The saline added in the blood-citrate-saline mixture alters the final osmotic pressure of the original dilutions and this must be allowed for in plotting the graph, as shown in Table II.

DISCUSSION

The micromethod described has shown an acceptable degree of accuracy and is useful for the detection of abnormal red cell fragility in children.

The buffers recommended by Dacie (1956) have not been used in these experiments; instead the pH of the distilled water and saline used have been checked, as recommended by Suess *et al.* (1948). While 0.1 ml. of capillary blood has been used in these experiments, slight variations in this volume should not modify the results, and this has in fact been found to be the case.

My thanks are due to Dr. A. A. Sharp for advice and encouragement, and to Mr. G. J. Draper of the Department of Biometry, University of Oxford, for checking the statistical analysis.

REFERENCES

- Creed, E. ff. (1938). *J. Path. Bact.*, **46**, 331.
 Suess, J., Limentani, D., Dameshek, W., and Dolloff, M. J. (1948). *Blood*, **3**, 1290.
 Dacie, J. V. (1956). *Practical Haematology*, Churchill, London.

LOCUM BUREAU

The locum bureau provides useful work for pathologists who have retired or who are temporarily out of work.

Anyone interested in being a locum or in employing a locum should get in touch with:

Dr. Anne Gibson,
 The South London Hospital,
 Clapham,
 London, S.W.4.
 Tel. no. KELvin 1221.

A simple method for the use of water melon seed preparations in the estimation of blood urea

K. A. KHALEQUE, M. G. MUAZZAM, and P. ISPAHANI
From Dacca Medical College, Pakistan

Soya and jack beans are commonly used for the preparation of urease in the estimation of blood urea but Damodaran and Sivaramakrishnan (1937) demonstrated that the seeds of water melon (*Citrullus vulgaris*) had a high urease content. The raw water melon seeds without any treatment have been used as a source of urease in this work. The potency of urease was high in all healthy seeds as compared with Merck urease. Both these preparations of urease gave results with standard urea solutions and blood, which were within the range of error as advocated by King and Wootton (1956).

The presence of urease in the soya bean (Soja) was demonstrated as early as 1909. Annett (1914) found the high urease potency in the family *Papilionaceae* including jack bean and horse gram (*Dolichos biflorus*) but the activity of the latter deteriorated rapidly on storage. Klein (1933) demonstrated the presence of urease within the family *Cucurbitaceae*. Damodaran and Sivaramakrishnan (1937) concluded that the urease from the seeds of the water melon are superior to those from the soya and jack beans as it is free from the errors that pertain to the latter ones.

MATERIALS AND METHODS

PREPARATION OF UREASE FROM WATER MELON (*CITRULLUS VULGARIS*) SEEDS. The seeds which did not look healthy were discarded. The healthy seeds were washed in tap water to remove the sticky juice of the fruit from the surface and then dried in the air in the shade. They were then kept at room temperature. For use, seeds were cut in the longitudinal axis with a fine knife along the borders. Three preparations were made:—

- 1 Half of the pulp of a seed chopped into fine particles;
- 2 a number of pulps cut into fine particles and preserved at room temperature;
- 3 a number of pulps cut into fine particles and preserved in the refrigerator.

UREASE (MERCK, E) This is manufactured from jack bean meal. The reagents and method of urea estimation were the same as described by King and Wootton (1956) except for the Nessler reagent which was made up by the method of Bock and Benedict (Harrison, 1957).

RESULTS AND DISCUSSION

The common mode of preparation (Van Slyke and Cullen, 1914) of urease requires a large quantity of acetone.

Received for publication 4 June 1963.

Damodaran and Sivaramakrishnan (1937) used petroleum in the preparation of urease from water melon seeds but the technique has not been popular in routine laboratories. Water melon seeds were used without any treatment in this laboratory and consequently preparation has been very simple without any extra expenditure.

To study the potency of seeds, 200 analyses of known urea solutions (100, 200, and 300 mg. per 100 ml.) were carried out with random melon seed preparation (1), i.e., with half of a seed.

In Fig. 1A, results of 20 investigations with each urea solution are recorded as the percentage deviation, which was 4% or less. It can safely be concluded that the potency of urease in different seeds did not vary to a great extent. It may also be noted in Fig. 1B that there was a variation of 4.8% or less with Merck urease.

FIG. 1A and 1B. Results of investigations with each solution of urea.

Analyses of 250 blood samples were carried out with water melon seed preparation 3 in parallel with Merck urease. The results ranged from 18 to 366 mg. of urea per 100 ml. Of these, identical results were obtained in 50 and different results in 200 samples. The Merck urease gave higher results in 100 samples, ranging from 6.6 to 8%, and a lower result in 100 samples, ranging from 8.5 to 0.9%.

Analyses with water melon seed preparations 2 and 3 and preparation 1 preserved for a month and Merck urease was carried out on five random samples of blood

on the first, eighth, fifteenth, twenty-second, and thirtieth day of the month. The differences between various results were within 6%. Similar analyses were carried out using standard urea solutions. The variation was within 8%. From these results it may be concluded that the water melon seed preparations can be preserved for about a month for analysis, but on prolonged storage they became contaminated, usually with fungi, more so at room temperature than in the refrigerator.

Water melon seed preparations 1 and 3 prepared each month from preserved seeds and Merck urease were used for analysis of standard urea solutions (100 mg. per 100 ml.) for a period of 20 months. Each month 20 investigations were carried out. The variation was never more than 7%.

Parallel tests were done with preserved seeds of the previous year, with fresh seeds, and with Merck urease against 40 samples of standard urea solutions (20 samples of 100 mg., 10 samples of 200 mg., and 10 samples of 300 mg. per 100 ml.) and 50 blood samples. The difference between these findings was not more than 3.5 mg. %, and the variation in the tests with Merck urease was within 0 to 5%. It may be concluded from these results that the urease activity in the seeds preserved at room temperature remains potent for at least 20 months. As preparation 2 becomes contaminated, the seeds should be preserved at room temperature and preparation 3 used for routine work.

It may be of interest to note that the husks from which the inner coatings have been removed possess no urease activity. Urease from the inner coating possesses useful potency.

More than 2,000 samples of human blood from patients have been analysed with preparation 3. All abnormal values in more than 1,500 samples were consistent with the clinical diagnosis.

SUMMARY

A simple, reliable, and quick method of preparing urease from water melon seed is described. The method is convenient and cheap and may be carried out in all laboratories. The potency of urease is high in all healthy seeds. Water melon seed preparations have been used for 20 months though water melons are available for only a few months in the year.

REFERENCES

- Annett, H. E. (1914). *Biochem. J.*, **8**, 449.
- Damodaran, M., and Sivaramakrishnan, P. M. (1937). *Ibid.*, **31**, 104.
- Harrison, G. A. (1957). *Chemical Methods in Clinical Medicine*, 4th. ed. Churchill, London.
- King, E. J., and Wootton, I. D. P. (1956). *Micro-Analysis in Medical Biochemistry*, 3rd, ed. Churchill, London.
- Klein, G. (1933). *Handbuch der pflanzenanalyse*, vol. 4, pt. 2, p. 889. Springer, Vienna.
- Van Slyke, D. D., and Cullen, G. E. (1914). *J. biol. Chem.*, **19**, 211.