METHODS FOR THE INVESTIGATION OF THE COAGULATION MECHANISM IN SMALL CHILDREN

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The major problem which presents itself in any investigation of blood clotting in small children and infants is that of obtaining adequate specimens of plasma and serum. In the older child, as in the adult, blood may be taken by venepuncture in large amounts; the operation is technically easy and can readily be repeated if necessary. In the small infant the only veins readily available are those in the groin and the neck and the sagittal venous sinus. Venepuncture in these sites is much more difficult technically; it is not without hazard to the child and cannot readily be repeated. Therefore it has been necessary to develop methods for obtaining plasma and serum by skin puncture.

The first test of blood coagulation which was devised for use in clinical practice employed capillary blood (Wright, 1893), a matter of some interest in view of the poor opinion which is to-day held of techniques using capillary blood. Stefanini and Dameshek (1955), in their book on the haemorrhagic disorders, state that micro methods exist for the one-stage prothrombin time but that they are to be used only when blood cannot be obtained by venepuncture and the results obtained by these methods are to be regarded with grave suspicion. Objections to techniques involving the use of capillary blood are threefold. First, the use of capillary blood is opposed on the grounds that, in taking blood by this technique, the shed blood is to some extent mixed with tissue juices and the results are vitiated by the introduction of thromboplastic substances from this source. Merskey (1950) in a study of a series of haemophilic patients found that the consumption of prothrombin was reduced when blood for the test was taken by venepuncture, but that when blood for the test was taken by skin prick only 27 of 42 cases showed abnormal prothrombin consumption. Fantl and Sawers (1954), however, published a study of the blood clotting mechanism in a series of patients suffering from haemophilia

and deficiency of plasma thromboplastin component (Christmas disease) in which they found that, while in patients suffering from classical haemophilia the consumption of prothrombin was reduced both in blood taken by venepuncture and by finger prick, in patients suffering from deficiency of plasma thromboplastin component the consumption of prothrombin was reduced in blood taken by venepuncture but not in blood taken by finger prick. These authors attributed this difference to the effect of admixture of tissue juices when blood was taken by finger prick, and suggested that tissue juices were capable of replacing plasma thromboplastin component but not antihaemophilic globulin. The method by which capillary blood was obtained from these patients was not stated, so that the extent of contamination of the specimens with tissue juice cannot be assessed, and it is difficult to understand how, if the tissue fluids, which are in a constant state of interchange with the plasma by the lymphatic circulation, contain a substance that can replace plasma thromboplastin component, the plasma can be deficient in this factor.

Technical objections to the use of capillary blood are that the amounts of plasma and serum available are small and that clotting takes place during the collection of the blood so that plasma free from serum cannot be obtained. These two objections can be considered together as they both depend on the method of obtaining capillary blood. If skin puncture is performed unskilfully and the finger or heel is subjected to compression in an effort to obtain an adequate flow of blood, the amount of blood obtained will be disappointingly small; it will be contaminated with tissue fluids and clotting at the end of the tube and on the skin surface will be troublesome. At the Alder Hey Children's Hospital, Liverpool, a wide variety of investigations, chemical, haematological, and serological, are carried out on small children and it is often necessary to repeat the tests at

frequent intervals for the control of treatment. Under these conditions methods using capillary blood have been developed for all investigations on babies and for many on older children; the collection of blood by skin puncture has been developed to a high degree of efficiency, and capillary blood is, in many instances in childhood, to be preferred to blood obtained by venepuncture from a small, struggling child.

The third objection to the use of capillary blood is that the methods of testing which are used are special micro methods and are basically unsound. Kato (1940) and Innes and Davidson (1941) described methods of determining the one-stage prothrombin time in which whole blood is mixed with an anticoagulant, either in a special slide or in a white cell pipette, and the test is carried out by mixing the whole blood with thromboplastin and calcium chloride in a watch glass or on a porcelain spot plate. Biggs and Macfarlane (1953) describe a method in which dilution of capillary blood is carried out in a white cell pipette. Approximately equal volumes of blood and anticoagulant are employed, the mixture is centrifuged, and the supernatant fluid used as diluted plasma. Both of these methods have disadvantages; in the former the test is a special "micro method" using whole blood, and it is difficult to read the end-point; in the latter the degree of dilution of the plasma depends to a considerable extent on the haematocrit, a higher concentration of plasma being present in the supernatant from anaemic patients.

The third type of method described is that of Bray and Kelley (1940): 1 ml. of blood is taken from the heel or finger and is allowed to fall into a tube containing anticoagulant. The plasma obtained by this means is adequate in amount and the dilution factor is the same as that when venous blood is used. Although Bray and Kelley described the method for use in the one-stage prothrombin time test, it has been used also in the thromboplastin generation test (van Creveld, Nagel, Nijenhuis, Miranda, and Tjon Sien Kie, 1954; van Creveld, Paulssen, Ens, and van der Meij, 1954), and its use has now been extended to the other tests of blood clotting.

The volume of blood which can be obtained by these methods is small by comparison with the volume obtainable by venepuncture. For routine laboratory tests micro methods may, however, safely be used (King, 1951) and the volume of blood plasma or serum is adequate for the tests without further modification: similarly, single tests of blood clotting in diagnosis and in the

control of anticoagulant therapy can be carried out by the orthodox "macro" methods. When several tests have to be carried out on one specimen some further modification of the standard tests is necessary.

Methods

Collection of Specimens.—It is essential, when using blood taken by skin prick, to collect the specimens with great care. The main points in technique are: first, that the needle used must be sharp and the stab deep, in which case there is very little pain and only a minimum of restraint is required; secondly, the finger or heel must be warm, an important point in small babies in whom the peripheral circulation is very sensitive to a reduction in the external temperature; and thirdly, the blood should be allowed to fall directly into the test-tube or be collected into a capillary tube, accumulation of blood on the skin being avoided. Where these points of technique are followed a surprisingly large amount of blood can be obtained in a short time. If there is an adequate free flow of blood from a skin puncture, without compression of the part, the admixture of tissue fluids, after the first drop has been wiped away, is very small and may be disregarded for all practical purposes.

The instrument used is a clean Hagedorn needle which is sharpened frequently on a smooth stone, is wiped clean after use, and is kept in a bottle containing spirit. The needle is discarded when the edge cannot be resharpened or if any form of hook develops at the tip. The stab must be delivered sharply and cleanly at right angles to the skin surface and must penetrate deeply into the subcutaneous tissues. Any pushing or sawing action will cause trauma to the skin, will make the procedure needlessly painful, and will impair the quality of the specimen obtained.

In older children and in adults the subcutaneous pad of the tip of a finger or of the thumb is sufficiently large and vascular for capillary blood to be obtained in adequate amounts; in small infants the fingers are too small and the heel must be used. The skin of the heel remains soft and easily punctured until the child begins to stand and to walk about the cot and can readily be used up to about the age of 12 months.

Special preparation of a finger is usually unnecessary as constant hand movements in a warm environment maintain an adequate blood flow; in cold weather washing the hands in hot water will stimulate vasodilatation.

In the case of small infants all preparations for the collection of blood are made before the child is removed from its bed-clothes, the child is wrapped in a blanket with one foot exposed, and is held firmly by a nurse. This ensures that reflex peripheral vasoconstriction is kept to a minimum. Very small, immature, infants are warmed either in an incubator or with a hot-water bottle in the cot.

TABLE I
COMPARISON OF VENOUS AND CAPILLARY SPECIMENS

Test	Blood	Case							
		A	B	C	D	E	F	G	H
One-stage prothrombin time (whole plasma % prothrombic activity)	Venous	100	100	120	120	100	120	65	100
	Capillary	100	120	100	50	100	100	65	100
One-stage prothrombin time (diluted plasma % prothrombic activity)	Venous	112	107	63	107	120	100		
	Capillary	107	113	61	78	100	92		
Factor V assay (%)	Venous	80	100	75	110	130	100		
	Capillary	60	90	67	90	110	100		
Factor VII assay (%)	Venous	85	120	120	120	140	120		
	Capillary	120	125	85	130	135	110		
Combined assay of prothrombin and factor VII (%)	Venous	110	105	105	110	110	110		
	Capillary	105	105	70	105	110	105		
Prothrombin assay (%)	Venous	80	90	65	100	100	100		
	Capillary	70	75	55	90	100	112		
Thromboplastin generation test (plasma %)	Venous	110	75	110	67	110	74	105	78
	Capillary	85	69	110	73	87	64	100	68
Thromboplastin generation test (serum %)	Venous	69	69	82	89	95	85	97	105
	Capillary	72	80	84	85	94	105	90	120

The skin is cleansed with spirit and allowed to dry and a sharp stab is made with only sufficient pressure on the part for the skin to be kept taut. The first few drops of blood are wiped away with sterile wool and discarded. Serum is obtained by holding the finger or heel so that the stab wound is dependent and allowing the drops of blood to fall directly into a plain glass test-tube or by allowing the blood to run into a glass tube of 2 mm. drawn out to a capillary point at one end. The blood is allowed to clot and serum is separated by centrifugation. Serum obtained by this technique is clear and free from haemolysis.

Clotting is prevented in blood from which plasma is to be obtained by the addition of 3.8% sodium citrate solution. A small tube (50×10 mm.) is marked at 1.0 ml., and 0.1 ml. of citrate solution is placed in it. Drops of blood are allowed to fall directly into the anticoagulant and are mixed by shaking the tube until the 1.0 ml. mark is reached. Blood is not allowed to fall on to the sides of the tube and the tube is not allowed to touch the skin. The specimen is discarded if any clotting takes place. Approximately 0.5 ml. of plasma can be separated from this specimen by centrifugation.

Tests of Coagulation.—Sufficient blood may be obtained by this method for the conventional one-stage assays of prothrombin activity (Quick, 1951), factor V (Stefanini, 1951; Wolf, 1953), factor VII (Owren and Aas, 1951), the combined effect of prothrombin and factor VII (Dreskin, 1952), and prothrombin alone (Owren and Aas, 1951). If several test must be carried out on one child the plasma can be diluted 1 in 5 with 0.85% sodium chloride solution and compared with diluted normal plasma.

The thromboplastin generation test (Biggs and Douglas, 1953) in its original form requires relatively large volumes of plasma and serum. It has therefore been modified in two respects. The incubation mixture of alumina plasma, serum, platelets, and calcium chloride need only be sampled once, at the end of six or 10 minutes (Biggs, Eveling, and Richards, 1955). This variation in technique permits

the use of smaller volumes of each of the constituents of the reaction mixture, 0.1 ml. of each is adequate. Secondly, the method of preparation of alumina plasma is altered, the plasma may be diluted 1 in 5 with 0.85% sodium chloride solution before absorption of prothrombin and factor VII with alumina. These two modifications make it possible to carry out several assays of thromboplastin generation on a small blood sample.

A small series of experiments were undertaken to demonstrate the validity of these methods.

Specimens of plasma and serum were obtained from a series of volunteer donors by venepuncture and by skin prick. The one-stage prothrombin time was determined both on whole plasma and on plasma diluted 1 in 5 with saline. Assays of factor V, factor VII, prothrombin, and the combined activity of prothrombin and factor VII were carried out by the methods described above. The thromboplastin generation test was also carried out. The results of these experiments are set out in Table I.

Comparison of the results obtained in each of the tests using plasma or serum obtained by venepuncture and by finger prick show that there is little difference between the results and that the coagulation factors in the plasma and serum are normal by both methods. The results for the one-stage prothrombin time, though not for the other tests, in Case D show a significant difference between the values obtained on venous plasma and those obtained on capillary plasma. In this case the procedure was made difficult by the lack of co-operation from the donor, and by the thickness of the skin of the finger which made an adequate stab difficult. This illustrates the importance of good technique.

In addition two cases of haemophilia in small boys have been identified and confirmed by this technique.

A series of 17 specimens of plasma, three from normal individuals and 14 from newborn children, were used in the thromboplastin generation test. All were treated with alumina both before and after dilution. The results of this experiment, which are

TABLE II
COMPARISON OF RESULTS OF DILUTING PLASMA FOR
THROMBOPLASTIN GENERATION TEST BEFORE AND
AFTER ABSORPTION WITH ALUMINA

	Plasma Treated before Dilution (%)	Plasma Treated after Dilution (%)
Normal 1	84	95
2	90	100
3	100	108
Abnormal 1	74	82
2	58	62
3	80	130
4	58	39
5	95	106
6	69	61
7	73	90
8	76	73
9	73	65
10	95	117
11	82	81
12	74	73
13	79	61
14	80	71

set out in Table II, show that good concordance between the two methods is obtained.

Summary

The difficulty of obtaining blood from small infants is discussed, and a method for obtaining citrated plasma by skin prick is described. The value of micro methods for tests of clotting function is discussed and it is concluded that the

standard tests should be used rather than the micro methods.

Modifications to the thromboplastin generation test are described.

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REFERENCES

- Biggs, R., and Douglas, A. S. (1953). *J. clin. Path.*, **6**, 23.
 ———, Eveling, J., and Richards, G. (1955). *Brit. J. Haemat.*, **1**, 20.
 ———, and Macfarlane, R. G. (1953). *Human Blood Coagulation and its Disorders*, 1st ed., p. 351. Blackwell Scientific Publications, Oxford.
 Bray, W. E., and Kelley, O. R. (1940). *Amer. J. clin. Path.*, **10**, 154.
 Creveld, S. van, Nagel, C. G. M., Nijenhuis, J. H., Miranda, S. I., and Tjon, Sien Kie (1954). *Ét. néo-natal*, **3**, 135.
 ———, Paulssen, M. M. P., Ens, J. C., and Meij, C. A. M. van der (1954). *Ibid.*, **3**, 53.
 Dreskin, O. H. (1952). *Amer. J. clin. Path.*, **22**, 140.
 Fantl, P., and Sawers, R. J. (1954). *Aust. Ann. Med.*, **3**, 245.
 Innes, J., and Davidson, L. S. P. (1941). *Brit. med. J.*, **1**, 621.
 Kato, K. (1940). *Amer. J. clin. Path.*, **10**, 147.
 King, E. J. (1951). *Micro-Analysis in Medical Biochemistry*, 2nd ed. J. & A. Churchill, London.
 Merskey, C. (1950). *J. clin. Path.*, **3**, 301.
 Owren, P. A., and Aas, K. (1951). *Scand. J. Lab. clin. Invest.*, **3**, 201.
 Quick, A. J. (1951). *The Physiology and Pathology of Haemostasis*. Kimpton, London.
 Stefanini, M. (1951). *Lancet*, **1**, 606.
 ———, and Dameshek, W. (1955). *The Haemorrhagic Disorders*. Grune & Stratton, New York.
 Wolf, P. (1953). *J. clin. Path.*, **6**, 34.
 Wright, A. E. (1893). *Brit. med. J.*, **2**, 223.