Technical Methods

A micromethod for estimating osmotic fragility of erythrocytes

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The conventional methods described for estimating osmotic fragility of red cells (Creed, 1938; Suess, Limentani, Dameshek, and Dolloff, 1948) require about 0.5 ml. of venous blood. Since this investigation is often required in the investigation of anaemia in infants a method has been developed which uses a volume of capillary blood which can be easily obtained from a heel prick.

METHOD

Capillary blood, 0.1 ml., is mixed with 2.5 ml. of citrate saline (1 part of 3.8% sodium citrate to 9 parts of 0.9% NaCl, pH 7.0). Tubes are prepared containing 3 ml. of the following concentrations of NaCl:—0.1%, 0.20%, 0.30%, 0.35%, 0.40%, 0.45%, 0.50%, 0.55%, 0.65%, 0.75%, 0.85%. To each of these dilutions is added 0.2 ml. of the blood-citrate-saline mixture. The tubes are allowed to stand at room temperature for 30 minutes, and are then agitated and centrifuged at 2,000 r.p.m. for 10 minutes. The contents of each tube are then decanted into a 1 cm. cuvette, and the haemoglobin in the supernatant measured in a spectrophotometer at 580 mμ.

RESULTS

The osmotic fragility of the red cells was measured using blood derived from three patients with congenital spherocytosis, two with acute acquired haemolytic anaemia, and 40 patients with no obvious spherocytes in

their blood (Table I). The means and standard deviations for each concentration in the 'normal' series have been calculated and the normal ranges ($\bar{x} \pm 2\sigma$) are shown together with the abnormal curves, in Figure 1.

TABLE I

MEDIAN FRAGILITY (50% HAEMOLYSIS)											
40 Patients without Spherocytosis				Two Patients with Acquired Haemolytic Anaemia				Three Patients with Congenital Spherocytosis			
0.46	0.45	0.46	0.41	0.50	0.51			0.54	0.55	0.60	0.69
0.42	0.46	0.48	0.45								
0.46	0.46	0.45	0.47								
0.43	0.48	0.45	0.44								
0.44	0.46	0.45	0.42								
0.45	0.43	0.41	0.46								
0.42	0.47	0.46	0.46								
0.45	0.43	0.43	0.46								
0.41	0.49	0.43	0.42								
0.45	0.46	0.45	0.45								

TABLE II

MEAN PERCENTAGE OF HAEMOLYSIS AND STANDARD DEVIATIONS FOR VARIOUS STRENGTHS OF SALINE IN 40 PATIENTS WITHOUT SPHEROCYTES

% Saline	Mean % Haemolysis	S.D.
A 0.150	100.0	—
B 0.245	99.4	1.1
C 0.338	96.0	3.0
D 0.384	89.6	6.4
E 0.430	63.9	16.5
F 0.479	28.9	17.4
G 0.526	5.0	5.7
H 0.572	0.3	0.6
I 0.620	0.0	0.0
J 0.665	0.0	0.0
K 0.760	0.0	0.0
L 0.851	0.0	0.0

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.

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

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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.

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