervals of 1 min, 0.1 ml vols of the appropriate reagent and test plasma are added. Each mixture is incubated for 10 min, and the reaction is then completed by the addition of 0.1 ml Thromboplastin and 0.1 ml warm 25 mM- CaCl_2 solution.

Note that the reagent and test plasma are preincubated with kaolin together.

tube and mixed. Similarly, at the third and fourth minutes 0.1 ml adsorbed plasma diluted 1 in 5 in tris was added to each of the next pair of tubes, with 0.1 ml control or patient's plasma respectively. Similarly, the final pair of tubes received 0.1 ml of the eluate at the fifth and sixth minutes, and 0.1 ml control or patient's plasma respectively. As the watch approached 10 min the reaction mixture in the first tube was completed by adding 0.1 ml Thromboplastin and, at exactly 10 min, 0.1 ml 25 mM- CaCl_2 solution, and clotting was timed on another watch. At successive minutes, the mixtures in the subsequent tubes were similarly completed and clotting was timed. The whole manoeuvre was repeated a second time in the opposite order to exclude temporal drift due to changes in the reagents, and the means of the duplicates were calculated. From these, the difference patient's time minus control time was tabulated for each of the additions (buffer, adsorbed plasma 1 in 5, and eluate).

Correction of Giddings' reagent was tested by mixing equal volumes of the test or control plasmas with this reagent and then performing the KPTT on 0.1 ml of the mixture; the degree of correction in each case was assessed by comparison with the result of performing the KPTT on 0.1 ml of Giddings' reagent alone.

The effect of contact product was assessed by performing the PTT on a mixture of 0.1 ml volumes of test or control plasma, phospholipid, CaCl_2 -solution, and contact product, added in succession in that order. Before use, the contact product was diluted so that the clotting time obtained from the control plasma in the PTT with contact product was the same as that obtained on the same plasma in the KPTT, to make it possible to compare the patient-control differences in the two tests.

CONTACT TESTS

The contact activation test was modified from Margolis (1957), after Hardisty and Ingram (1965), by exposing samples of the patient's or control plasma to Celite 512 and observing the degree of shortening afforded by these plasmas when added in various dilutions to uncontacted normal plasma. In the Celite-6 test for factor XI, quoted by Hardisty and Ingram (1965), normal plasma was depleted of factor XI by prior incubation with Celite 512; and mixtures of the control of patient's plasmas with this plasma were tested for their ability to shorten the clotting time of intact normal plasma. Since several dilutions were tested in both procedures, it was possible to calculate from the results the activity of the patient's plasma as a ratio to that of the control plasma as a parallel-line bioassay.

Patients

Patient B, male (factor-XI deficiency) aged 38, had been detected in routine testing before intended haemorrhoidectomy. He had previously suffered excessive bleeding after an operation for hernia and after an injury in a road traffic accident. He gave no history of haemarthrosis and did not suffer from abnormal spontaneous bleeding. The original diagnosis had been made by Dr P. Aggler; he was subsequently investigated at the Haemophilia Centre at Oxford where his KPTT was 147 s but was completely corrected by the addition of contact product: no factor XI-activity could be detected.

Patient H, male (factor-XI deficiency) aged 46, had bled for two to three days after dental extractions aged 30 and 37, although he had survived tonsillectomy in childhood and did not recall prolonged postoperative bleeding. His factor-XI activity assayed in Giddings' system was <1% of control; but the contact activation and Celite-6 tests gave activities of 17 and 21% respectively; whatever may explain the discrepancy between the results in these tests and in Giddings' reagent, the agreement between the results in the two simpler systems supports the conclusion that H's contact defect is explained by his deficiency of factor XI.

Patient P, male (factor-VIII deficiency) aged 30, is a severely affected haemophiliac who has frequently attended this Haemophilia Centre during the last 15 years and was previously treated at The Hospital for Sick Children, Great Ormond Street, London. His blood shows no factor-VIII activity; he does not have an antibody to factor VIII. His plasma has often been used as the basis for factor-VIII assays.

Patient R, female (factor-XII deficiency) aged 38, had not bled abnormally after an operation for perforated duodenal ulcer aged 14 nor after a

nasal plastic repair operation in adult life. She did not appear to bruise unduly easily but had suffered from troublesome epistaxes until recently. Her platelet count, bleeding time, and prothrombin time were normal but the whole-blood clotting time and kaolin partial thromboplastin time were prolonged. Using a modification of the latter test on the mixtures of the patient's plasma and various reference plasmas, the patient's plasma had been shown at the Oxford Haemophilia Centre to be grossly deficient in factor XII. On the present occasion the whole-blood clotting time was *ca* 23 min in glass tubes and *ca* 45 min in silicone-coated tubes.

Patient S, female (factor-XII deficiency) aged 28, was a blood donor whose deficiency had been detected through the delayed clotting of a blood sample; it was later learned that bleeding had been excessive after tonsillectomy and after dental extraction, but transfusion had not been required (Marsh and Jenkins, 1961). The diagnosis of factor-XII deficiency had been suggested by the correction of the clotting time after S's blood was tested in a glass tube which had first been rinsed with normal plasma and then several times with saline. On the present occasion S's plasma showed 2% of the control activity in the contact activation test, but in the Celite-6 test for factor XI her plasma showed over 100% of control activity; these results are compatible with an isolated deficiency of factor XII.

Results

ADDITION OF $\text{Al}(\text{OH})_3$ -PLASMA OR $\text{Al}(\text{OH})_3$ -ELUATE TO PTT BEFORE PRE-INCUBATION WITH KAOLIN

Table II shows the effect of adding diluted normal plasma, diluted $\text{Al}(\text{OH})_3$ -adsorbed normal plasma, and $\text{Al}(\text{OH})_3$ -eluate to the plasmas of H and S, before preincubation with kaolin. It is seen that both are corrected by the $\text{Al}(\text{OH})_3$ -adsorbed plasma (in H's case, as well as by whole normal plasma), but that there is much less correction with $\text{Al}(\text{OH})_3$ -eluate.

Table III shows the effect of adding a 1 in 55 dilution of normal $\text{Al}(\text{OH})_3$ -plasma either before or after preincubation to factor-VIII-deficient, factor-XI-deficient and factor-XII-deficient plasma. It is seen that good correction is obtained with the factor-VIII-deficient plasma, irrespective of when the addition is made, but that with the contact-factor-deficient plasmas the correction is notably greater when the $\text{Al}(\text{OH})_3$ plasma is added before preincubation with kaolin. We have not examined a sufficient number of contact-factor-deficient patients to know whether the difference in the

Reagents Added	Difference, Patient's KPTT Minus Control KPTT (s)	
	H (XI-deficient)	S (XII-deficient)
Kaolin + tris buffer	65.3	360
+ 1 in 5 whole normal plasma	29.5	nt
+ 1 in 5 Al(OH) ₃ -adsorbed plasma	29.3	18.3
+ Al(OH) ₃ eluate	62.1	81.3

Table II Partial thromboplastin time differences in plasmas from patients H and S

nt = not tested.

For simplification, the table shows only the differences between paired readings obtained in tests in which the same reagents were added to both control and patient's plasmas before preincubation with kaolin. Each difference is based on means of duplicate clotting times.

The control times lay between 39.0 and 49.6 s after the various reagents had been added.

degree of correction shown in the plasmas of B and S is the general rule with XI-deficient and XII-deficient plasmas respectively.

Table IV shows the effect of adding contact product to the partial thromboplastin time tests on plasma samples from B and R (both factor XII-deficient) by comparison with the effect of pre-incubation with kaolin. It is seen that contact product completely corrects the defect in both cases. For comparison, the three plasmas of table III were similarly tested: on the addition of contact product to patients' and control plasmas, in place of kaolin preincubation and addition of Al(OH)₃-plasmas, the patient minus control differences for the three plasmas were: P (factor-VIII-deficient), 82.0 s; B (factor-XI-deficient), 4.0 s; and S (factor-XII-deficient), 6.7 s. There is thus a clear differentiation between haemophilia and the contact factor defects; and the correction of the contact-factor-defective plasmas

Reagents Added	Patients	
	B	S
Tris	nt	422
Kaolin	146	615
Contact product	29	29

Table IV Partial thromboplastin times on XII-deficient plasmas (s)

nt = not tested.

The marked defect in the patient's plasma is corrected by contact product.

Clotting times are the means of two or three readings.

by this technique is as good as the correction of VIII-deficient plasma by the addition of Al(OH)₃-plasma after incubation with kaolin (table III).

EXPERIENCE WITH CONTACT PRODUCT IN SCREENING FOR CONTACT FACTOR DEFECTS

Table V shows the results from seven patients who have been routinely investigated in this laboratory and in whom a long KPTT was found which was corrected by the addition of contact product and in whom the contact factors were subsequently assayed. The degree of correction with contact product is measured as the difference between the KPTT and the PTT with contact product, and this difference is tabulated against the factor XI or factor XII activity (whichever was the lower) assayed in plasmas from B, H, or S. Dilutions of test and control plasmas were preincubated with kaolin in the presence of specifically deficient plasma, and then recalcified with phospholipid in the ordinary way; the results were calculated according to the standard principles of parallel-line bioassay.

Experiences with Giddings' Reagent

We have prepared several batches of Giddings' (1971) artificial XI-deficient substrate, and data on our preparations may be of interest. The levels

Time of Adding Normal Al(OH) ₃ -adsorbed Plasma 1 in 5	Difference, Patient's KPTT Minus Control KPTT (s)		
	P (VIII-deficient)	B (XI-deficient)	S (XII-deficient)
Before preincubation with kaolin	8.3	33.8	10.0
After preincubation with kaolin	4.7	58.5	156.2
Addition of tris buffer instead of Al(OH) ₃ -adsorbed plasma, as control	62.3	95.8	219.5

Table III Partial thromboplastin time differences on plasmas from patients P, B, and S

As in table II, this table shows only the differences between paired readings obtained in tests in which the same reagents were added to both control and patient's plasmas under the stated condition. Each difference is based on means of duplicate clotting times. The mean control times lay between 37.6 and 38.0 s in the tris control tests, between 39.3 and 40.0 s when the diluted Al(OH)₃-adsorbed plasma was added before preincubation with kaolin, and between 43.5 and 47.8 s when added after preincubation.

Case No.	Age	Sex	Patient minus Control Difference (s)		Difference (s)	Factor %	
			KPTT	Contact Product PTT		XI	XII
1	47	M	13.7	0.5	13.2	72	
2	8	M	15.9	8.7	7.2	57	
3	9	F	13.8	3.2	10.6	31	
4	3	M	6.2	-4.6	10.8		24
5	43	F	11.6	-1.7	13.2	5	
6	17	M	40.4	3.6	36.8	4	
7	24	F	40.4	3.4	37.0	3	
Patient B	38	M	98.7	1.3	97.4	0	
Patient S	28	F	219.5 ¹	6.7	212.8		0

Table V Findings in seven cases of contact factor defect

¹Tests contained in addition a vol of tris buffer

Factors XI and XII were both assayed but only the lower value is tabulated.

These cases were identified in the course of routine work by the screening procedure using contact product. The test was applied because the KPTT difference was greater than the critical value of 6 s (Matchett and Ingram, 1965); note that the difference between the KPTT and contact product PTT differences was also greater than 6 s. Case 1 is obviously doubtful, with 72% factor XI (factor XII, 82%), but no other abnormality could be found and there was a clear history of a number of occurrences of abnormal post-traumatic bleeding. Only case 2 was asymptomatic; he was tested because he was the brother of case 3. The other cases are all from separate families. In general, there is a relationship between the magnitude of the difference in col 6 and the degree of abnormality in cols 7 or 8. Patients B and S are included as zero values.

of factors V, VIII, XI, and XII we have obtained in five batches are shown in table VI, and table VII shows the KPTT results on the fifth batch from table VI, compared to the times from the control plasma and from natural XI- and XII-deficient plasmas, and from various mixtures of them. We have found difficulty in maintaining adequate levels of factor XII in our Giddings' reagent, but the mixture experiments of table VII suggest that *ca* 50% (the level in batch 5) is sufficient to give reasonable discrimination in mixtures of patient's and test plasmas. Our evidence suggests (batches 2-4) that 20%, rather than 3-4%, trisodium citrate 2H₂O must be used in the preparation as the anti-coagulant, as directed. It was noted that factors V and VIII were completely lost after double celite absorption of the plasma employed, so that it was

necessary to assay the material at this stage to be able to calculate the required supplements of V and VIII.

Simple Screening Procedure for the Diagnosis of Isolated Clotting Factor Defects

USE OF A DICHOTOMOUS KEY

The evidence summarized in the introduction, together with the data presented in this paper, permits the construction of a dichotomous key for the determination of isolated clotting factor defects, based on the PT and PTT with the ancillary reagents here described and two other tests which are now widely available.

The key is shown in table VIII. The initial procedure is to perform the PT and the KPTT on the

Batch	Tri-sod. Cit. Dihydr. Reagent, %	Constituent Concentrates				Completed Reagent Activities			
		Factor V%		Factor VIII (iu/ml)		Factor V%	Factor VIII (iu/ml)	Factor XI%	Factor XII%
		Pre-celite	Post-celite	Pre-celite	Post-celite				
1	20	665	4010 ¹	12.3	11.0	173	2.4	6.6	Present
2	3.2	692	433	30.0	15.5	117	1.3	<1	37
3	3.2	nt	nt	nt	nt	nt	nt	<1	12
4	20	nt	nt	nt	nt	nt	nt	<1	72
5	20	1034	826	nt	28.0	121	0.7	<1	54

Table VI Clotting activities in five batches of Giddings' reagent and in the constituent concentrates of factors V and VIII

¹The factor V appeared to have activated in this sample.
nt = not tested.

Plasma	Clotting Times (Means of Two Readings) (s)		
	Tested Alone	Tested in Equal Mixture with	
		Control	Natural XI-deficient Natural XII-deficient
Control	33		
Natural XI-deficient	119	41	
Natural XII-deficient	188	41	45
Giddings' reagent	76	40	81 44

Table VII Kaolin partial thromboplastin times with control plasma, naturally occurring factor XI- and factor XII-deficient plasmas and Giddings' reagent, alone and in equal mixtures. The results on the mixtures show that Giddings' reagent clearly distinguished the factor XI-deficient from the factor XII-deficient plasma

patient's plasma and a normal control plasma in parallel and to note whether the patient's time is prolonged in either the PT, or the PTT, or in both tests, and then to proceed through the table accordingly. Note that the key may fail in the face of more than one defect, and also if the initial difference between the patient's and the control times in the PT is not greater than 10 s or in the KPTT 15 s. The key is also inapplicable if the patient's clotting defect is due to an inhibitor or an inactivator.

DETECTION OF INHIBITOR OR INACTIVATOR

Before proceeding through the key, it is therefore appropriate to eliminate the possibility of an

inhibitor by preparing a 50:50 mixture of the patient's and the control plasma and testing this mixture in whichever of the two procedures had yielded an abnormal result on the patient's plasma alone; if the patient's defect is due to a clotting factor deficiency, the clotting time of the mixture should approximate to that of the control, whereas if the defect is due to an inhibitor, the clotting time of the mixture should be nearer to that of the patient (Hardisty and Ingram, 1965). Note that this test becomes discriminating only when the initial difference between the patient's and the control clotting times is at least 10 s in the PT and 15 s in the KPTT.

1	a	PT alone prolonged, corrected by Al(OH) ₃ eluate or by serum	Factor-VII Defect
	b	KPTT alone prolonged	2
	c	Both tests prolonged	3
2	a	KPTT corrected by Al(OH) ₃ -adsorbed plasma added before kaolin preincubation	4
	b	KPTT corrected by Al(OH) ₃ eluate	Factor-IX Defect
3	a	Both tests corrected by Al(OH) ₃ -adsorbed plasma	6
	b	Both tests corrected by Al(OH) ₃ eluate	7
4	a	KPTT also corrected if Al(OH) ₃ -adsorbed plasma added after kaolin preincubation;	Factor-VIII Defect
	b	PTT not corrected by contact product	
	b	KPTT not corrected if Al(OH) ₃ -adsorbed plasma added after kaolin preincubation;	
5	a	Patient's plasma corrects Giddings' reagent	Factor-XII Defect
	b	Patient's plasma does not correct Giddings' reagent	Factor-XI Defect
6	a	Patient's plasma clots with thrombin; immunological tests for fibrinogen normal	Factor-V Defect
	b	Patient's plasma poorly clotted or incoagulable with thrombin; immunological tests for fibrinogen abnormal	Fibrinogen Defect
7	a	PT also corrected by serum	Factor-X Defect
	b	PT not corrected by serum	Prothrombin Defect

Table VIII Dichotomous key for severe, isolated clotting factor defects

Tests: Prothrombin time (PT); partial thromboplastin time with kaolin (kaolin-cephalin clotting time) (KPTT)

Supplementary reagents:

Aged normal serum

Al(OH)₃-adsorbed fresh normal plasma 1 in 5; Al(OH)₃ eluate (Knights and Ingram, 1967)

Contact product (Pérez-Requejo and Ingram, 1975)

Celite-adsorbed normal plasma (factor XI-deficient) with factors V and VIII added (Giddings, 1971)

Thrombin solution, ca 5 NIH u/ml

Immunological test for fibrinogen

Note that the key may fail if the defect is not sufficiently marked to prolong the patient's PT by at least 10 s or the patient's KPTT by at least 15 s over the control; if more than one clotting factor is defective; or in the presence of a clotting inhibitor or an inactivator of a clotting factor, both of which must first be excluded by the mixing tests described.

TEST FOR AN INACTIVATOR

When the initial difference between the patient's and control clotting times is very long, suggesting a major abnormality, the presence of an inactivator of a clotting factor (for example, an anti-factor-VIII antibody) should be sought by incubating equal volumes of the patient's and the control plasmas in sufficient quantity to withdraw aliquots periodically (say, over 2 hr) and repeat whichever test initially demonstrated the abnormality. Control readings are obtained by incubating in parallel separate samples of the patient's and the control plasmas, and testing freshly made 50:50 mixtures from them in parallel with the tests on the incubated mixture (Hardisty and Ingram, 1965). The presence of an inactivator is demonstrated if the prolongation of serial clotting times from the incubated mixture exceeds that from the freshly made mixtures.

ANCILLARY REAGENTS

In addition to those reagents already mentioned, the key includes the thrombin clotting time test and an immunological test for fibrinogen. Thrombin solution, which may be diluted to *ca* 5 NIH u/ml, may be obtained from various sources; and several commercial kits are now available for the immunological detection of fibrinogen or its derivatives. It has therefore been assumed that such reagents will be available if necessary.

References

- Giddings, J. C. (1971). Preparation and use of a new artificial system for factor XI assay. *Med. Lab. Technol.*, **28**, 284-299.
- Hardisty, R. M. and Ingram, G. I. C. (1965). *Bleeding Disorders: Investigation and Management*. Blackwell, Oxford.
- Hardisty, R. M. and MacPherson, J. C. (1962). A one-stage factor VIII (antihaemophilic globulin) assay and its use on venous and capillary plasma. *Thrombos. Diathes. haemorrh. (Stuttg.)*, **7**, 215-229.
- Knights, S. F. and Ingram, G. I. C. (1967). Partial thromboplastin time test with kaolin: diagnosis of haemophilia and Christmas disease without natural reference plasmas. *J. clin. Path.*, **20**, 616-619.
- Margolis, J. (1957). Initiation of blood coagulation by glass and related surfaces. *J. Physiol. (Lond.)*, **137**, 95-109.
- Marsh, W. L. and Jenkins, W. J. (1961). The recognition of Hageman deficiency in blood donors. *J. clin. Path.*, **14**, 550-551.
- Matchett, M. O. and Ingram, G. I. C. (1965). Partial thromboplastin time test with kaolin. *J. clin. Path.*, **18**, 465-471.
- Pérez-Requejo, J.-L. and Ingram, G. I. C. (1975). Preparation of 'contact product' as a reagent for coagulation tests. *J. clin. Path.*, **28**, 596-599.
- Wilson, W. J., Ingram, G. I. C., and Hills, M. (1971). The use of kaolin or contact product in the one-stage assay of factor VIII. *Coagulation*, **4**, 113-117.

Addendum

The new method of Blewitt (1975: *J. clin. Path.*, **28**, 332-336) for the preparation of an artificial plasma was published while the above paper was in press.