NORMAL VALUES Urines were collected from 12 normal males. Daily output was 33, 49, 51, 56, 67, 68, 70, 72, 92, 115, 153, and 205 mg. (mean 86 mg./day).

It has been suggested (Jagenburg, 1959; Gartler, 1959) that the population is divided into 'high' and 'low' excretors, the latter being in the majority, and that this distinction is genetically determined. Our preliminary results do not entirely bear this out; further work will be needed to demonstrate the importance of other factors, *e.g.*, total nitrogen excretion and the role of the spleen (Hillcoat, 1962), and thus whether two separate groups can indeed be distinguished.

SUMMARY

Methods available for the estimation of B.A.I.B. in urine are discussed, and a method that is cheap, reliable, and quick is described.

I would like to thank Dr. H. E. M. Kay, Director of the Department of Clinical Pathology, for his help and encouragement during the preparation of this paper.

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A gel diffusion precipitin method for the estimation of C-reactive protein

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The precipitation of C-reactive protein with the non-specific somatic pneumococcal C-polysaccharide was first described by Tillett and Francis (1930). The inability to demonstrate the protein in health and its appearance during pregnancy and immediately after immunization has formed the basis of a useful but non-specific test with implications similar to, but not identical with, the erythrocyte sedimentation rate. C-reactive protein is an 'acute phase' protein associated with infections, rheumatoid arthritis, neoplasms, and other inflammatory and necrotic conditions. Estimates of the concentration of this protein have been used to measure the course of disease processes and their response to treatment.

Current methods for the detection and estimation of C-reactive protein utilize a specific antiserum generally raised in rabbits (MacLeod and Avery, 1941). The capillary precipitin method (Anderson and McCarty, 1950; Daguet, 1960) is the one in most common use; however, gel-diffusion precipitin (Fukuda, Heiskell, and Carpenter, 1959), complement-fixation (Muschel and Weatherwax, 1954; Rapport and Graf, 1956), and latex-fixation (Singer, Plotz, Pader, and Elster, 1957) techniques have also been developed. These latter methods have not found general application because of their technical difficulties and the length of time required for their performance.

The gel-diffusion precipitin method devised by Gell (1957) for γ globulin, siderophilin, and coeruloplasmin, and expanded by Soothill (1962), appeared to be adaptable for C-reactive protein and the possibility of this application has been investigated.

METHOD AND MATERIALS

ANTISERUM C-reactive protein antiserum Schieffelin (Scheiffelin and Co. New York, N.Y.) is a rabbit anti-human C-reactive protein antiserum, absorbed with whole human serum to ensure specificity.

STANDARD SERUM Serum from a case of rheumatoid arthritis with a high level of C-reactive protein (++++ capillary precipitin), hereafter treated as 100% C-reactive protein, was divided into 1 ml. portions and stored at −20°C.

BUFFERED AGAR One per cent Oxoid ion agar No. 2 in phosphate buffer *p*H 7·0 (50 ml. $NaH_2PO_4 \cdot 2H_2O$ 90 g./l., 200 ml. Na_2HPO_4 60 g./l., 250 ml. NaCl 8·5 g./l.).

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Eight millilitres of the buffered agar is poured into petri dishes of 80 mm. nominal diameter and when set a pattern of six large holes, 7·5 mm. in diameter, each surrounded by a regular hexagon of six smaller holes, 5·0 mm. in diameter, is cut using the special cutter described by Soothill (1962). The large holes are for the antigen, three concentrations of the standard, 1, ½, and ¼, and three for the unknown, 4, 1, and ¼. The small holes are for the antiserum, a 'logarithmic' series of dilutions of 1/7, 1/11, 1/16, 1/22, 1/29, and 1/37 of C-reactive protein antiserum (Scheiffelin) giving satisfactory precipitin lines. All of the dilutions are made in saline using a dropper pipette. After setting up the petri dishes are left overnight (about 16 hours) at room temperature and the results read the following morning by comparing the shape, size, and appearance of the precipitin line hexagons of the unknown with those of the standard serum. Concentrations of the unknown intermediate between those of the standards are estimated. A range of from greater than 400% to less than 3% of the standard C-reactive protein is thus covered by one plate. Figure 1 illustrates the arrangement of the antigen and antibody holes, and also a typical precipitin pattern for a positive serum.

The error of the method was estimated by replicate estimations of C-reactive protein at five different concentrations and calculation of the coefficient of variation for the results obtained at each concentration. Each set of estimations was performed on a separate day without prior knowledge of the previous results. The error of the method is the mean of these values, *i.e.*, 15% (Table I).

The sensitivity of the method is 1 drop of a 1/40 dilution of standard serum.

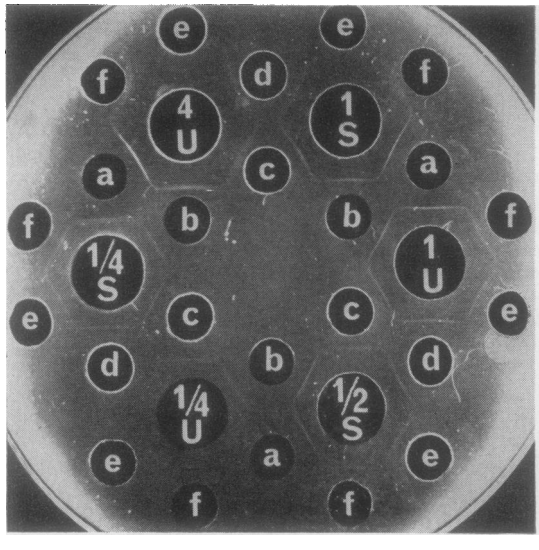

FIG. 1. *A gel-diffusion precipitin plate for the estimation of C-reactive protein. The standard serum is placed in the large holes labelled S in the dilutions shown and the unknown serum in the holes labelled U. The antiserum dilutions are placed in the small holes labelled a to f. In this plate 1 drop of the unknown produces a precipitin hexagon intermediate in size and shape between 1 and ½ of the standard*, i.e., *the unknown is equivalent to 75% of the standard.*

TABLE I

DETERMINATION OF PRECISION OF GEL-DIFFUSION PRECIPITIN METHOD

Serum	*Concentration of C-reactive Protein Found*					*Mean*	*Standard Deviation*	*Coefficient of Variation (%)*
	Test No.							
	1	*2*	*3*	*4*	*5*			
A	2	3	3	2	3	2·6	0·55	21
B	100	100	125	75	100	100	18	18
C	50	50	62	62	50	55	17·6	12
D	25	25	25	22	25	24	2·0	8·2
E	18	18	18	15	12	16	2·7	17

Mean coefficient of variation = 15%.
All estimations were performed on different days without knowledge of the previous findings. Values for concentration (and standard deviation) are expressed as percentages of that in the arbitrary standard (see text).

The method has been compared with the capillary precipitin method and with a latex slide test for C-reactive protein (the Hyland C.R. test). These results are summarized in Table II. It can be seen from these figures that the latex slide test gave the highest number of positive results and appears to be the most sensitive method, whereas the capillary precipitin method is the least sensitive but is roughly quantitative (Fig. 2). As the latex slide test was never negative when the gel-diffusion precipitin test was positive the slide test appeared to be a good screening method and has been used as such in subsequent studies, positive results being confirmed, and the concentration of the C-reactive protein estimated by the gel diffusion precipitin method.

TABLE II

COMPARISON OF RESULTS OF TESTING 265 SERA FOR C-REACTIVE PROTEIN BY LATEX SLIDE TEST, CAPILLARY PRECIPITIN METHOD, AND GEL-DIFFUSION PRECIPITIN METHOD

Method	*Number of Sera*	
	Positive	*Negative*
Latex slide test	82[1]	183
Capillary precipitin	53[2]	212
Gel diffusion precipitin	71	194

[1]Eleven of these results were positive by this method alone.
[2]Two of these results were negative by both of the other methods.

SUMMARY

A gel diffusion precipitin method with an error of 15% is described for the estimation of C-reactive protein. The

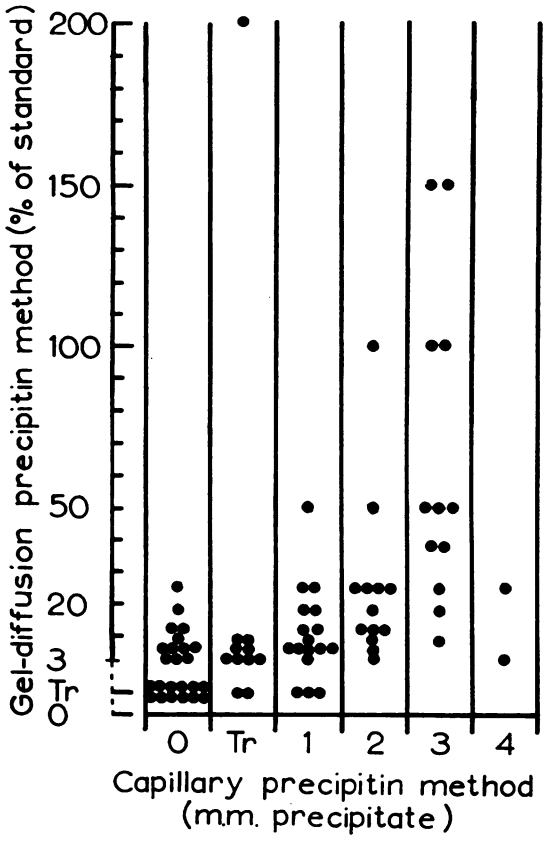

FIG. 2. *Comparison of the results of testing 79 C-reactive protein-positive sera by the capillary precipitin and gel-diffusion precipitin methods.*

method has been compared with the conventional capillary precipitin method and with a latex slide test and has been found to be intermediate in sensitivity.

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A method for the comparison of the antiplasmin activity of two groups of blood sera

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PRINCIPLE

The presence of antiplasmin activity in a blood sample can be shown by its ability to prolong the lysis time of a plasmin-enriched standardized fibrin clot, the extent of prolongation varying directly with the level of antiplasmin activity. Plasmin in such experiments is usually formed by the autoactivation of pure plasminogen, so that it is free from both activator and antiplasmin activity. Pure plasminogen is difficult to prepare and when obtained commercially is both expensive and unstable, so the following method has been devised for the measurement of antiplasmin activity using materials readily obtainable in any clinical laboratory.

Plasmin is produced by the streptokinase activation of the euglobulin fraction of normal serum. All serum plasminogen is precipitated in this fraction and the antiplasmins remain in the supernatant (Kowalski, Kopeč, and Niewiarowski, 1959; Flute, 1960). Epsilon-amino-caproic acid (E.A.C.A.) is added after activation to neutralize any remaining activator activity. As a source of fibrinogen for the clot, human plasma euglobulin is used. The clot is arranged to contain E.A.C.A. at a concentration of 10^{-3} molar. At this level it will inhibit further streptokinase activation of plasminogen contained in the plasma euglobulin without affecting the plasmin proteolysis of fibrin (Alkjaersig, Fletcher, and Sherry, 1959).

The actual tests are best carried out on serum, as the differing fibrinogen content of plasma samples may influence lysis times.

MATERIALS

1 PHOSPHATE BUFFER *p*H 7·4 Na_2HPO_4, 9·47 g., is dissolved in 1 litre of distilled water. To this is added 3·02 g. KH_2PO_4 dissolved in 250 ml. distilled water.

2 BOVINE THROMBIN (Leo Pharmaceutical Products) Dissolved in buffer to 50 N.I.H. units/ml.

3 STREPTOKINASE (Varidase, Lederle Laboratories) Dissolved in buffer, stable for at least one month when stored in aliquots at −30°C.

4 EPSILON-AMINO-CAPROIC ACID (Light and Co.) A 10^{-2} molar solution in buffer.

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