CCLXII. THE SODIUM CONTENT OF HUMAN ERYTHROCYTES

By MURIEL GRACE CRABTREE AND MONTAGUE MAIZELS

*From the Department of Clinical Pathology,
University College Hospital*

(Received 11 October 1937)

THE sodium contents of human erythrocytes recorded by various authors differ widely. Oberst [1935] gives an average of 12 m. eq. per l. of cells; Maizels [1936] also gives 12 m. eq., whilst according to Hald & Eisenman [1937] the average sodium content is 16 m. eq. McCance, on the other hand, in a recent paper [1937] gives considerably higher figures, ranging between 17 and 35 m. eq. Since calcium is absent from human erythrocytes and magnesium is present only in small amounts, cell sodium may be determined indirectly from the difference between total cell base and potassium. This indirect method of sodium estimation has been used by the present writers in order to determine which of the above ranges of sodium contents is most probably correct.

Method

Heparinized blood is centrifuged for 20 min. at 4000 r.p.m. in small tubes of about 1.5 mm. bore, sealed at one end. These tubes hold 0.1 ml. and are accurately graduated in 0.005 ml. divisions. The supernatant plasma and upper layers of packed cells are removed with a capillary pipette and the tubes again spun for 5 min. The volume of cells is then read, any residual plasma washed away and the cells analysed. Total base is estimated by the method of Adair & Keys [1934]; the error for 0.05 ml. is not greater than $\pm 2\%$. Potassium is measured by the method of Kramer & Tisdall [1921] slightly modified [Maizels, 1935], the error for 0.1 ml. cells being less than $\pm 2\%$. It will be realized that the difference values so obtained are not an accurate measure of cell sodium, but they may be employed to define the actual range of sodium content. The figures in Table I are derived from patients with normal blood counts.

Table I

Age years	Disease	Total base m. eq. per l. cells	Potassium m. eq. per l. cells	Difference m. eq. per l. cells
58	Arteriosclerosis	114.0 $\pm$ 1.25	102.5 $\pm$ 1.0	11.5 $\pm$ 2.25
26	Arthritis	108.5 $\pm$ 0.5	98.0 $\pm$ 0.0	10.5 $\pm$ 0.5
29	"	116.0 $\pm$ 0.0	102.5 $\pm$ 1.5	13.5 $\pm$ 1.5
58	Aneurysm	116.5 $\pm$ 1.0	97.5 $\pm$ 2.0	19.0 $\pm$ 3.0
24	Normal	116.0 $\pm$ 1.0	103.5 $\pm$ 1.5	12.5 $\pm$ 2.5
43	"	119.0 $\pm$ 1.5	105.0 $\pm$ 0.5	14.0 $\pm$ 2.0
23	"	104.0 $\pm$ 0.0	94.0 $\pm$ 0.0	10.0 $\pm$ 0.0

It will be seen that these figures for sodium confirm those of Oberst, Maizels and Hald & Eisenman. They are a good deal lower than McCance's. The first two writers have made their observations on packed centrifuged cells; the second pair have estimated cell sodium indirectly from the plasma and whole blood sodium contents and the red blood cell volume determined by haematocrit. In

both these methods the figure ultimately obtained is the sodium content of packed cells plus the sodium content of the intercellular plasma. Maizels has shown [1936] that the latter amounts to 3% by volume and we have confirmed this by suspending erythrocytes in saline containing purified haemoglobin (9%), centrifuging for 20 min. and washing away all the supernatant haemoglobin solution. The packed cells are then suspended in 2 ml. 0.9% NaCl and again centrifuged. The intercellular haemoglobin saline solution is washed out by the second suspension and after recentrifuging remains in the supernatant NaCl solution where it may be estimated colorimetrically. This method gives 3.05% intercellular haemoglobin saline solution. It therefore follows that the estimation of cell sodium by any method involves an error due to the presence of sodium in intercellular plasma amounting to about 4 m. eq., since plasma contains about 140 m. eq. sodium per l., and that true cell sodium is about 8 m. eq. per l.; this is the same as Oberst's figure obtained on cells washed with plasma from which all sodium had been removed by dialysis and replaced by potassium.

The indirect method of cell sodium estimation employed by Hald & Eisenman and by McCance has a special disadvantage in that it requires a very high degree of accuracy. If V is the percentage volume of cells relative to whole blood, then

$$\text{Blood Na} - \text{plasma Na} \times (100 - V)/100 = \text{cell Na} \times V/100.$$

The right-hand expression is relatively very small and is the difference of the two large values on the left-hand side of the equation, so that errors of $\pm 2\%$ in blood Na, plasma Na and V summate to $\pm 50\%$ in cell Na. Nevertheless although the method may be expected to give wide variations, the average figure of a large series should conform with those of other writers and this is the case with the data of Hald & Eisenman, but not with the consistently high values recorded by McCance. The reason for this may be as follows: Hald & Eisenman have estimated blood and serum sodium and cell volume on defibrinated blood and their data agree, but McCance has estimated whole blood Na on oxalated blood, serum Na in the fluid separated from clotted blood and cell volume on heparinized blood, so that his data are obtained under different physical conditions. If, during the process of clotting, cells were slightly compressed and lost fluid, serum Na would be too low and cell Na would appear too high. A shrinkage in cell volume of only 5% as compared with the cell volume in heparinized blood would explain McCance's high values.

SUMMARY

Direct measurement of cell sodium gives an average value of 12 m. eq. per l.; indirect determination from the difference between total cell base and potassium gives an average value of 13 m. eq.; or 11 m. eq. if an allowance of 2 m. eq. for magnesium is deducted. Of this, 4 m. eq. of sodium are contributed by sodium in the intercellular plasma, so that the true cell sodium averages about 7 m. eq. per l.

REFERENCES

- Adair & Keys (1934). *J. Physiol.* **81**, 162.
- Hald & Eisenman (1937). *J. biol. Chem.* **118**, 275.
- Kramer & Tisdall (1921). *J. biol. Chem.* **46**, 339.
- McCance (1937). *Biochem. J.* **31**, 1278.
- Maizels (1935). *Biochem. J.* **29**, 1970.
- (1936). *Biochem. J.* **30**, 821.
- Oberst (1935). *J. biol. Chem.* **108**, 153.