REFERENCES

Adams, D. H. & Thompson, R. H. S. (1948). *Biochem. J.* **42**, 170.
Barron, E. S. G., Bartlett, G. R. & Miller, Z. B. (1948). *J. exp. Med.* **87**, 489.
Berenblum, I. & Wormall, A. (1939). *Biochem. J.* **33**, 75.
Boursnell, J. C., Francis, G. E. & Wormall, A. (1946*a*). *Biochem. J.* **40**, 768.
Boursnell, J. C., Francis, G. E. & Wormall, A. (1946*b*). *Biochem. J.* **40**, 737.
Boyland, E. (1948). *Biochem. Soc. Symp.* **2**, 61.
Burnop, V. C. E., Richards, D. E., Watkins, W. M. & Wormall, A. (1951). *Nature, Lond.*, **168**, 251.
Burnop, V. C. E., Richards, D. E. & Wormall, A. (1952). To be published.
Butler, J. A. V., Gilbert, L. A. & Smith, K. A. (1950). *Nature, Lond.*, **165**, 714.
Butler, J. A. V. & Smith, K. A. (1950). *J. chem. Soc.* p. 3411.
Cole, S. W. (1933). *Practical Physiological Chemistry*, 9th ed. Cambridge: W. Heffer and Sons.
Dixon, M. & Needham, D. M. (1946). *Nature, Lond.*, **158**, 432.
Fleming, D. S., Moore, A. M. & Butler, G. C. (1949). *Biochem. J.* **45**, 546.
Fruton, J. S., Stein, W. H. & Bergmann, M. (1946). *J. org. Chem.* **11**, 559.
Fruton, J. S., Stein, W. H., Stahmann, M. A. & Golumbic, C. (1946). *J. org. Chem.* **11**, 571.
Gilman, A. & Philips, F. S. (1946). *Science*, **103**, 409.
Gjessing, E. C. & Chanutin, A. (1946). *Cancer Res.* **6**, 593.
Goldacre, R. J., Loveless, A. & Ross, W. C. J. (1949). *Nature, Lond.*, **163**, 667.
Gurin, S., Deluva, A. M. & Crandall, D. I. (1947). *J. org. Chem.* **12**, 606.
Haddow, A., Kon, G. A. R. & Ross, W. C. J. (1948). *Nature, Lond.*, **162**, 824.
Hartman, F. W., Mangun, G. H., Feely, N. & Jackson, E. (1949). *Proc. Soc. exp. Biol., N.Y.*, **70**, 248.
Holiday, E. R. (1942). By communication.
Hopkins, S. J. & Wormall, A. (1933). *Biochem. J.* **27**, 740.
Karnofsky, D. A. (1950). A chapter in *Advances in Internal Medicine*, vol. 4, ed. by Dock, W. & Snapper, I. Chicago: The Year Book Publishers.
Kidd, J. G. & Landsteiner, K. (1944). By communication.
Landsteiner, K. (1936). *New Engl. J. Med.* **215**, 1199.
Landsteiner, K. (1945). *The Specificity of Serological Reactions*, revised ed. Cambridge, Mass.: Harvard University Press.
Loveless, A. (1951). *Nature, Lond.*, **167**, 338.
Loveless, A. & Revell, S. (1949). *Nature, Lond.*, **164**, 938.
Markham, R. (1942). *Biochem. J.* **36**, 790.
Moore, A. M. (1944). By communication.
Moore, A. M. & Rockman, J. B. (1950). *Canad. J. Res.* (E), **28**, 169.
Philips, F. S. (1950). *Pharmacol. Rev.* **2**, 281.
Proom, H. (1943). *J. Path. Bact.* **55**, 419.
Thompson, R. H. S. (1947). *J. Physiol.* **105**, 370.
Van Slyke, D. D. (1912). *J. biol. Chem.* **12**, 275.
Watkins, W. M. & Wormall, A. (1948). *Nature, Lond.*, **162**, 535.
Watkins, W. M. & Wormall, A. (1952). *Biochem. J.* (in the Press).

The Determination of Plasma Fibrinogen by the Clot-Weight Method

By G. I. C. INGRAM
Department of Surgery, University of Edinburgh

(*Received* 20 *November* 1951)

The clot-weight technique is a convenient method for the determination of plasma fibrinogen. The result is obtained as fibrin with sufficient accuracy for clinical purposes. Nevertheless, certain possible causes of error appear not to have received attention and these have been investigated.

METHODS

Plasma. 9 vol. of human blood are received from a paraffined syringe into 1 vol. of sodium citrate solution containing 3·8 g./100 ml. and the mixture is centrifuged at 2000 rev./min. for 30 min.

Technique of fibrinogen determination. The citrated plasma is diluted with an approximately equal vol. of 0·025 M-$CaCl_2$ solution and allowed to stand at 37°. The fibrin is wound off on to a wooden applicator until no further clot forms, washed in distilled water, rolled off the rod, drained between filter papers, pulled out thin (when of sufficient bulk), dehydrated first in acetone and then in air at 105° for 3 hr., and weighed.

From the haematocrit of the citrated blood the calculation is made as follows:

$$\text{Plasma fibrinogen content} = \frac{\text{clot weight}}{\text{sample volume}} \times \frac{\text{volume of citrated plasma in 10 ml. citrated blood sample (from haematocrit)}}{\text{volume of citrated plasma} - \text{volume of citrate}}.$$

This correction ignores the variable effect of the citrate upon the cell-plasma fluid partition, but error from this cause is thought to be small compared with differences between persons.

EXPERIMENTAL

Formal experiments were made to determine the effect of certain variables in technique which in practice it may not always be possible to standardize.

Effect of sample storage. It may not always be possible to make determinations on fresh samples. Beginning, therefore, 3 hr. after the blood was drawn, triplicate determinations were made on 8 successive days (except the fifth) on 5·0 ml. samples from a quantity of plasma stored in glass at about 4°.

The data are shown in Table 1. Analysis of the results showed a significant linear regression increasing on days over the 8 days, but as no effect was apparent until the fourth day after venepuncture, storage errors would appear to be seldom of practical consequence.

Table 1. *Effect of storage on clot weight*

(Weights of clots from 5·0 ml. portions of a sample of plasma stored at 4° and tested 3 hr. after withdrawal (day 0) and on subsequent days for 1 week.)

	Replicate clot weights (mg.)			
Day	1	2	3	Mean
0	14·0	14·0	14·5	14·17
1	14·1	14·0	13·8	13·97
2	14·5	—	14·3	14·37
3	14·3	13·9	14·4	14·20
4	14·7	14·5	14·4	14·53
6	14·7	14·5	14·4	14·53
7	14·4	14·2	14·6	14·40

Effect of sample volume. From one sample of plasma, three replicate determinations were made on subsamples of 5·0, 4·0, 3·0, 2·0 and 1·0 ml. In eight clinical determinations replicates had also been made on unequal volumes.

The data from the formal experiment are shown in Table 2. Comparative analyses were made on the two groups of results. The analysis of the fifteen experimental determinations showed a significant

Table 2. *Effect of sample volume on clot weight*

	Replicate fibrin determinations (mg. fibrin/ml. plasma)			
Sample vol. (ml.)	1	2	3	Mean
5·0	2·08	2·18	2·16	2·14
4·0	2·18	2·15	2·25	2·19
3·0	2·20	2·20	2·20	2·20
2·0	2·25	2·25	2·25	2·25
1·0	2·20	2·20	2·30	2·23

negative linear regression on volume, equivalent to an addition of about 1·3 % to the clot weight for each ml. decrease in volume below 5·0 ml. A similar (but non-significant) association was observed in the eight field determinations, differing from subject to subject, but equivalent, on the average, to an addition of 3·6 % wt./ml. decrease in volume.

Comparison of recalcification with added thrombin for fibrin isolation. As it is theoretically possible that sufficient thrombin to clot its fibrinogen content might not be available from a given sample of plasma, it might be preferable to cause clotting by the addition of thrombin (Jones & Smith, 1930). Nineteen parallel determinations were therefore made on fifteen plasma samples (none from patients known to be suffering from one of the haemorrhagic diseases) by the recalcification technique given above and by diluting with 2 or 3 vol. of saline and then adding thrombin solution until no further clot formed.

Table 3. *Comparison of fibrinogen concentration determined with thrombin and by recalcification*

	Fibrinogen concentration (g./100 ml.)	
Plasma samples	Fibrin obtained by adding thrombin	Fibrin obtained by recalcification
1	0·37	0·37
2	0·74	0·67
3	0·44, 0·45, 0·42	0·46, 0·46, 0·41
4	0·63	0·60
5	0·38	0·36
6	0·37	0·41
7	0·57	0·49
8	0·61, 0·61	0·64, 0·59
9	0·36	0·32
10	0·48, 0·45	0·59, 0·48
11	0·48	0·39
12	0·43	0·46
13	0·29	0·39
14	0·48	0·55
15	0·59	0·59

The data are shown in Table 3. The range of values obtained on the fifteen samples was 0·29–0·74 g./100 ml. and inspection shows no systematic difference between the series at high or at low levels. The mean difference between the two series was 0·006 g./100 ml. in favour of thrombin; $t = 0·042$ ($P > 0·9$). The two methods are clearly equivalent on the basis of this experience.

Effect of dilution before recalcifying. It is usually recommended that the plasma be diluted with 10 or 20 vol. of saline before recalcifying, but this might lead to significant loss from the solubility of fibrin in physiological salt solutions. Six 5·0 ml. replicates were determined by the described procedure in parallel with six similar replicates from the same sample diluted to 45 ml. with 0·9 % (w/v) sodium chloride and then recalcified with 5·0 ml. 0·025 M-calcium chloride.

The data are shown in Table 4. The mean clot weight of the six diluted replicates was 13·73 mg. and of the six undiluted replicates, 14·03 mg.; the difference in means was $0·30 \pm 0·11$, which is significant ($0·02 < P < 0·05$). The lower, diluted value, a decrease of about 2 % on the undiluted value,

suggests that a little fibrin was lost by solution; the described procedure was therefore preferred: in this also, the manipulation of fibrin was found to be easier in the smaller volume.

Table 4. *Effect of dilution on clot weight*

(Weights of clots from twelve 5·0 ml. portions of one sample of plasma: in six instances the recalcified mixture was diluted five times.)

Replicates	Clot weight (mg.) Diluted	Undiluted
1	14·1	14·1
2	13·9	14·0
3	13·6	13·8
4	13·6	14·1
5	13·8	14·1
6	13·4	14·1
Mean	**13·73**	**14·03**

Ash weight of clot. To exclude systematic error from mineral content (Foster & Whipple, 1922), two groups of three clots from 5·0 ml. normal plasma samples were pooled and weighed. Thereafter they were ashed and re-weighed. In neither instance did the combined weight of ash from the pooled clots exceed the limit of balance error (± 0·1 mg.).

DISCUSSION

Effect of sample volume. The inverse relation of fibrinogen concentration to the plasma sample volume is curious. It is interesting that a similar inverse relation is apparent in the fibrin dry-weight data of Foster & Whipple (1922) and of Howe (1923), though in neither report does it receive comment; in Howe's data the magnitude of the effect is very small. The error, about 5% or less per ml. below 5 ml., is obviously small compared with differences expected between persons, if approximately the same volume of plasma, 3–4 ml., is always used.

Effect of sample dilution. The findings suggest that plasma samples should not be much diluted at recalcification, although again the error, a loss of 2% on clot weight with fivefold dilution of the reaction mixture, is small compared with differences to be expected between persons.

SUMMARY

1. Daily determinations on a stored plasma sample showed a significant increase of clot weight from the fourth day after withdrawal.

2. There is an inverse relation between sample size and determined fibrinogen concentration.

3. Clot weights obtained by recalcification agreed well with those obtained by adding thrombin.

4. Dilution of the sample at recalcification reduced the weight of clot obtained.

5. The ash weight of the clot was negligible.

The author is indebted to the Medical Research Council for a grant. It is a pleasure to acknowledge the assistance of Dr P. Armitage of the Medical Research Council Statistical Research Unit, London School of Hygiene and Tropical Medicine, in the analyses of the numerical data.

REFERENCES

Foster, D. P. & Whipple, G. H. (1922). *Amer. J. Physiol.* **58**, 365.

Howe, P. E. (1923). *J. biol. Chem.* **57**, 235.

Jones, T. B. & Smith, H. P. (1930). *Amer. J. Physiol.* **94**, 144.

Bacterial Arylsulphatase

By J. E. M. WHITEHEAD

Department of Bacteriology, St Thomas's Hospital Medical School, London, S.E. 1

and A. R. MORRISON and L. YOUNG

Department of Biochemistry, St Thomas's Hospital Medical School, London, S.E. 1

(*Received* 31 *December* 1951)

It is known that in plants and animals there occur enzymes which catalyse the hydrolysis of sulphuric acid esters. These enzymes, the sulphatases, have recently been reviewed by Fromageot (1950). The sulphatases differ with respect to the type of sulphuric acid ester upon which they act, and they include arylsulphatase (phenolsulphatase), which hydrolyses arylsulphuric acids. The existence of arylsulphatase was first demonstrated by Derrien (1911) who observed that extracts of the shell fish, *Murex trunculus*, hydrolyse potassium indoxyl sulphate. An enzyme with similar activity was later found to be present in *Aspergillus oryzae* by Neuberg & Kurono (1923), and when evidence became available concerning its specificity it was named 'phenolsulphatase' (Neuberg & Simon, 1932).