rapid guide to the serological behaviour of a particular extract or method of preparation. Typical results are shown in Table I. The results on fresh extracts are reproducible with an error of $\pm 10\%$, but deterioration is rapid at 20°C ., and is still noticeable at 4°C .; extracts stored at

TABLE I
Milk Clotting 'Units'

Papain Preparation	Milk Clotting 'Units'	
	Fresh Extract Alone	Extract + Serum 2:1
Stapleton and Moore	28	15
Papain-EDTA	53	30
Either, Seitz clarified	6	2
Papain-cysteine (Löw)	128	75
Seitz clarified	113	50

-20°C . lose activity more slowly. As might be expected papain-cysteine (Löw, 1955) is both the most active and the most stable preparation.

Serological tests confirmed the impression gained from the milk-clotting tests, and showed that papain-EDTA produced higher titration scores and more avid reactions than the 1% papain of Stapleton and Moore (1959). No difficulty was experienced with 'non-specific' reactions, nor did the presence of bovine albumin in concentrations of 7.5-15%, the range present in our antisera, result in any lack of specificity. Diluted 10 times in buffer-saline, papain-EDTA gave almost as high titration 'scores' as the undiluted product, whereas a prozone and a low titration 'score' occurred when papain 1% (Stapleton and Moore, 1959) was diluted three times. The stability of papain-EDTA at 20°C . was greater than that of the simpler extract, particularly in the presence of serum.

This new extract has now been in routine use in this service for several months with selected anti-D, -C+D, and -c sera. During this time no discrepancies have been noted between the latest results and those of former tests on the same persons. Preliminary studies have shown that papain-EDTA 1% also forms a satisfactory reagent for the detection of Rh antibodies by a modification of the two-stage technique of Lang and Lodge (1961).

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An improved plasma recalcified clotting test and its modification as a simple rapid heparin retarded clotting test

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Uncontrolled 'contact' activation and subsequent decay of activated plasma coagulation factors and variation in platelet substrate action during clotting are the two main factors which make the simple plasma calcium clotting time very variable (Margolis, 1957). Soulier (1959) found that as little as 1 mg. of bentonite resulted in rapid and complete activation of 1 ml. of plasma. Soya bean extract has been found to be a very satisfactory platelet substrate substitute both in the clotting of recalcified plasma and in thromboplastin generation tests (Connor and Carter, 1958; Hyun, Dawson, Butcher, and Custer, 1960).

It has been possible to develop a simple, accurate and reproducible plasma calcium clotting test, using optimum concentrations of soya bean extract and optimum amounts of bentonite. After exposure to bentonite fresh plasma is completely activated within three minutes, and by performing the test within the subsequent 10 minutes, activated factors do not decay. Since no tissue thromboplastin is added to this system, presumably mainly intrinsic thromboplastin is responsible for a normal plasma clotting time of between 34 and 43 seconds by this method. When heparin is added to the system in amounts sufficient to interfere with intrinsic thromboplastin generation, a simple rapid heparin retarded clotting test results, which takes less than three minutes to perform after the initial three minutes' activation of plasma.

PROCEDURE

REAGENTS The following are made up:—

Heparin solution Heparin solution (5 international units per ml.) is made up from a commercial preparation of initial strength of 5,000 units per ml. in 0.13 g. % parachlormeta-cresol in normal saline and kept at 4°C . (stable for at least one month).

Bentonite suspension Bentonite suspension (20 mg./ml. in normal saline) is stable at room temperature. Mix thoroughly before use. The working suspension is 2 mg./ml. in saline. Mix before use.

Soya bean extract Inosithin¹, 5 g. per 100 ml. in normal saline, is kept at -40°C . A working solution of 0.2 g. per 100 ml. saline is made up in a series of small tubes and kept frozen until required.

Heparin—bentonite working suspensions These are two, A and B.

- A 1 ml. stock bentonite suspension
- 1 ml. stock dilute heparin solution
- 8 ml. 0.85% saline.

¹The agents for Inosithin are V. A. Howe and Co. Ltd., 64 Pembridge Road, London, W.11.

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TABLE
RESULTS

Normal Values

<i>Plasma Recalcified Clotting Times (sec.)</i> (20 results)	<i>Recalcified Clotting Times (sec.)</i> + 0.5 i.u. Heparin (17 results)	<i>Recalcified Clotting Times (sec.)</i> + 1 i.u. Heparin (12 results)
37.9 ± 1 (range 34 to 43)	76.3 ± 6 (range 59 to 93)	124.3 ± 11 (range 103 to 156)
<i>Reproducibility in normal plasma (24 replicates)</i> 39.1 ± 1 (range 37 to 41)	80.9 ± 2 (range 74 to 98)	134.2 ± 5 (range 111 to 156)
<i>Reproducibility in Dindévan plasma (24 replicates)</i> 44.4 ± 1 (range 43 to 47)	107.5 ± 2 (range 103 to 118)	245.8 ± 12 (range 213 to 324)

This gives a final suspension of 2 mg. bentonite per ml. and a final heparin concentration of 0.5 i.u. per ml.

- B 1 ml. stock bentonite suspension
2 ml. stock heparin solution
7 ml. 0.85% saline.

This gives a final suspension of 2 mg. bentonite per ml. and a final heparin concentration of 1.0 i.u. per ml.

All blood samples are collected in siliconed syringes into polystyrene containers containing 1 part of 3.8% sodium citrate to 9 parts of whole blood.

TESTS Three tests were performed, the second and third being modifications of the first, basic procedure.

1 *Plasma recalcified clotting time, with optimal 'contact activation' and optimal platelet substrate substitute concentration* Bentonite suspension, 0.1 ml. (2 mg./ml.), and 0.1 ml. Soya bean extract (0.2 g.%) are placed in a 0.5 by 7.5 cm. tube at 37°C. for a few minutes. Add 0.2 ml. high-spun citrated plasma. Mix and leave to complete activation for three minutes. Recalcify with 0.2 ml. M/40

CaCl₂, reading the time of clotting in an EEL prothrombin meter, which reads to the nearest one second.

2 *As 1 but effect of 0.5 i.u. heparin/ml. added to bentonite* As 1 but substitute heparin-bentonite suspension A for plain bentonite suspension.

3 *As 1 but effect of 1.0 i.u. heparin/ml. added to bentonite* As 1 but substitute heparin-bentonite suspension B for the plain bentonite suspension.

I am grateful to Messrs. Bayer Products who generously purchased an EEL prothrombin meter for these studies.

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