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THE CHANGES IN WATER AND CHLORIDE DISTRIBUTION DURING HEAVY SWEATING

By W. S. S. LADELL

From the Colonial Medical Research Committee Laboratory for Hot Climate Physiology, Medical School, Yaba, Lagos, Nigeria

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Correlated observations on man between changes in salt and water balance and changes in the blood electrolytes during the acute salt loss that occurs during heavy sweating have not been made to the same extent as corresponding observations on animals during acute salt loss otherwise induced. Lee, Murray, Simmonds & Atherton (1941) discussed the strain which working in the heat imposes upon the salt/water balance, but they did not report any figures for the blood electrolyte concentration; their subjects were not sweating at very high rates, the maximum being 10·4 c.c./min., and the salt losses were all small, often less than 1 g. and never more than 2 g. in an 8 hr. test, considerably less than the losses in the urine over the same period. McCance's studies (1936, 1937, 1938) were on subacute rather than on acute salt deficiency, and sweating was only one of the means by which he induced the deficiency; the blood electrolytes were estimated, but, as the samples were not taken in close relation to the sweating periods, it is not possible to evaluate the effect of sweating as compared with other factors. Nadal, Pedersen & Maddock (1941) compared the effect of salt loss with that of water deprivation, and their results indicated that water is lost from the extracellular compartments in salt deprivation, and from the extra- and intracellular compartments in water deprivation; but as their deprivations were induced relatively slowly it would not be admissible to deduce from them the effects of a few hours of very profuse sweating.

Elkinton, Danowski & Winkler (1946*a*, *b*) studied acute salt deprivation in dogs induced by intraperitoneal injection of hypertonic glucose with subsequent removal of the intraperitoneal fluid. They correlated the changes in the blood chemistry and in the clinical condition of the animals with the changes in salt and water balance and calculated the movements of fluid between the intra- and extracellular compartments.

In the series of experiments reported elsewhere (Ladell 1947, 1948), and in others of a similar nature, there were a number of occasions in which the salt

and water balances were known accurately and when blood samples were taken immediately before and after two or more hours of very heavy sweating. During this time as much as 20 g. of sodium chloride were sometimes lost, while urine flow was practically suppressed (Weiner, 1945). From changes in the chloride content of the blood and plasma, and from the salt and water balance figures, sufficient data were available to calculate the fluid shifts according to the methods of Elkinton & Winkler (1944).

METHODS

Men were subjected to the routine of alternate work and rest described previously (Ladell, 1947) for periods of from 110 to 170 min. in an air-conditioned room maintained at 100° F. dry-bulb temperature and 94° F. wet-bulb. The subjects were weighed before and after each bout of work, and sweat samples were obtained at the time of each weighing from impermeable bags worn on one arm; these bags give a true sample of 'mixed body sweat' (Ladell, 1948). Any urine passed was collected. The amount of water drunk and of salt ingested, as 10% sodium chloride solution, was accurately measured. Blood samples were taken immediately before and immediately after exposure to the heat by venepuncture with the minimum of stasis. The samples were collected under oil, and true plasma was obtained by centrifuging under paraffin wax.

The chloride content of sweat and urine samples was estimated by the Whitehouse method, and the blood and plasma chloride by the Volhard method on the Folin-Wu filtrate (Peters & Van Slyke, 1932). In a number of experiments the plasma protein and the haematocrit value were estimated by Van Slyke's copper sulphate, specific gravity method (Phillips *et al.*, 1942).

The sweat loss was calculated from the change in body weight, the water uptake and the urine passed. No correction was made for respiratory exchange; the weight change due to this is less than 10 g./hr., which is negligible in comparison with the total sweat loss, and is less than could be detected over short periods. The chloride losses were calculated for each period between weighings from the sweat water loss and the chloride concentration in the sweat sample produced during that period. When water or salt was being replaced the calculated losses during one period were given to the subject for consumption during the next period, so there was always a slight lag in the replacement; this was allowed for by giving the subject a small 'advance' of salt or water or both, as the experiment required, as soon as he entered the room; this 'advance' was included in the final balance, as was the last dose given before leaving the room, but not that given as he left the room. There was usually an interval of at least 20 min. between the last dose of salt or water included in the balance and the taking of the post-exposure blood sample.

RESULTS

Actual observations. Three young male subjects, all fit and fully acclimatized to work in the heat, completed in all thirty tests under varying conditions of salt and water intake. Sweat rates varied from 17·7 c.c./min. for 137 min. (Exp. 20) to 38·2 c.c./min. for 130 min. (Exp. 2); the greatest sweat volume lost was 5497 c.c. in 165 min. (Exp. 1) and the greatest salt loss was 25·02 g. in 162 min. (Exp. 15). The replacements, where carried out, were effective; for example, in Exp. 16, the final water balance was +28 c.c. and in Exp. 27 the final salt balance was −0·99 g. In the other experiments there were considerable disturbances of the salt and water balances, which were reflected by changes in the chloride contents of the blood and plasma, and in the haematocrit readings and in the plasma protein concentration; these changes are shown in Table 1.

TABLE 1. Summary of experiments showing the changes observed in the blood chemistry (Exps. 1–10: subject LAD. Exps. 11–19: subject BRA. Exps. 20–30: subject GOL

Exp. no.	Duration of exposure (min.)	Sweat loss		Intake		Final balance (including urine losses)		Whole blood chloride		Plasma chloride		Overall change in	
		Water (c.c.)	Chloride (as NaCl) (g.)	Water (c.c.)	Chloride (as NaCl) (g.)	Water (c.c.)	Chloride (as NaCl) (g.)	Before exposure (m.eq./l.)	After exposure (m.eq./l.)	Before exposure (m.eq./l.)	After exposure (m.eq./l.)	Plasma protein (g./100 c.c.)	Haematocrit reading (%)
1	165	5497	21·68	4540	Nil	− 957	−21·68	82·2	76·8	97·2	91·2	—	—
2	130	4969	22·73	3100	Nil	−1869	−22·73	78·0	74·2	96·3	90·9	—	—
3	160	4835	20·73	2370	Nil	−2465	−20·73	68·4	65·1	96·0	85·8	—	—
4	165	4963	24·58	234·5	23·45	−5101	− 1·89	84·3	87·6	103·2	109·8	+2·944	+ 7·5
5	135	4726	20·84	3667	Nil	−1276	−20·93	78·9	74·4	99·0	92·1	+0·40	− 1·1
6	135	4205	20·17	4717	Nil	+ 355	−20·67	78·6	71·1	97·8	90·6	+1·04	+ 7·2
7	135	4199	22·10	4633	Nil	+ 85	−22·75	79·0	69·3	97·6	90·1	+1·40	+ 8·0
8	135	3238	16·70	3180	Nil	− 138	−16·95	77·4	70·8	104·4	97·2	+0·35	0
9	135	3574	19·11	4270	Nil	+ 546	−19·43	78·4	66·6	100·8	88·4	+0·70	+ 3·6
10	150	4296	23·20	4428	25·58	+ 87	+ 2·27	79·6	78·0	98·8	99·2	0	+ 2·7
11	162	3401	17·43	3835	Nil	+ 47·5	−18·17	78·3	66·0	93·6	87·0	—	—
12	162	3521	14·68	Nil	Nil	−3829	−15·21	80·4	80·1	99·6	102·3	—	—
13	162	4420	18·40	4723	15·63	+ 118	− 3·68	82·2	79·5	101·4	99·9	—	+ 1·1
14	162	4797	18·50	4631	Nil	+ 324	−19·35	79·2	73·3	99·0	95·4	—	+ 2·2
15	162	4933	25·02	4619	21·88	− 577	− 3·60	76·2	76·8	93·3	97·2	—	+12·0
16	162	4333	19·97	4633	36·47	+ 28*	+15·59*	84·0	88·8	96·0	96·0	+0·23	− 2·1
17	110	2651	14·22	761	12·11	−1880*	− 3·18*	80·4	75·6	100·2	96·0	+0·26	+ 3·8
18	110	2908	14·93	Nil	Nil	−3288	−16·57	75·6	78·6	101·4	104·4	+0·94	+ 6·2
19	110	2845	15·08	136	13·63	−2933*	− 2·98*	77·4	75·0	99·0	97·0	+0·56	—
20	137	2425	9·87	3054	8·43	+ 514	− 3·12	80·1	74·4	99·3	96·3	+0·54	− 1·0
21	137	2737	12·58	3478	10·73	+ 651	− 2·62	79·2	79·2	98·4	98·4	+0·14	− 1·4
22	137	2939	13·05	3647	11·81	+ 633	− 2·61	73·8	79·6	102·6	98·7	+0·22	− 1·5
23	137	3036	10·87	3564	9·67	+ 426	− 2·21	77·7	77·7	93·6	93·0	+0·36	− 4·8
24	162	4450	18·92	4405	Nil	− 139	−19·87	78·0	72·0	98·4	92·1	+0·06	+ 0·8
25	110	2961	12·58	Nil	Nil	−3001	−12·74	74·8	78·2	97·2	100·6	+0·84	+ 3·8
26	162	4476	13·32	4447	Nil	− 118	−13·79	76·8	73·2	98·2	94·2	+0·88	+ 0·8
27	162	4074	14·10	3826	14·03	− 356	− 0·99	78·0	74·4	97·8	95·4	0	+ 2·0
28	162	5032	19·83	4898	Nil	− 214	−20·34	78 9	71·4	96·3	90·0	+0·54	+ 5·8
29	162	4802	23·89	4781	21·87	− 181	− 2·41	78·0	75·2	99·4	97·0	+0·60	− 2·8
30	110	3282	13·92	Nil	Nil	−3345	−14·29	75·2	78·0	94·6	97·2	+0·80	+ 3·2

* Balances unreliable as subject vomited and absorption was defective; but vomit collected as far as possible and included in the balance.

In general the plasma chloride fell concomitantly with the development of a negative chloride balance, and it rose or remained the same when the chloride losses were made good or when water losses as well as salt losses went unrelieved. On three occasions only did the plasma chloride fall below 90 m.equiv./l. The changes in the whole-blood chloride were usually, but not invariably, in the same direction as the plasma chloride changes; on four occasions the whole-blood chloride was reduced below 70 m.equiv./l.

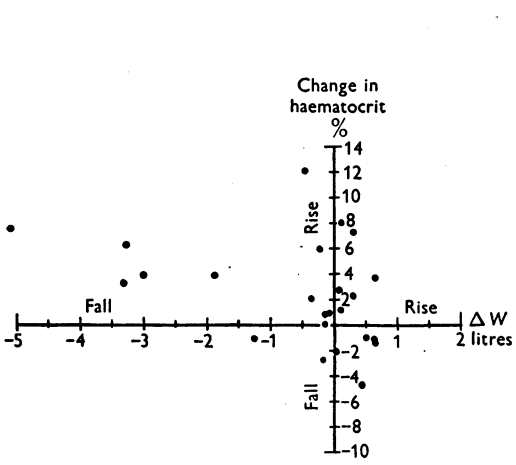

Fig. 1.

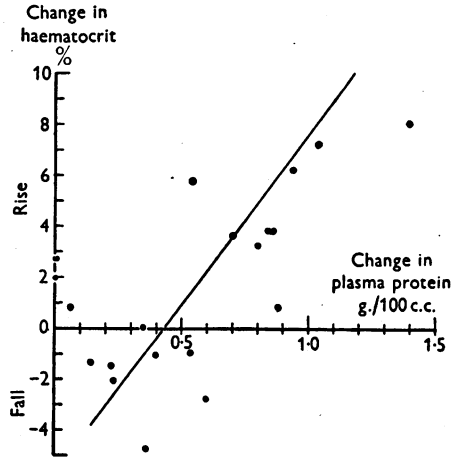

Fig. 2.

Fig. 1. Scatter diagram showing changes in haematocrit readings and changes in total body water (ΔW). No correlation was observed.

Fig. 2. Changes in haematocrit readings correlated with changes in plasma protein. Correlation coefficient, 0·671; $t = 3{\cdot}84$; P less than 0·01.

Plasma-protein changes were followed on twenty-two occasions; twice there was no change and on twenty occasions there was a rise. The changes in the haematocrit readings were not related to the changes in water balance (see Fig. 1), but were well correlated with the plasma-protein changes (see Fig. 2). Excluding two pairs of values, where the protein estimations were possibly faulty (Exps. 4 and 19) for technical reasons, there was a correlation coefficient of 0·671 ($t = 3{\cdot}84$, P less than 0·01) between the two.

Derived values. Using the method of Elkinton & Winkler (1944) the changes in extracellular fluid volume were calculated for each experiment, from the body weight, the chloride balance, and the change in plasma-chloride concentration. In Exps. 16, 17 and 19 the salt replacement by means of 10% sodium chloride was not well tolerated, the subject vomiting, and subsequently having saline diarrhoea; accurate chloride balances were not, therefore, available for these experiments. The changes in the extracellular fluid volumes calculated for the remaining twenty-seven tests are shown in Table 2. From the change

in the total body water, i.e. the water balance (ΔW), and the change in the extracellular fluid volume (ΔE), the change in the intracellular fluid volume was obtained by difference for each experiment; these changes (ΔI) are also shown in Table 2.

TABLE 2. Changes in chloride balance, body water, extracellular fluid and intracellular fluid

Exp. no.	Chloride balance (as NaCl) (g.)	Calculated change in: Body water ΔW (l.)	Extracellular fluid ΔE (l.)	Intracellular fluid ΔI (l.)
		(*a*) Chloride losses not replaced		
1	−21·68	−0·957	−2·936	+1·979
2	−22·73	−1·869	−2·257	+0·388
3	−20·73	−2·465	−2·137	−0·328
5	−20·93	−1·276	−2·620	+1·344
6	−20·67	+0·355	−2·618	+2·973
7	−22·75	+0·085	−2,871	+2·966
8	−16·95	−0·138	−1·778	+1·640
9	−19·43	+0·546	−1·583	+2·129
11	−18·17	+0·047	−2·110	+2·157
12	−15·21	−3·829	−2·924	−0·905
14	−19·35	+0·324	−2·708	+3·032
18	−16·57	−3·288	−3·179	−0·019
24	−19·87	−0·139	−2·285	+2·146
25	−12·74	−3·001	−2·763	−0·238
26	−13·79	−0·118	−1·528	+1·410
28	−20·34	−0·214	−2·447	+2·233
30	−14·29	−3·345	−2·871	−0·474
		(*b*) Chloride losses replaced		
4	− 1·89	−5·101	−1·247	−3·854
10	+ 2·27	+0·087	+0·321	−0·234
13	− 3·68	+0·118	−1·659	+1·777
15	− 3·60	−0·477	−0·227	−0·250
20	− 3·12	+0·514	+0·055	+0·459
21	− 2·62	+0·651	−0·445	+1·096
22	− 2·61	+0·633	+0·310	+0·323
23	− 2·21	+0·426	−0·272	+0·698
27	− 0·99	−0·356	+0·311	−0·667
29	− 2·41	−0·181	+0·058	−0·239

There was no direct relationship between ΔW and ΔE. The ΔI values, however, fell into two groups, depending on whether the chloride losses had been replaced or not; with replacement the mean ΔI was +40 c.c. and without replacement +1450 c.c.; this difference is significant, t being 9·85 and P less than 0·01. In both cases ΔI and ΔW ran together (see Fig. 3); significant regression and correlation coefficients were obtained for both groups, and from these regressions it appeared that when chloride was not replaced ΔI remained positive until ΔW exceeded 2·7 l.; the regression passed very near to the origin, however, when there was replacement.

The changes in plasma-chloride concentration were correlated with ΔI (Fig. 4); the correlation coefficient was −0·65 ($t = 4·31$, P less than 0·01). From the changes in the whole blood and in the plasma-chloride concentrations, and

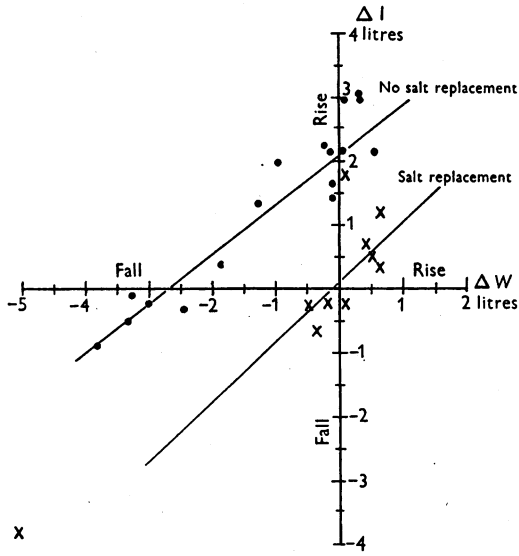

Fig. 3. The changes in intracellular fluid volume (ΔI) correlated with changes in total body water (ΔW), when salt was not replaced (spots) and when salt was replaced (crosses). Both regressions are significant, with correlation coefficients of 0·865 ($t=6·65$, P less than 0·01) and 0·807 ($t=3·86$, P less than 0·01) respectively.

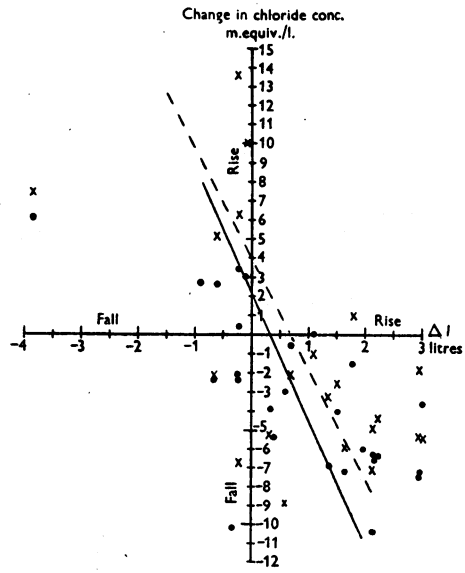

Fig. 4. Changes in the chloride content of the plasma (spots and solid line) and of the red blood cells (crosses and broken line) correlated with the changes in the intracellular fluid volume (ΔI). Both regressions are significant: for plasma correlation coefficient is $-0·65$, $t=4·31$; for cells coefficient is $-0·53$, $t=2·84$; P less than 0·01 and 0·01 respectively.

from the haematocrit values before and after exposure to the heat, the chloride concentration in the cells was calculated. The mean fall in twenty-two experiments was 4·45 m.equiv./l.; the mean fall in plasma chloride for the same series was 3·21 m.equiv./l. The cell chloride change was also correlated with ΔI, a coefficient of $-0{\cdot}53$ ($t=2{\cdot}84$, $P=0{\cdot}01$) was found. The scatter and the regression is shown in Fig. 4.

DISCUSSION

Fluid movements. Although the changes in the concentration of only one ion have been followed in these thirty experiments, the results indicate in what direction and to what extent transfer of water takes place between the extra- and intracellular compartments when men sweat heavily. The immediate source

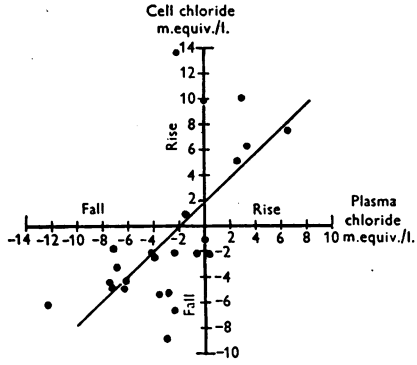

Fig. 5. Changes in the chloride content of the red blood cells correlated with the changes in the chloride content of the plasma. Correlation coefficient 0·67, $t=4{\cdot}24$, P less than 0·01.

of the water lost in the sweat must be the extracellular fluid, which also provides the chloride; depending upon the extent to which these losses are made good there might be expected a greater or less disturbance of the osmotic equilibrium between the extra- and intracellular compartments. The results of these experiments suggest that such disturbances do not develop to any great extent, the equilibrium being quickly restored by simple fluid transfer. When chloride is not replaced water flows from the extra- to the intracellular compartment, until, presumably, osmotic equilibrium is obtained; the fall in the chloride concentration of the extracellular fluid is paralleled, on the one hand by a fall in the osmotic pressure of the intracellular fluid, which is reflected by the fall in the chloride content of the blood cells (Fig. 5), and on the other hand by a rise in the intracellular fluid volume (Fig. 4). The magnitude of the fluid transfer depends upon the amount of water that is replaced and the amount of salt that is lost. In these tests the mean salt loss was 19 g.; the regression shows that, with this loss, if the subject remained in water balance the mean ΔI was $+2{\cdot}1$ l., and with over-hydration it was greater, and that a negative water

balance of 2·7 l. had to be incurred before any water was lost from the intracellular compartment. But when salt was replaced as fully as was possible and water was either not replaced, or replaced relatively more slowly than the salt, the water loss was borne almost entirely by the intracellular compartment, and the osmotic pressure in both compartments rose, as shown by the rise in plasma and blood-cell chloride concentrations in Exps. 4, 18, 25 and 30.

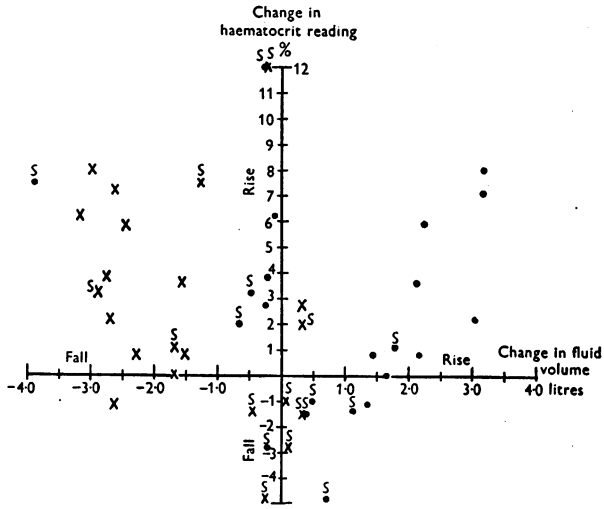

Fig. 6. Scatter diagram to show how, in general, the haematocrit reading rises as the extracellular fluid volume falls, indicated by crosses, and as the intracellular fluid volume rises, indicated by spots. There is no statistical correlation. The letter *S* above a point indicates that in that test salt was replaced.

The changes in the haematocrit readings merely reflect the relative changes in the cell and plasma volumes, and these in turn may reflect the changes in the intra- and extracellular fluid volumes. Fig. 6 shows that in general, as the intracellular fluid volume rose and the extracellular volume fell, the haematocrit reading rose; so many other factors are involved, however, that a statistical correlation was not obtainable. As no red cell counts were made the mean cell volumes could not be calculated, and so the question of the behaviour of the red blood cells as simple osmometers (McCance, 1937) remains open.

Electrolyte movements. McCance (1937) concluded that, in a salt deficiency state lasting a number of days, potassium, chloride and possibly sodium ions passed out of the cells into the plasma. The passage of base out of the cells in conjunction with cell water has also been described during water deprivation by Newburgh & Johnston (1934), who noted the rise in urinary potassium towards the end of a period of dehydration; while Johnson, Pitts & Consolazio (1944) reported a rise in the potassium content of the sweat during heavy

sweating, the source of this potassium presumably being the cells. Elkinton & Winkler (1944) considered that potassium loss from the cells was a general and compensating reversible physiological response of the organism to severe depletion of water. The passage of base out of the cells is, therefore, well established; but the movements of chloride have not been followed in such detail, nor have the movements of either base or chloride been considered during sudden and severe derangements of water and salt balance, such as are described in this communication.

In the present series of tests, if the changes in the chloride concentration in the red blood corpuscles, apart from the chloride shift, were due solely to the passage of water in and out of the cells, as part of the general transfer of fluid from the extra- to the intracellular compartments, then

$$\text{R.B.C. vol. X R.B.C. Cl conc.} = \text{constant},$$

but R.B.C. vol. is proportional to I.C.F. vol.,

therefore $$\text{I.C.F. vol. X R.B.C. Cl conc.} = K \text{ (a constant)}; \qquad (1)$$

differentiating gives

$$\frac{d\,(\text{I.C.F. vol.})}{d\,(\text{R.B.C. Cl conc.})} = -\frac{K}{(\text{R.B.C. Cl conc.})^2}.$$

A mean value for $\frac{d\,(\text{I.C.F. vol.})}{d\,(\text{R.B.C. Cl conc.})}$ may be obtained from the regression equation for ΔI on cell chloride:

$$\Delta I = 627{\cdot}565 - 140{\cdot}107\ (\text{change in R.B.C. Cl conc.}),$$

and by substituting this and the mean value for (R.B.C. Cl conc.), the value of K was obtained, and thence, from equation (1) and the mean (R.B.C. Cl conc.), a value for intracellular fluid volume. The figure calculated in this way was 37·85 l. This figure is a little high (cf. Marriott, 1947), but in view of the approximations involved, especially that of treating the relationships as linear, it may be considered a good result; it may therefore be concluded that, if the changes in the chloride concentration in the red blood cells may be taken as an example, the observed changes in the fluid distribution and in the chloride concentrations during a short period of severe sweating may be accounted for without postulating transfer of chloride ions.

General. Nadal *et al.* showed in 1941 that the peripheral circulatory failure seen in salt deficiency was due to the reduction in the extracellular fluid volume, and that in water deficiency water is lost from both compartments; Marriott (1947) also explained the clinical picture of dehydration, from whatever cause, on the basis of loss of extracellular fluid. But Gamble (1944) and Winkler, Elkinton, Hopper & Hoff (1944) have shown that the real danger and the ultimate cause of death in water deficiency is intracellular desiccation and the consequent rise in intracellular osmotic pressure. The beginnings of this were

seen in the present series of experiments; when salt was replaced fully and water either not at all or inadequately, the chloride concentrations inside the cells rose and the intracellular compartments were drawn upon for water; this shows the danger of replacing salt unless water can be replaced as well.

The intracellular over-hydration that occurs when salt is not replaced and water losses are either replaced or not very high is analogous to that described by Danowski, Winkler & Elkinton (1946) in over-hydrated dogs. These animals developed convulsions, the so-called water intoxication; it is possible that 'heat cramps' in man may be the same condition; in the long series of tests, of which the thirty here described are a small proportion only, the conditions for intracellular over-hydration were frequently fulfilled and 'heat cramps', fully developed or incipient, were a regular complaint. The water intoxication hypothesis of 'heat cramps' (Moss, 1922; Haldane, 1923; Hunt, 1912) has been doubted by Talbott (1935), but our experiments show that there is intoxication by water, though of the intracellular compartments and not, as earlier thought, of the extracellular.

Some similarity was looked for between the condition of the subjects at the end of an exposure in the climatic chamber and the clinical acute condition of type I heat exhaustion (Ladell, Waterlow & Hudson, 1944); the heat exhaustion cases did not sweat so profusely as the men in the climatic chambers, but they sweated for longer, and the falls in blood and plasma-chloride concentrations were correspondingly greater, and cramps were common; one biochemical feature was the proportionately greater fall of the whole-blood chloride concentration relative to the fall in the plasma chloride; this was shown by the reduction ratio

$$\frac{\text{Final blood Cl conc.}}{\text{Initial blood Cl conc.}} : : \frac{\text{Final plasma Cl conc.}}{\text{Initial plasma Cl conc.}},$$

the mean value for this being 0·95, and all values being below 1 except in mild cases. The mean ratio for the men in the climatic chamber was 0·9933 ($\pm$ 0·007 S.E.), but when the ratios were plotted against the final plasma-chloride concentration on eleven out of seventeen occasions when salt was not replaced the point came below or within the standard error of the arbitrary line, which Ladell *et al.* found separated the points plotted for type I from those plotted for type II cases; the equation for this line is

$$P + 75R = K,$$

where P = plasma-chloride concentration, R = reduction ratio, K = a constant value 165·4. (In the communication by Ladell *et al.* an error in the original manuscript resulted in the value of K being given incorrectly as 106·8.) There is, therefore, some similarity between the fluid and electrolytic changes in the acute condition seen in the climatic chamber, and those in the less acute, but more severe heat exhaustion, type I.

In the present series of tests exposure to the heat resulted in an increase in the protein content of the plasma, irrespective of the final water balance; this contrasts with the observation by Lee *et al.* (1941); they found a decrease in the serum-protein concentration, even with deficits of body water as high as 2%. The difference might be due to the very much higher rates of sweating in the present tests.

SUMMARY

1. The changes in the chloride concentration of the blood and plasma which occur when men sweat heavily as the result of exercise in a hot humid atmosphere are described.

2. When these changes are considered in relation to the alterations in the salt and water balance it can be shown that, if the salt losses in the sweat are not replaced, there is a transfer of water from the extracellular to the intracellular compartments.

3. These findings are discussed in the light of reports by other workers on salt and water movements in men and in animals, under varying conditions.

This work was done at the Medical Research Council Neurological Research Unit, National Hospital, Queen Square, London, under the direction of Dr E. Arnold Carmichael, to whom I am greatly indebted for advice and encouragement. I also wish to thank my subjects for their loyal co-operation in carrying out these tests.

REFERENCES

Danowski, T. S., Winkler, A. W. & Elkinton, J. R. (1946). *J. clin. Invest.* **25**, 130.
Elkinton, J. R., Danowski, T. S. & Winkler, A. W. (1946*a*) *J. clin. Invest.* **25**, 120.
Elkinton, J. R., Danowski, T. S. & Winkler, A. W. (1946*b*) *J. clin. Invest.* **25**, 130.
Elkinton, J. R. & Winkler, A. W. (1944). *J. clin. Invest.* **23**, 93.
Gamble, J. L. (1944). *Proc. Amer. Phil. Soc.* **88**, 3.
Haldane, J. S. (1923). *Brit. med. J.* i, 986.
Hunt, E. H. (1912). *J. Hyg., Camb.*, **12**, 479.
Johnson, R. E., Pitts, G. C. & Consolazio, F. C. (1944). *Amer. J. Physiol.* **141**, 575.
Ladell, W. S. S. (1947). *J. Physiol.* **106**, 237.
Ladell, W. S. S. (1948). *J. Physiol.* **107**, 465.
Ladell, W. S. S., Waterlow, J. C. & Hudson, M. F. (1944). *Lancet*, ii, 491, 527.
Lee, D. H. K., Murray, R. E., Simmonds, W. J. & Atherton, R. G. (1941). *Med. J. Aust.* **2**, 249.
Marriott, H. L. (1947). *Brit. med. J.* i, 246, 285, 328.
McCance, R. A. (1936). *Proc. Roy. Soc.* B, **119**, 245.
McCance, R. A. (1937). *Biochem. J.* **31**, 1278.
McCance, R. A. (1938). *J. Physiol.* **92**, 208.
Moss, K. N. (1922). *Proc. Roy. Soc.* B, **95**, 181.
Nadal, J. W., Pedersen, S. & Maddock, W. G. (1941). *J. clin. Invest.* **20**, 691.
Newburgh, L. H. & Johnston, M. W. (1934). *J. Nutrit.* **7**, 107.
Peters, J. P. & Van Slyke, D. D. (1932). *Quantitative Clinical Chemistry*, 1st. ed. **2**. London: Baillière, Tindall and Cox.
Phillips, R.A., Van Slyke, D. S., Dole, V. P., Emerson, K., Hamilton, P. B. & Archibald, R. M. (1942). *Copper Sulphate Method for Measuring Specific Gravity of Whole Blood and Plasma.* Washington cmr. report: Bureau of Medicine and Surgery.
Talbott, J. (1935). *Medicine*, **14**, 321.
Weiner, J. S. (1945). *J. Physiol.* **103**, 36 *P.*
Winkler, A. W., Elkinton, J. R., Hopper, J. & Hoff, H. E. (1944). *J. clin. Invest.* **23**, 103.