CLX. THE CHANGES IN THE PLASMA AND CELLS DURING EXPERIMENTAL HUMAN SALT DEFICIENCY

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THE inorganic elements of the blood serum have been extensively studied in recent years and it has been shown that during health the concentration of sodium (and indeed of all the bases) is maintained within narrow limits. The concentration of chloride tends to vary inversely with that of bicarbonate, so that the sum of the two remains almost as constant as the concentration of sodium. In trying to produce a state of salt deficiency in normal men, a deliberate attempt was made to alter the composition of these stable body fluids, and the present paper describes the immediate results. No previous work appears to have been done on the serum or on the red blood corpuscles (R.B.C.) in experimentally produced human salt deficiency, but studies have been made with animals and also with patients suffering from diseases incorporating this syndrome. The general changes in the serum are not disputed, but, although there have been numerous investigations, there is still considerable controversy over the behaviour of the R.B.C. A study of the literature indicates that (1) the technical difficulties involved in the withdrawal and analysis of blood are very considerable; (2) there are undoubtedly species differences in the ways in which the R.B.C. react to changes in their saline environment; (3) there may be considerable differences in the ways the R.B.C. react *in vivo* and *in vitro*; (4) in spite of making allowances for (1), (2) and (3), there are still unexplainable discrepancies between the results of different (and even of the same) investigators. The problem is clearly a very intricate one.

PREVIOUS INVESTIGATIONS

Three main questions have occupied people's minds:

- (1) Do the R.B.C. behave as simple osmometers so far as volume is concerned when their environmental osmotic pressure (O.P.) is altered?

According to Peters [1935] and other workers at Yale the R.B.C. probably do behave in this way both *in vitro* and *in vivo* [Eisenman *et al.* 1937]. Darrow & Yannet [1935] working on dogs, rabbits and monkeys found that NaCl deficiency produced by intraperitoneal glucose led to a fall in concentration of proteins in the R.B.C. of about the magnitude to be expected from osmotic considerations. Working on dogs, the same authors [1936] drew similar conclusions from their experimental results. Their figures, however, show that while this may have been true after removing NaCl, no reverse changes took place on restoring NaCl to the circulation. These reverse changes should have occurred according to their theory. Bellis & Scott's [1935] results suggest that the cells, at any rate of the ox, may lose protein when subjected to environmental alterations of O.P., so that the determination of cell protein does not seem such a

reliable guide to cell size as the determination of haemoglobin. Some of Darrow & Yannet's findings may be due to this. Harrop [1936] concluded from his work on dogs that a fall in plasma Na and Cl led to a rise in the mean corpuscular volume, i.e. a fall in the concentration of haemoglobin in the R.B.C. A study of his figures for haemoglobin and cell volume, however, particularly those of his "glucose" experiments, suggests that this conclusion was not justified. The work of Swingle *et al.* [1936] on dogs provides no evidence that salt deficiency produces a fall in the concentration of haemoglobin in the R.B.C. The same may be said of Herrin's [1935] experiments in which the salt deficiency was produced in quite a different way, namely by the continuous removal of succus entericus. The balance of evidence would seem to be that dogs' R.B.C. do not behave as simple osmometers *in vivo*. Rabbits' R.B.C. have been extensively investigated *in vitro* by Ponder and his associates [for references *v.* Ponder & Robinson, 1934], and it is clear from their work that the conditions are very complicated and that even *in vitro* the cells may not behave as true osmometers.

- (2) Can base be transferred across the corpuscular membrane in response to a change in concentration of base outside the cells?

This has been frequently investigated, and there is a general consensus of opinion that dog and ox R.B.C., which contain much more Na than K, may alter their Na content to conform with the O.P. of the surrounding medium [Davson, 1934; Yannet *et al.* 1935-36]. There is therefore no difficulty in explaining why dog cells remain the same size *in vivo*, and it is indeed rather surprising that they should have been found by some observers to alter their size. Rabbit and monkey R.B.C., which contain much more K than Na, were considered by Yannet *et al.* to be much less permeable to base than dog R.B.C. Nevertheless Ponder & Robinson [1934] and Henriques & Orskov [1936] considered rabbits' R.B.C. to be permeable to K under appropriate conditions. Eisenman *et al.* [1937], moreover, found that although human R.B.C. appeared to act as perfect osmometers *in vivo* so far as volume was concerned, yet base was transferred across their membranes in a wholly capricious manner and in one not dictated by osmotic considerations. There is in short considerable evidence that base may cross the membranes of the R.B.C., but the ease with which it does so appears to vary with the species and the ionic constitution of the cells.

- (3) Does chloride pass across the cell membranes in response to a rise or fall in plasma NaCl?

Very few attempts have been made to answer this question experimentally. Indeed, the well-known fact that Cl can pass in and out of cells without passage of base in response to changes of O₂ tension and pH, seems to have led scientific opinion to attach too little importance to changes in cell Cl brought about by other environmental changes. Yannet *et al.* [1935-36] concluded from their experiments that changes in cell base would be accompanied by corresponding changes in the sum of the cell Cl and HCO₃, and this is what one would expect. More clinical work has been done on the Cl ion than on any other, and there is no doubt that the cell Cl falls when the serum Cl falls [Vignes & Levy, 1934; Chabanier *et al.* 1934]. One of the most interesting cases may be quoted. Bernard *et al.* [1929] found the plasma Cl in one patient to be 216 mg./100 ml. and the HCO₃ 25.2 vol. %. The cell Cl at this time was 142 mg./100 ml. Saline was administered and after a slow recovery which was carefully followed stage by stage the plasma Cl was found to be 379 mg./100 ml. and the HCO₃ 58 vol. %. The cell Cl had now risen to 206 mg./100 ml. It is certain that the cell HCO₃ rose

with the plasma HCO_3 , and therefore these results are very strong indirect evidence that a large increase in the concentration of cell base had also taken place. It is true that unless simultaneous measurements of haemoglobin had been made, changes in the concentration of cell ions, even of this magnitude, do not prove that passage of inorganic ions took place across the cell membranes—but they strongly suggest it.

PRESENT INVESTIGATION

Blood was taken four times from each of five subjects, once during the preliminary control period, twice when they were salt-deficient and once after the main experiments had been concluded. From four of these subjects the bloods during deficiency were taken on successive days, but in R.M.L.'s experiment on the morning and evening of the same day. The last blood was generally collected 5–8 days after the subject had begun to take NaCl once more. From one subject, W. M. G., who could only be made partially deficient, two comparable blood samples were taken. The salt deficiency was produced by diet and sweating [McCance, 1936, 1, 2]. Blood was taken from a vein in the arm without constriction. One portion was collected anaerobically under paraffin in a centrifuge tube with sodium oxalate as anticoagulant. The plasma from this was used for Cl and HCO_3 determinations. A second small test-tube was almost filled without anaerobic precautions, using ammonium oxalate to prevent coagulation. This tube was corked up at once and the contents used for all the whole blood determinations and haemoglobin, and in the first two subjects for cell volumes. A third portion was allowed to clot in a stoppered tube, the serum separated and used for the determination of proteins, Na and K , while in the last four subjects a fourth portion was collected anaerobically under paraffin with heparin as anticoagulant and used for the cell volumes. Serum proteins were determined on 0.5 ml. samples as described by McCance & Watchorn [1930–31]. For the serum sodium duplicate portions of 0.5 ml. of serum were treated with 8 ml. of water and 1.5 ml. of trichloroacetic acid. The Na in 2 ml. of the protein-free solution was precipitated as sodium zinc uranyl acetate and washed as described by McCance & Shipp [1931]. The triple salt was then either transferred to a 100 ml. flask with water and determined colorimetrically as described by these authors or titrated with standard alkali in the centrifuge tube as described by Weinbach [1935]. The serum K was determined by Kramer & Tisdall's [1921] method, special precautions being taken to standardize the cobaltinitrite against solutions of pure potassium salts. Chloride in plasma and whole blood was determined by Volhard's principle [Van Slyke, 1923–24, McCance & Shipp, 1933]. Plasma bicarbonate was determined by the Van Slyke gasometric method [Harrison, 1930]. The whole bloods were dry ashed for Na and K , the former being estimated as described above and the latter by Hubbard's [1933] method. The cell volumes were determined by haematocrit in tubes of approximately 1 mm. internal diameter. For two of the subjects larger tubes of approximately 3 mm. were used. The tubes were centrifuged till the percentage of the column occupied by the cells was constant. Haemoglobin was estimated by Haldane's CO method, 100% being equivalent to 13.8 g. of haemoglobin per 100 ml. of whole blood. No determinations except of haemoglobin and cell volumes were made until the four samples of blood had been collected from each individual and all were then carried out together, using the same measuring apparatus for each of the four samples. Thus, from the first, each experiment was arranged to provide its own control and to make the comparative results as reliable as possible. In the later experiments standard solutions of Na , K , and Cl were determined at the same time and with the same apparatus as the unknowns.

RESULTS AND DISCUSSION

The results are shown in Table I and the following points call for comment.

(1) The centre of interest in this investigation has been a comparison of the findings in the same person under two different conditions. There is no need therefore to discuss at length the normal findings in the light of other results but it is as well to mention their relationship to those of three recent investigators. Hald & Eisenman [1937] found a range of 129–139 m.eq./l. for serum Na, and Schmitt [1936, 1] 130–143. Maizels [1936] found a range of 136–142 m.eq./l. The present figures average 145.5 m.eq. with a range of 138–155, but it is probable that all D. W.'s serum figures are slightly too high since a redetermination of his normal serum Na 18 months after his original experiment showed it to be 144 m.eq./l. R. B. N.'s and J. T. B.'s serum sodiums were unchanged at that time and R. A. M.'s slightly higher than his previous level. The true range of normality as found by these methods therefore may be considered to be 138–150 m.eq./l. with an average of 143 m.eq./l. Maizels [1936] found an average of 102 m.eq. of Cl/l. of plasma and Schmitt a range of 98–105. The present average of 100 is in good agreement.

In the cells Hald & Eisenman [1937] found an average of 16 m.eq. of Na/l. of cells, and Maizels [1936] one of 12 m.eq. The present results are higher and range from 17 to 35 m.eq., but are much lower than those of Schmitt [1936, 1] which were 62–69. This last range seems to be too high. Hald & Eisenman [1937] found a range of 72–102 m.eq. of K/l. of cells, Schmitt [1936, 1] 74–82 and Maizels [1936] 101–110. The present results agree well with those of Maizels. Maizels found an average of 51 m.eq. of Cl/l. of cells with which the present results are in fair agreement. Schmitt's results are much higher and it is probable that there was some technical error in this author's determination of the cellular ions, since there are other points in which the results are at variance with those of other investigators [Schmitt, 1936, 1, 2, 3]. Schmitt for example found almost as much Ca in the cells as in the serum and as much or more Cl in the cells than in the plasma during diabetic acidosis.

(2) The present experiments are unanimous in showing a fall in serum Na and Cl during salt deficiency and a return to normal afterwards. W. M. G., who could only be made partially deficient, showed less fall than the others. There can be no doubt at all that the period of deficiency was accompanied by a fall in the osmotic pressure of the extracellular fluids. This has been discussed in previous publications [McCance, 1936, 1, 2]. The concentration of serum proteins, which in this respect are to be considered with the haemoglobin and cell volume of the whole blood, rose during salt deficiency, indicating that there was less water per 100 ml. of plasma at that time and probably a reduction in the total plasma volume. This last shows that fluctuations in the concentration of sodium in the serum are in themselves no measure of the total sodium ions in the circulating blood. These signs of dehydration are well known to follow supra-renalectomy or experimental removal of the extracellular ions in animals. They seem in most species to be an essential part of the salt-deficient syndrome even when, as in the present instance, there has been a free intake of water and a normal daily output of urine. After the deficiency the concentrations of haemoglobin and plasma proteins fell, not merely to their predeficient (normal) level but to a lower one. This change is far too large to have been due to the removal of blood for tests, so that temporary over-compensation must have taken place. Darrow & Yannet [1936] have reported the same phenomenon in dogs. The cause of it is unknown but it may be due to over-stimulation of the suprarenal cortex

Table I

Subject	During health												During salt deficiency											
	Serum or plasma						Whole blood			R.B.C.			Serum or plasma						Whole blood			R.B.C.		
	Before or after deficiency	Na m.eq./l.	K m.eq./l.	Cl m.eq./l.	HCO ₃ m.eq./l.	Pro-teins g./l.	Cell vol. %	Haemo-globin g./l.	Na m.eq./l.	K m.eq./l.	Cl m.eq./l.	Haemo-globin g./l.	Test no.	Na m.eq./l.	K m.eq./l.	Cl m.eq./l.	HCO ₃ m.eq./l.	Pro-teins g./l.	Cell vol. %	Haemo-globin g./l.	Na m.eq./l.	K m.eq./l.	Cl m.eq./l.	Haemo-globin g./l.
R. A. M.	B	138	4.9	99	30	72	—	138	—	—	—	—	1	122	4.8	87	30	84	58.0	174	26	79	45	300
	A	138	3.4	102	30	60	43.0	119	31	111	64	276	2	122	6.1	85	31	86	57.0	164	26	79	49	288
R. B. N.	B	148	5.0	102	31	64	44.0	139	24	111	58	316	1	134	4.9	82	33	80	56.0	172	21	90	47	310
	A	149	4.7	102	30	59	40.0	128	17	103	61	320	2	132	4.2	78	30	77	52.0	162	14	88	45	311
D. W.	B	154	4.2	101	28	61	45.6	140	35	103	53	308	1	144	4.2	86	31	73	57.4	177	33	95	43	310
	A	155	3.9	104	29	58	40.0	132	40	104	57	330	2	139	4.2	80	29	73	56.3	174	36	98	40	310
W. M. G.	B	138	4.8	101	31	71	45.1	137	—	108	56	306	1	132	4.4	96	30	79	51.0	156	—	98	52	306
J. T. B.	B	150	4.8	100	33	—	48.0	141	24	103	55	294	1	138	5.1	82	33	—	60.0	182	22	96	41	304
	A	149	5.1	102	33	—	42.8	132	19	105	56	308	2	128	5.1	78	33	—	58.5	174	17	89	41	298
R. M. L.	B	141	3.5	94	29	73	46.2	145	22	108	53	314	1	131	5.4	77	29	83	55.3	174	14	93	50	315
	A	138	4.6	93	29	69	40.9	131	(5?)	110	65	321	2	126	4.9	75	29	83	54.2	170	13	96	48	313
Average (total)		145.5	4.4	100.0	30	65	43.5	135	—	106.5	58.0	309		131.5	4.8	83.5	31	80	56.0	170	—	91.0	45.5	306
Average (before deficiency excluding W. M. G.)						68	46.0	141	—	—	—	—		—	—	—	—	—	—	—	—	—	—	—
Average (after deficiency)						62	41.3	128	—	—	—	—		—	—	—	—	—	—	—	—	—	—	—
Fall (after or during deficiency)						6	4.7	13	—	—	—	—		13.5	—	16.5	—	—	—	—	—	15.5	12.5	3
Rise (during deficiency, reckoned on average before deficiency)						—	—	—	—	—	—	—		—	—	—	—	12	10	29	—	—	—	—

or other regulating organ by the prolonged period of anhydraemia. It is worth noting that in man very low blood ureas accompany these post-deficient falls in serum proteins and haemoglobin.

Salt deficiency brought about no change in the plasma bicarbonate and no consistent change in the serum K.

(3) Turning to the cells, it will be observed in the first place that there was no consistent change in the concentration of haemoglobin in the R.B.C. The average difference was less than 1%. Had the cells behaved throughout as simple osmometers there should have been a fall in the concentration of haemoglobin/litre of cells of the order of 10%, quite a large enough fall to have been detected with certainty in the averaged results and measured with a reasonable degree of accuracy even in the individual subjects. One must conclude therefore that the cells did not alter in size. That is to say, if they did temporarily alter in size for osmotic reasons following each daily removal or restoration of NaCl, subsequent adjustment enabled them to return later to their original size. In the second place, although the changes were less regular than the corresponding ones in the serum (see later), there was a consistent fall in the concentrations of K and Cl within the cells. Coupled with the fact that the R.B.C. did not alter in size, these findings suggest that in these subjects permanent osmotic equilibrium was achieved by a transference of ions rather than water across the cell membranes. It must be emphasized that the present results do not constitute any direct contradiction of those of Eisenman *et al.* [1937], for the conditions of experimentation were quite different. In the present work for example the cells were allowed a very much longer time in which to reach equilibrium with their surroundings.

The changes in the cell Na are much more irregular than those of the other ions. The details are given in Table I but the results have not been considered sufficiently consistent to average. There appears, however, to be a tendency for the cell Na to fall slightly during the period of deficiency. Nevertheless it seems that in man adjustment of the cells to an NaCl deficiency in the plasma is not brought about only by a passage of Na and Cl ions across the membranes. K is also transferred. Even apart from Na, which is admittedly difficult to determine with accuracy in the cells, the present results include some curious individual inconsistencies. Take for example the normal findings on R. M. L. before and after deficiency. In the presence of the values for Na and Cl found in serum and the consistent cell K, how can one explain on technical grounds the preliminary low cell Cl or the subsequent low cell Na? The same figures for whole blood Cl were obtained by two different persons making independent analyses on separate days. The Na and K were determined on the same ashed samples. The work of Eisenman *et al.* [1937] suggests that these curious inconsistencies are not technical but metabolic in origin, and if so they demonstrate how little we know about the mineral exchanges of the circulating red blood corpuscle.

SUMMARY

1. Salt deficiency in man, brought about by diet and sweating, leads to a fall in the concentration of Na and Cl in the serum, i.e. to a fall in its osmotic pressure.
2. The red blood corpuscles do not permanently increase in size and although they may do so temporarily this has not been observed. Osmotic equilibrium with the hypotonic plasma is brought about by a passage of K, Cl and possibly Na ions out of the cells.
3. The deficiency of NaCl also brings about a rise in the serum proteins, haemoglobin and cell volume of the whole blood. Restoration of a free NaCl

intake causes the serum proteins, haemoglobin and cell volume to fall below their initial levels.

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