612 . 392 . 61

Experimental Sodium Chloride Deficiency in Man

By R. A. McCance, Biochemical Laboratory, King's College Hospital

(*Communicated by E. Mellanby, F.R.S.—Received November* 4, 1935)

Introduction

Dehydration plays an important part in the pathology of a number of diseases. The loss of water may take place through the kidney (as it does in diabetic coma), the gut (as in pyloric stenosis, paralytic ileus, acute diarrhoea, and vomiting or cholera), the lungs and skin (as in hot dry atmospheres) or occasionally through other channels. This loss of water has been known for a very long time to be associated with loss of salt, but a study of the literature reveals considerable confusion of thought as to the relationship of one to the other. This is particularly well illustrated by the discussions of some of the experimental work on Addison's disease. Much experimental work has been done on the lower animals to elucidate the pathology of the intestinal obstructions; diabetic coma has been studied to some extent, and in the last 5 years Addison's disease has been produced and controlled experimentally in numerous rats, cats, and dogs. In every case, however, the salt or water deficiency is but a part of the picture. From the acute and dangerous nature of the human diseases and the complications they introduce it is particularly difficult to study the effects of severe water or salt deficiency in patients. Some work has been carried out on diabetic coma, which, however, presents water and salt loss in its most complicated form. No papers of any value have been found on severe uncomplicated salt deficiency. It was therefore decided to make a direct experimental attack on the question and normal human adults were selected as the most suitable subjects. The deficiency was produced by a salt free diet combined with sweating.

Nature and Arrangement of the Experiments

(*a*) *The Subjects*—One of the women students of this Hospital volunteered to be the subject for the first experiment, which was of a semi-quantitative nature and was intended to try out methods rather than get results. Mild deficiency only was produced because E. ceased to lose significant amounts of NaCl in her sweat after 4 or 5 days. Nevertheless,

some interesting observations were made which will be discussed in their appropriate place, and the fact that this subject reacted to the experimental regime so differently from the others is a matter which may be of some importance and will be further investigated. The second and third experiments were done on two males, R.A.M. age 36, and R.B.N. age 24. In both experiments every reasonable precaution was taken to make the whole investigation as quantitative as possible. Both subjects were in good health when the experiments began and the observations were not upset by any " colds " or other minor pathological interferences. R.A.M. is normally an active man, fond of exercise, and gets enough of it throughout the year to keep himself physically fit. R.B.N. is a South African, who came to this country with a Rhodes Scholarship and is now a medical student. He takes enough regular exercise to keep in fair training.

(*b*) *Sweating*—This was carried out in a radiant heat bath. A mattress with two pillows at one end was placed on the floor and covered by a strip of mackintosh sheeting 8 feet long. The bath, which consisted of a metal and wood tunnel closed at one end and fitted internally with three rows of electric hot lamps, was placed on the mattress and the open end covered by hanging a small piece of sheeting over it. When the temperature was fully raised the subject entered feet first through the open end and lay there. While in the air bath he drank a measured amount of water, made notes and regulated his own temperature by raising or lowering the flap of sheeting. After 15–25 minutes sweat poured off and an assistant was always within call to help to bail this off the sheeting. 800 cc were often collected in this way. After 2 hours the heating was switched off and 10 minutes later the subject crawled out and was washed down quantitatively with a jet of warm distilled water into an enamel washing tub. The sheetings and parts of the air bath in contact with them were subsequently washed down with distilled water into the tub. The contents of this were evaporated down at a neutral or slightly acid reaction, combined with the liquid sweat which had been bailed off the sheeting during the time the subject had been in the bath, and made up to a convenient volume, generally 2 litres. The condition of the subject in the bath is best described by one of the records made at the time:—

Subject R.A.M., 10.1.35—

2.16 p.m.—Naked weight 9 st 12 lb 0 oz.
2.18—Into bath. Temperature 98·0°. Begin drinking 800 cc water.
2.25—Moist all over, lying on face.

2.33—Temperature 99·2° sweating well. Drinking.
2.45—Temperature 100°, sweating very well. Always seem to sweat well when temperature rising. First bail out.
2.55—Temperature 100·8°. Drinking.
3.10—Temperature 101°. Sweating very well.
Half-time—Temperature 101°. Comfortable.
3.30—Temperature 101·6°. Too hot, uncomfortable, pulse banging. Open flap.
3.35—Temperature 101·4°. Water nearly finished.
3.45—Temperature 101°. Water all done. Not sweating so well, still uncomfortable. Sickish.
3.50—Better, sweating well. Temperature 100·8°.
4.10—Temperature 101°. O.K. but cramps.
4.20—Heat off.
4.30—Out, feel rotten few minutes, washed down. Then O.K. Weight before dressing 9 st 9 lb 0 oz. Total loss of weight = 3 lb + 800 gm = 2150 gm.

(*c*) *Diet and mode of life*—R.A.M. lived at his own home and R.B.N. stayed there also during his experiment. Both subjects shaved every day, taking great care to remove all the lather, but no other "domestic" washing or soap of any kind was indulged in during the experiment except for the hands. Their food, which was carefully prepared and accurately weighed, consisted of salt-free "casein" bread, synthetic NaCl free milk, salt-free butter, fruit, thrice boiled vegetables, jam, home-made salt-free shortbread and toffee. The milk and bread were devised for these experiments (Widdowson and McCance, 1935) to enable a reasonably high protein intake to be combined with a salt-free diet, and both proved most satisfactory. R.A.M. experimented with washed mince made up with curry as a variety, but this proved so nauseating that he fell back entirely on to the casein bread and special milk for his supply of protein. Water was taken as desired by all the subjects.

At the end of her deprivation period E. took 28 gm NaCl in a few hours, drank copiously for the next 12 hours, and was back to normal activity and weight the following morning. This meant an increase of 7¼ lb. R.A.M. brought himself slowly back to health by eating regulated quantities of anchovies, bacon, liver, measured amounts of cow's milk, and small weighed amounts of NaCl. Every third rasher of bacon was taken for analysis in the raw state, and to prevent loss, all bacon was eaten from the frying pan used for its preparation, and the pan washed out with hot water which was drunk. Samples of the

anchovies were also analysed. All other food was maintained practically salt free, but ordinary salt-free bread was taken instead of the casein-enriched bread, since plenty of protein was now being eaten. R.B.N. used bacon, roast beef, and small amounts of NaCl to remove his salt deficiency. He observed the same precautions as R.A.M. and changed also from " casein " to ordinary bread.

Except for these salted foods used during the recovery period and special foods such as the washed mince, which were all analysed for nitrogen, potassium, sodium, and chloride, the composition of the foods eaten was calculated from the tables of McCance and Shipp (1933) and unpublished data of McCance, Widdowson, and Shackleton.

(*d*) *Collection and treatment of excreta*—Urines were collected and analysed in 24-hour periods, or shorter if kidney function tests were being made. Faeces were collected and analysed in 3- or 4-day periods. To obviate loss of nitrogen, the portion of moist faeces which had been weighed out for analysis was well mixed with glacial acetic acid before being placed in the oven at 50° C to dry. The portion to be used for chloride determinations was dried without any additions. The sweat and bath washings were collected for analysis as already described. The insensible perspiration was collected in the following way:—A set of underclothes was washed in distilled water till no reaction for chlorides was obtained from the washings. They were then dried and put on when the experiment began. All R.A.M.'s clothes were washed in this way, but only underclothing in the case of R.B.N. These underclothes were worn night and day throughout the period of deprivation and were only removed for the periods of sweating in the air bath. They were then washed out as before, dried in a few hours and worn continuously till the recovery period was over, when they were washed again. The washings were evaporated and analysed. At the conclusion of the recovery period the subjects were washed down with distilled water and the washings combined with those from the underclothes. Owing to the regular washings after the sweat baths this additional wash down was omitted at the close of the deprivation period.

Methods

1. *General*—

Blood pressure. Sphygmomanometer of usual type with mercury column.

Eye tension. McLean's tonometer.

2. *Chemical methods for urine*—

Total nitrogen. Kjeldahl (McCance and Shipp, 1933).
Urea. A modification of Beattie's (1928) xanthydrol procedure.
Creatinine and creatine. Folin (Hawk, 1923).
Chlorides. Volhard's principle; the urine was heated at 100° C with HNO_3 and excess $AgNO_3$ (McCance and Shipp, 1933).
Metallic radicles. After incineration. McCance and Shipp (1933). When a large excess of potassium over sodium was present the former was removed with perchloric acid before determining the latter (McCance, Widdowson, and Shackleton).

3. *Blood*—

Cell volume. Haematocrit, coagulation prevented by minimal amounts of ammonium oxalate.
Haemoglobin. Haldane's CO technique.
Urea. A modification of Beattie's (1928) method.
Serum proteins. Kjeldahl (McCance and Watchorn, 1931) or micro-Kjeldahl (Pregl).
Alkali reserve. Van Slyke gasometric (Harrison, 1930).
Plasma chlorides and whole blood chlorides. Volhard's principle, Van Slyke (1923–24) and McCance and Shipp (1933). Plasma for chlorides and alkali reserve was taken under paraffin without constriction.
Sodium in serum. A less micro form of the method described by McCance and Shipp (1931).
Potassium in serum. Kramer and Tisdall (1921), Hubbard (1933).
Osmotic pressure of the serum by depression of freezing point using a standard Beckmann thermometer.
Metallic radicles in whole blood. Incineration (McCance and Shipp, 1933).

4. *Sweat, washings, etc.*—As urines.

Symptoms and Signs

As the deficiency developed all three subjects lost weight (*see* later). Their cheeks fell in and they began to look ill. People who had no idea that they were being used as experimental animals commented on their appearance. Their sense of flavour and taste was affected. E. interpreted this aberration or lack of sensation as thirst. She complained of it constantly and drank freely but without obtaining any relief. R.A.M.

recognized the feeling as distinct from thirst. His mouth was not unduly dry but food was tasteless, even highly flavoured food, and this was the more noticeable because such foods were eagerly sought to make the meals more appetizing. Chewing fried onions, for example, evoked only a sensation of greasy sweetness which was extremely nauseating. The distaste, however, was not confined to meals and was a feature of every waking hour " Even cigarettes don't taste."* R.B.N. was not so much troubled by this symptom but felt it from time to time. He noted once that he was " Thirsty all morning—drank a lot but water seems to make little difference " and on another day reported that he had a " funny feeling in the mouth."*

On the whole all slept well but R.A.M. and R.B.N. were apt to be roused by attacks of nocturnal diuresis, and both were troubled by nightmares. Both were probably in a fairly exhausted state by the end of the deprivation period, and R.A.M. went to sleep in the laboratory about an hour and a half after his first meal containing salt. R.B.N. and R.A.M. both found it very difficult to get through their prescribed rations. R.A.M. was " never hungry "* but preferred the bread to anything else, while R.B.N. found it almost uneatable towards the end. " It's the bread that gets me down."* Nausea accompanied almost every meal but neither subject had indigestion after meals or any constipation. R.A.M. suffered considerable abdominal discomfort during and after the hot air baths on several days, but food tended to relieve it and this was certainly not the cause of the nausea.

Both the male subjects suffered considerably from cramps, but E. escaped, probably because her deficiency was relatively slight. These muscular cramps were not of the very severe localized type which are said to affect stokers and miners (Moss, 1923–24), but were widespread, frequent, not very painful, and generally controllable. Any muscle in the body was liable to go into spasmodic contraction, especially if some little effort was demanded of it (Moss, 1923–24). " Coughed a little over something, cramps round chest, mild."* R.B.N. experienced occasional cramps in the muscles lining the floor of the mouth but the extremities were most affected, and it was possible sometimes by suitable movement of the toes to alternate the spasms for hours on end between the extensor and flexor muscles. Perhaps the most characteristic of all were the manual cramps. R.A.M. experienced " constant mild cramps of the fingers and thumb when using forceps at the balance,"* and R.B.N. found that he " cramped quickly when working, telephoning, or using pipettes."*

* Diary.

Physical symptoms predominated in the case of R.A.M. but all experienced them in some degree. A mild breathlessness at first and sense of fatigue gave place later to general exhaustion and distress on the least exertion. The effort of dressing, breakfasting, and getting into and out of a car was so great that R.B.N. "lay exhausted on a couch in the common room on arriving."* R.A.M. found that going up two flights of stairs to the laboratory was a serious undertaking, causing a sense of breathlessness and a most unpleasant feeling of constriction across the sternum which compelled him to stop and rest. Throughout the experiment he used to go for a measured walk of about a mile after breakfast. Towards the end of the deficient period the breathlessness and sense of constriction forced him to sit down and rest two or three times at a hill, for which he would ordinarily not have slackened pace. Little acts of the daily routine produced a localized sense of fatigue; his "arm got tired shaving"* and finally his "jaw got tired eating toast."*

Mentally R.A.M. felt normal but R.B.N. felt "slow in the head"* and showed it in his behaviour. He would remain in a chair for periods of 30–60 minutes "content to sit and do nothing."* He never slept on these occasions and was always willing to talk, but spoke slowly and rarely initiated conversation. For several days he experienced at frequent intervals sensations of "*déjà vu.*" He became apathetic and his mental processes appeared to be dulled. R.B.N. longed for salt and often went to sleep thinking about it. R.A.M. felt no specific craving for salt and had difficulty in convincing himself that taking salt would at once make him feel all right again.

In both subjects the resting pulse rate remained normal, but the volume became very small. The pulse during exercise was not investigated. The blood pressure was taken on numerous occasions in the case of R.B.N. and rather less frequently in the case of R.A.M. Both subjects had normal blood pressures and maintained them within narrow limits throughout the experiment. No fall of systolic or diastolic pressure accompanied the salt deficiency and actually the lowest figure in each subject was obtained under normal conditions.

The eye tension of all three subjects was investigated when they were normal and at the height of the deficiency. The results which are shown in Table I indicate no consistent change. Temperatures were taken in the mouth before and during the hot air baths. They were always within normal limits before entering the bath (Marriott, 1923).

Recovery was quite dramatic. Half an hour after eating 15 gm of

* Diary.

NaCl with bread, butter, and an egg E.'s sense of flavour and taste had returned, although no fluid had been taken. This she spoke of as a quenching of her thirst. Genuine and almost unbearable thirst supervened later and was only satisfied by copious draughts of water. R.A.M. found his sense of flavour returned before he had finished his first salt meal. About an hour later he felt hot all over, he experienced a deep

TABLE I

	Normal mm Hg	During deficiency mm Hg
E.	35	25
R.A.M.	32	30
R.B.N.	25	32

seated pricking and tingling in his extremities and was conscious of bounding pulsation. In a few hours he was much more comfortable in mounting the stairs, and by evening was " no longer aware of his legs as he moved about the room."* His carriage and movements were observed to be more normal. He felt more " himself," and on the next day although not yet normal, he experienced an " exhilarating sense of well-being "* from morning till night.

R.B.N. ate his first meal containing salt in the evening and made no comment on the return of flavour and taste, but this is not surprising because this symptom had never worried R.B.N. very much. He did, however, feel as though " his heart was beating all over him " and his vaso-motor reactions were no doubt very similar to those of R.A.M. During the night he had some colic and diarrhoea, and was still breathless and very easily tired the next day. After 48 hours he " jumped off the 'bus while it was going, and ran up the stairs "*—simple pleasures, but keenly enough appreciated to make him record that he had " had a grand day."*

The Blood

All three subjects have shown similar changes, the main differences being those of degree. The appearance and behaviour of the venous blood altered as the experiment proceeded. It became dark and viscous and would not flow satisfactorily even through a large bore needle, so that some difficulty was experienced in getting the necessary samples. The red cell counts, the haemoglobin and the plasma proteins rose, and with them presumably the colloidal osmotic pressure of the plasma. The

* Diary.

concentrations of sodium and chloride in the plasma fell, and also the total osmotic pressure as indicated by a smaller depression of the freezing point. Such a syndrome has become a familiar one since experimental suprarenalectomies have been undertaken. Further, the concentration of urea in the blood rose, but not enough to maintain the total O.P. of the plasma. The alkali reserve did not change, nor did the serum potassium. There was a decrease in the corpuscular chlorides. With recovery, all the crystalloids were restored to their initial values, but the cell counts, haemoglobin and plasma proteins became subnormal for a time. The actual changes observed in R.B.N.'s experiment are shown in Table II. The depression of the freezing point was not determined in his serum. In R.A.M. it rose from $-0{\cdot}596^{\circ}$ C before the experiment began to $-0{\cdot}547^{\circ}$ at the close of the deprivation period, and fell again to $-0{\cdot}615^{\circ}$ during recovery. In E. the changes were in the same direction but smaller, and since the chemical changes of R.B.N. resembled closely those of E. and R.A.M. the determination of the F.P. was omitted. In one small respect the serum changes of the three subjects have not agreed. Considered in terms of equivalent weights R.B.N.'s chlorides fell more than his sodium. With R.A.M. and E. the reverse occurred. The greatest care was taken over the determinations and no technical reason for these differences could be found.

The Nitrogen and Potassium Balances

Balances of nitrogen, potassium, sodium, and chloride were determined on R.A.M. and R.B.N. but not on E. In both, sodium chloride deficiency produced a negative nitrogen balance which was partially masked by the retention of nitrogenous waste products as exemplified by the rise of blood urea. R.B.N.'s nitrogen balance as determined by experiment is given in Table III and it will be noticed that there was a negative balance of some 36 gm which was converted to a small positive balance during recovery. It must be emphasized that both subjects were on a carefully weighed diet and that ample food was eaten to supply their calorific requirements, so that undernutrition may be excluded as a cause of the loss of body nitrogen. The balance corrected for urea retention is shown in Table IV and indicates that 45 gm of nitrogen were lost during the deprivation and between 12 and 13 gm regained during the 7 days' recovery. Table V shows the apparent (uncorrected) nitrogen balance of R.A.M. made up in two- and three-day periods and this illustrates rather well the fact that the balance steadily became more negative as NaCl was withdrawn from the body. The corrected balance would demon-

TABLE II—R.B.N.

Period	Cell count mil/cmm	Haemoglobin % of normal	Cell vol %	Serum proteins %	Urea mg/100 cc	Chlorides mg/100 cc		Alk. res. vols %	Serum mg/100 cc	
						Plasma	Corpuscles		Na	K
Before experiment ..	5·2	101	44	6·4	31	361	204	69	340	19·4
Day of deprivation—										
9th	6·4	125	56	8·0	71	291	168	73	308	19·3
10th	5·9	115	56	7·7	84	275	160	67	302	16·2
11th and last	6·1	117	52	7·2	69	—	—	—	—	—
After recovery	4·5	93	40	6·1	25	361	218	66	342	15·0

strate this even more clearly. R.A.M. lost 33·6 gm of body nitrogen (corrected) during the deprivation period as against R.B.N.'s 45 gm and showed a small negative balance instead of a positive one over the

TABLE III—NITROGEN BALANCE. R.B.N.

Day	Intake	Output				
		Urine	Faeces	Sweat	Insensible perspiration	Blood (experimental)
	gm	gm	gm	gm	gm	gm
Deprivation Period						
1	16·3	14·8	1·05	0·88		
2	12·9	14·4	1·05	0·52		
3	13·7	13·1	1·05	0·59		
4	13·7	13·7	1·05	0·48		
5	12·6	13·6	1·2	0·68		
6	13·1	15·8	1·2	0·62		
7	13·4	10·6	1·2	0·81		
8	13·7	16·3	1·1	0·67		
9	15·3*	17·6	1·1	0·85		
10	15·2*	21·2	1·1			
11	17·0*	23·0	1·2			
		174·1	12·3	6·1	0·55	8·5
Total.....	156·9	193·1				
Recovery Period						
1	14·5	19·0	1·20			
2	15·1	16·6	1·20			
3	15·0	14·0	1·22			
4	17·6*	14·2	1·44			
5	15·6	10·4	1·44			
6	15·1	10·0	1·44			
7	15·6	10·6	1·44			
		95·4	9·4		0·6	1·2
Total.....	108·5	105·4				

* 1·8 gm of the intake on these days consisted of nitrogen taken for purposes of studying kidney function.

whole recovery period. This last is explained by the fact that R.A.M.'s recovery period lasted only 5 days while R.B.N.'s lasted 7. In all other respects the balances closely resembled each other, and may be considered in good agreement as regards essentials.

The extra nitrogen appeared to be excreted as urea. A full nitrogen partition of the urine was never investigated, but the ammonia was determined on 7 random days during R.A.M.'s experiment and found to

TABLE IV—CORRECTED NITROGEN BALANCE. R.B.N.

	Intake total gm	Output observed gm	Catabolized but retained gm	Catabolized and excreted gm	Balance gm
Deprivation period....	156·9	193·1	8·8* Excreted but not catabolized	201·9 Excreted less non-catabolized nitrogen	−45·0
Recovery period	108·5	105·4	9·7*	95·7	+12·8

* These figures are arrived at by assuming (*a*) that 60% of the total weight of the body is water; (*b*) that the concentration of urea found in the blood holds throughout all the water in the body.

TABLE V—APPARENT NITROGEN BALANCE. R.A.M.

Days	Daily intake (average) gm	Daily output* (average) gm	Balance gm
Deprivation Period			
1–3 inclusive	13·5	13·4	+0·1
4–6 inclusive	12·9	14·4	−1·5
7–9 inclusive	14·1	16·5	−2·4
10–11 inclusive†	13·2	16·5	−3·3
Recovery Period			
1–3 inclusive	18·5	22·4	−3·9
4–5 inclusive	17·9	16·3	+1·6

* The output includes all sources except blood taken for analytical purposes.

† The last day of the deprivation period was not a full 24 hours. For the construction of this table the actual output for 19 hours has been multiplied by 24/19. The intake for the missing meal has been assumed to be the same as the intake on the previous day.

Applying a correction for the retained urea would increase the negative balance of the latter part of the deprivation period and decrease the negative balance of the early part of the recovery period.

lie between 0·5 and 0·8 gm per day, which is a normal range. The creatinine was determined every day. The results are given in Table VI as creatinine N. and show that the daily excretion maintained its normal constancy. Hartwell and Hoguet (1912) had recorded that dogs with

intestinal obstruction excreted large amounts of creatine, and this substance therefore was looked for on several days, but was found to be absent, or present in traces only. Much more urea, however, was excreted as the deficiency developed, and further, the urea nitrogen expressed as percentage of the total nitrogen tended to rise as the deficiency developed and fall again with recovery.

TABLE VI—DAILY EXCRETION OF CREATININE N. R.A.M.

Deprivation Period				*Recovery Period*	
Day	Creatinine N excreted gm	Day	Creatinine N excreted gm	Day	Creatinine N excreted gm
1	0·60	6	0·55	1	0·62
2	0·56	7	0·62	2	0·57
3	0·58	8	0·57	3	0·62
4	0·55	9	0·61	4	0·62
5	0·58	10	0·54	5	0·60
		11*	0·49		

* Excreted during 19 hours.

A large negative nitrogen balance would almost certainly be accompanied by a negative potassium balance. A loss of 45 gm of nitrogen from muscle might be expected to set free about 4500 mg of potassium, an amount rather difficult to detect under these experimental conditions, where the intake was of the order of 33,000 mg. R.B.N.'s potassium balance is shown in abridged form in Table VII and while no stress can be laid upon the result it shows a negative balance of 3600 mg during the experimental period and a positive balance of 580 mg during recovery. These figures are of the right order to tally with the nitrogen balances. R.A.M.'s potassium balance did not show such close agreement with his nitrogen balance. There was a negative balance of 560 mg during the deprivation period and a positive balance of 1660 mg during recovery, results qualitatively agreeing with those of R.B.N. but possibly of no significance. The question of the relation of the potassium to the nitrogen balance must be left for the time being *sub judice*.

The Sodium and Chloride Balances

Table VIII shows the sodium balance obtained on R.A.M. During the deprivation period the intake was about 40 mg a day. The output from all measured sources is shown in detail and the relative importance

TABLE VII—POTASSIUM BALANCE. R.B.N.

	Total intake mg	Output mg						Balance
		Urine	Faeces	Sweat	Insensible perspiration	Blood for experimental purposes	Total (excluding blood)	
Deprivation period (11 days)	33,244	24,645	7250	4550	417	370	36,862	−3618
Recovery period (7 days)..	24,342	18,060	5010	—	692	72	23,762	+580

of each may be seen by inspection. The net loss was 17,540 mg. It is unfortunately very difficult to assess the total amount of sodium in an adult's body, but there is no doubt that 17,540 mg is a large fraction of the whole (*vide infra*). The net gain during the recovery period exceeded the loss during the deprivation period by 3525 mg. R.A.M., however,

TABLE VIII—SODIUM BALANCE. R.A.M.

Deprivation Period

Day No.	Intake mg	Output, mg				
		Urine	Faeces	Sweat	Insensible perspiration	Blood (for tests, etc.)
1	58	3160	125			
2	40	1300	125	2680		
3	40	378	125	3000		
4	51	133	125			
5	51	66	50	2500		
6	34	31	50			
7	35	26	50			
8	41	25	50	1900		
9	38	11	76			
10	36	17	76	880		
11	34	10	76			
Totals	458	5157	936	10,960	690	255
				17,998		

Net loss: 17,540.

Recovery Period

1	5911	22	43			
2	5599	22	43			
3	5408	370	43			
4	5899	1450	43			
5	5218	4520	44			
	28,035	6384	216	—	370	—
				6970		

Net gain: 21,065.

was 1·03 kg heavier at the close of the experiment than he was when it began. It is most unlikely that this was a gain in weight of muscle or of any other organ owing to the simultaneous loss of nitrogen. It may have been fat but there is no evidence for this and was much more probably extra-cellular water (Adolph, 1921) and this is supported by the dilution

of the blood towards the close of the recovery period. A gain of 1·03 kg of extra-cellular water would require a retention of about 3400 mg of sodium, so that the corrected balance sheet agrees to a few mg. Thus:—

	mg		mg
Net loss	17,540	Net gain during recovery	21,065
		Deduct for gain in weight of 1·03 kg	3,400
	17,540		17,665

R.B.N.'s balance was also a good one. A net loss of 22,516 mg took place during the deprivation period which was greater than R.A.M.'s but considered in relation to his greater body weight, the two results are nearly the same. A gain of 21,514 mg accompanied recovery. There was, therefore, a negative balance of 1002 mg on the whole experiment. R.B.N. lost 1·06 kg during this time, but since there was also a loss of 30 gm of nitrogen, some of this loss of weight may have been loss of muscular tissue. No correction, therefore, for the change in weight has been applied.

Table IX shows R.A.M.'s chloride balance expressed in terms of Cl. A loss of 20,577 mg of chloride during deprivation was followed by a gain during recovery of 30,296, and even correcting the latter for the increase in weight, the gain during recovery comes to 26,696 mg, considerably more than the previous loss. Considering the care with which the experiment was conducted and the proximity of the sodium balance, this discrepancy cannot be attributed to experimental error. R.B.N.'s chloride balance, moreover, was essentially the same in all important respects and showed a similar discrepancy. The facts may be presented in another way as has been done in Table X. There it will be seen that in both subjects the *losses* of the two elements proceeded *pari passu* with their relative concentrations in serum, *i.e.*, more sodium than chloride was lost. During recovery neutral sodium chloride was taken by mouth and it was this salt, *i.e.*, sodium and chloride in equivalent amounts, which was taken up and retained by the tissues. Thus it follows that 160 milli equivalents of sodium in R.A.M.'s body, which had at the beginning of the experiment been combined with ions other than chloride, were combined with this ion as the neutral salt when the experiment terminated. The excess of chloride was no doubt excreted as the ammonium salt as time went on. It has been pointed out by a number of people that the basic ions are of much greater

TABLE IX—CHLORINE BALANCE. R.A.M.

Deprivation Period

Day No.	Intake as Cl mg	Output as Cl, mg				
		Urine	Faeces	Sweat	Insensible perspiration	Blood
1	90	3900	84			
2	224	1200	84	3460		
3	165	550	84	3800		
4	359	79	84			
5	284	42	55	3430		
6	141	24	55			
7	173	30	55			
8	212	26	55	2770		
9	153	12	42			
10	244	24	42	1230		
11	118	11	42			
	2163	5898	682	14,690	1080	390
				22,740		

Net loss: 20,577

Recovery Period

1	9185	22	79			
2	8474	110	79			
3	8224	760	79			
4	9137	3400	79			
5	8163	7530	79			
	43,183	11,822	395	—	670	—
				12,887		

Net gain: 30,296.

TABLE X

All figures for losses and gains are given in milli equivalents

	Deprivation Period				*Recovery Period*			
	Na lost	Cl lost	Na/Cl ratio	Na/Cl ratio in serum	Na gained	Cl gained	Na/Cl ratio	Na/Cl ratio in NaCl
R.A.M.....	765	590	1·30	1·41	770	750	1·02	1·00
R.B.N.....	980	760	1·29	1·41	940	980	0·96	1·00

significance to the body than the acidic, and these results provide further evidence that this is so.

The Water Balance

Water was taken as desired by all three subjects, and the urine volumes were from 1500 to 2000 cc per day. No attempt was made to measure the intake and output of water owing to the difficulty in assessing the amount taken with the food and of measuring the amount lost through the lungs, but in short term experiments such as these the fluctuations in

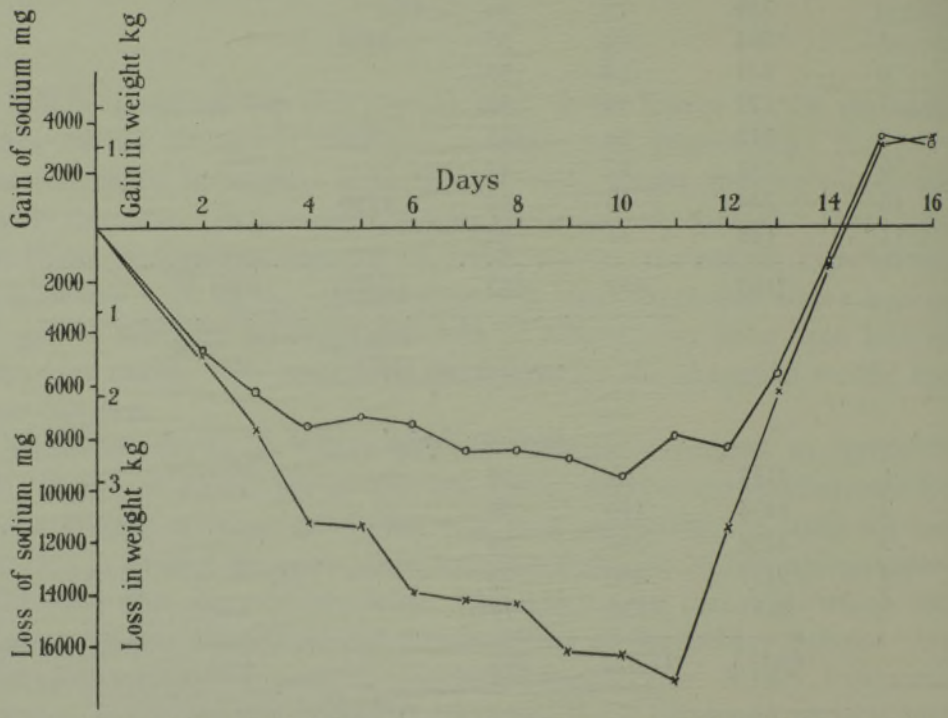

FIG. 1—The weight scale and the sodium scale are so constructed that 1 kg in weight ≡ 3300 mg of sodium. ○ weight; × sodium.

body weight may be taken as a rough measure of the variations in body water. The weights were always taken for this purpose under standard conditions—just before lunch, naked, and with an empty bladder—and all three subjects showed, as was to be expected, a considerable loss. Since this loss of water was brought about solely by a loss of salt (or more correctly of sodium ions) the relation of the former to the latter is important, and is shown in fig. 1. The two scales are so constructed that a loss of 1 kg of water is equal to a loss of 3300 mg of sodium, which is the amount of sodium present (approximately) in a litre of serum or other extra-cellular fluid. It will be noted that at first a removal of sodium was

followed *pari passu* by a loss of an equivalent amount of water. So long as this was so, red cell counts may have risen and other signs of anhydraemia appeared, but *no change in the osmotic pressure* of the body fluids had taken place. Further forced removal of sodium was *no* followed by an equivalent loss of water. The volume of the body fluids was relatively well maintained but *only at the expense of the osmotic pressure*, which fell. Normal fluid regulation (Adolph, 1921) during this stage was noted by both R.A.M. and R.B.N. to be very defective. Copious drinks of water were apt to be followed by no diuresis at the expected time. Hours later, during the night or even the next day, the diuresis would begin. This gave rise to curious weight fluctuations of which the rise in weight between days 10 and 11 is an example. R.B.N.'s weight altered widely from day to day during the worst of his deficiency and the changes bore no relation to those of the sodium. The first effect of restoring sodium was to produce a loss of weight (day 12). R.B.N.'s loss was much greater at this stage and his lowest weight was recorded the day after he began taking salt. Further restoration of salt led to minor retentions of water, till the level was reached at which the salt and water losses had parted company. After this point their restoration proceeded together.

Discussion

Before opening any discussion it is most important to emphasize once again that water was freely taken by all the subjects of these experiments, and that the urine volumes were large, considering the amount of water lost in the sweat. Loss of water therefore was fully compensated and the experiments show the effects of salt loss without water loss. Such clear cut experimental conditions have never been met with in man and have seldom been achieved in animals, and it is clear from the occasions when they have been, that there are probably important differences in the way in which lower orders react. Rabbits, for example, lose no weight (Michelsen, 1933). The mental confusion that undoubtedly exists over the relationship between salt loss and water loss and their effects must be attributed to the fact that most attempts to study the problem have been made on patients or animals suffering from conditions in which there was a forced loss both of salt and water and in which the latter was not fully replaced. It is hoped to review the whole literature bearing on this question in the Goulstonian lectures in 1936.

From fig. 1 it is clear that in man the removal of salt leads first to a loss of body water with little change in extra- or intra-cellular osmotic pressure. Some rise of red cell count and concentration of the organic

constituents of the plasma probably take place. Further withdrawal forces the body into a compromise between (*a*) the maintenance of the total osmotic pressure of the extra-cellular fluids, which must lead to progressively severe anhydraemia and a steady rise in the colloidal O.P. of the plasma; (*b*) the maintenance of the plasma volume at the expense of a fall in its total O.P., which may lead to a fall in the total and colloidal O.P. of the cell fluids. The red blood cells may swell owing to their hypotonic surroundings (Lavietes *et al.*, 1935) and further embarrass the capillary circulation, and, so far as space allows them, other cells of the body may also swell. Thus in experimental suprarenal deficiency (Loeb *et al.*, 1935), when the blood changes are very similar to those met with in the present experiments, the muscles and other organs have been found to contain more water than the controls and the excretion of ingested water to be very much delayed and abnormal (Silvette and Britton, 1933; Silvette, 1934; Swingle *et al.*, 1934). It may have been changes of this sort which caused the large weight fluctuations during the worst period of R.B.N.'s deficiency and it may have been the return of intracellular fluid to the vessels and its excretion which caused both subjects to lose weight on first taking salt. If the total osmotic pressure of the plasma falls and the cells are unable to swell, then an O.P. gradient must be set up between the cells and their surroundings (assuming for the moment no passage of kations takes place across the cell membranes). Neither the anhydraemia nor the loss of weight are in themselves any measure of the degree of a sodium deficiency. The anhydraemia may even improve for a time (and the weight increase) if the organism is prepared to put up with a further fall in its total extracellular, and possible intracellular, osmotic pressure.

These results have practical application. The loss of body weight which can be produced by sweating is limited in amount. Boxers and rowing coxes who may resort to this method should not only realize this, but also that even a small reduction produced in this way may be very detrimental to efficiency.

The Symptoms and Signs

The rapid restoration of the sense of flavour on taking salt points to its loss being one of the effects of hyponatraemia or hypochloraemia. It is probably not connected with the anhydraemia. Clinical observation supports this. Patients with intestinal obstruction complain of dreadful thirst. It is said that small injections of hypertonic saline,* too small to

* The author is indebted to Mr. J. B. Hunter for this observation.

have any effect on the general anhydraemia, may relieve the patient's symptom which may have been not so much thirst as this aberration of sensation. The cramps are probably allied to the so-called stokers' cramps and may also be attributed to the low osmotic pressure of the serum. The cardiovascular symptoms should probably be regarded as the result of the anhydraemia, but very little is known as yet of the effects of anhydraemia without simultaneous sodium deficiency. The reduction in blood volume, however, and the increased viscosity of the blood must decrease the efficiency of the gas exchanges, for (1) the circulation rate may be lowered, a matter which is being investigated; (2) only a fraction of the normal number of capillaries may be in action; (3) there may be some anoxic anoxaemia. The oxygenation of the venous blood is certainly subnormal (Haden and Orr, 1927, *b*). The arterial blood has not yet been investigated in man, but Herrin (1935) has found it to be incompletely oxygenated in dogs under similar conditions. The breathlessness, distress, and fatigue can be explained along these lines. It is interesting to speculate how much of the Addisonian asthenia and fatigue may be due to the sodium deficiency and how much, therefore, of the Addisonian syndrome may be experimentally reproduced in this way in man. The symptoms and many of the signs are undoubtedly very similar but there are also points of difference which are equally significant. Experimentally, for example, the blood pressures do not seem to fall. Symptoms of minor salt deficiency appear to be common enough in the tropics (McEwen, 1935).

The Negative Nitrogen Balance

This is probably the result of the cardiovascular embarrassment, but there is no proof that the fall in osmotic pressure may not, *per se*, so upset the metabolism of the cells that their protoplasm begins to disintegrate.

A negative balance was claimed by early workers, on slender evidence (Spiegler, 1901; Marriott, 1923), to follow water deprivation. It has repeatedly been shown to accompany experimental intestinal interference in animals (Whipple, Cooke, and Stearns, 1917; Haden and Orr, 1927, *a*, and Brandberg, 1929) and often attributed to toxaemia. It has been reported clinically in cases of gastric tetany (Steinitz, 1928), but the difficulty of maintaining an adequate nitrogen and calorie intake in such cases must not be overlooked. The nitrogen balance was investigated in these experiments because (Michelson, 1933) it was thought that a simple salt deficiency might make it negative, and since it did so there does not seem to be any need to invoke a toxaemia to explain the negative balance in the intestinal conditions.

The Percentage Loss of Sodium and Chloride

The difficulty in estimating this lies in the uncertainty about the total amount of these elements in an adult's body. No direct determinations seem to have been made. According to Darrow and Yannett (1925) and Lavietes *et al.* (1935), there is little sodium, if any, except in the extracellular fluids which constitute 20% of the body weight. The same is true of chlorine if one excepts the red blood corpuscle. Calculating, therefore, that 1 litre of extracellular fluid contains 3·3 gm of sodium, R.A.M. contained 43 gm of sodium when the experiment began. He lost 17·5 gm, *i.e.*, 41%. Calculating the chloride in the same way R.A.M. contained 47 gm of chloride, say 50 gm making a small allowance for the chloride in the red cells. He lost 20·8 gm, *i.e.*, 41·5%. According to Winter (1934) rats contain about 0·12% of chloride (as Cl). Rosemann (1910) whose figures were accepted by Lim and Ni (1925–26) found that adult dogs contained 0·11% of chloride and new born dogs rather more than 0·2%. Human foetuses contained between 0·197 and 0·29% and several workers quoted by Rosemann found new born infants to contain 0·146–0·189% of chloride, but adults certainly contain less. Magnus Levy (1910) suggested 0·1227% from an analysis of individual organs and Rosemann endorsed this. Assuming these figures to be true, R.A.M. would contain about 81 gm of chloride and would have lost on this basis of calculation 26% during his experiment.

The lower would seem to be the more probable answer, so that R.A.M. may be said to have lost between 25 and 30% of his extracellular ions. R.B.N.'s losses were of the same order but very slightly higher.

The Function of the Kidney

A rise in the blood urea in cholera was suggested in 1832 (O'Shaughnessy, 1831–32); it was noted in diabetic coma by Colin in 1868 and in intestinal obstruction in 1914 by Tileston and Comfort. It has been extensively investigated clinically and experimentally in the last 20 years and is known to be a part of these and similar diseases (Addison's disease) in which loss of extracellular base and (or) water are prominent features. It was hoped to reproduce this rise in blood urea by simple salt deprivation and one of the main objects of the present experiments was to do so and to study its causation. Table II shows that the primary object was achieved, and the whole question has accordingly been investigated. The results are now being collected for publication, but since it is

impossible to discuss them without extensive reference to the literature it is proposed to publish them in a separate paper.

Every thanks are due to Miss Edwards and R. B. Niven for their whole-hearted participation in these experiments. The author is also indebted to Miss Widdowson for a great deal of valuable help at almost every stage and to his wife and Mrs. MacGregor for their meticulous care in the preparation of the food. Professor J. B. S. Haldane has been good enough to go through the manuscript.

Summary

Forced loss of sodium and chlorine was produced by a very low NaCl intake and sweating. At least 25 to 30% of the body's extracellular ions were removed in this way. The fluid intake was not limited.

Such deprivation led to aberrations of flavour, cramps, weakness, lassitude, and severe cardio-respiratory distress on exertion.

The nitrogen balance became negative and the blood urea rose.

When subjected to such treatment the human body compromised between (*a*) maintenance of its total osmotic pressure at the expense of anhydraemia a reduction of blood volume, rise of haemoglobin, proteins and colloidal osmotic pressure in the serum, and (*b*) maintenance of its plasma and extracellular fluid volumes at the expense of a reduction in the concentrations of sodium and chloride in the serum, with a fall in its total osmotic pressure. Some evidence was obtained that (*b*) was followed by a fall in the total and colloidal O.P. of the cells.

References

Adolph, E. F. (1921). 'J. Physiol.,' vol. **55**, p. 114.
Beattie, F. (1928). 'Biochem. J.,' vol. **22**, p. 711.
Brandberg, R. (1929). 'Act. chir. scand.,' vol. **65**, p. 415.
Colin (1868). 'Gaz. hebd. méd. Chir. Ser. 2,' vol. **5**, p. 467.
Darrow, D. C., and Yannet, H. (1935). 'J. Clin. Invest.,' vol. **14**, p. 266.
Haden, R. L., and Orr, T. G. (1927, *a*). 'J. Exp. Med.,' vol. **45**, p. 433.
—— (1927, *b*). 'J. Exp. Med.,' vol. **46**, p. 709.
Harrison, G. A. (1930). "Chemical Methods in Clinical Medicine," 1st ed.
Hartwell and Hoguet (1912). 'J. Amer. Med. Ass.,' vol. **59**, p. 82.
Hawk, P. B. (1923). "Practical Physiological Chemistry," 8th ed.
Herrin, R. C. (1935). 'J. Biol. Chem.,' vol. **108**, p. 547.
Hubbard, R. S. (1933). 'J. Biol. Chem.,' vol. **100**, p. 557.
Kramer, B., and Tisdall, F. K. (1921). 'J. Biol. Chem.,' vol. **46**, p. 339.
Lavietes, P. H., D'Esopo, L. M., and Harrison, H. E. (1935). 'J. Clin. Invest.,' vol. **14**, p. 251.

Lim, R. K. S., and Ni, T. G. (1925–26). 'Amer. J. Physiol.,' vol. **75**, p. 475.
Loeb, R. F., Atchley, D. W., and Stahl, J. (1935). 'J. Amer. Med. Ass.,' vol. **104**, p. 2149.
McCance, R. A., and Shipp, H. L. (1931). 'Biochem. J.,' vol. **25**, pp. 449, 1845.
—— (1933). 'Special Rep. Med. Res. Coun. Lond.,' No. 187.
McCance, R. A., and Watchorn, E. (1931). 'Quart. J. Med.,' vol. **24**, p. 371.
McCance, R. A., Widdowson, E. M., and Shackleton, L. R. B. *Unpublished.*
McEwen, O. R. (1935). 'Lancet,' vol. **1**, p. 1015.
Magnus Levy, A. (1910). 'Biochem. Z.,' vol. **24**, p. 363.
Marriott, W. M. (1923). 'Physiol. Rev.,' vol. **3**, p. 275.
Michelsen, J. (1933). 'Arch. exp. Path. Pharmak.,' vol. **173**, p. 737.
Moss, K. N. (1923–24). 'Proc. Roy. Soc.,' B, vol. **95**, p. 181.
O'Shaughnessy, W. B. (1831–32). 'Lond. Med. Gaz.,' vol. **9**, p. 486.
Rosemann, R. (1910). 'Pflügers Arch.,' vol. **135**, p. 177.
Silvette, H. (1934). 'Amer. J. Physiol.,' vol. **108**, p. 535.
Silvette, H., and Britton, S. W. (1933). 'Amer. J. Physiol.,' vol. **104**, p. 399.
Spiegler, A. (1901). 'Z. Biol.,' vol. **41** (N.F., vol. **23**), p. 239.
Steinitz, H. (1928). 'Z. klin. Med.,' vol. **107**, p. 560.
Swingle, W. W., Pfiffner, L. J., Vars, H. M., and Parkins, W. M. (1934). 'Amer. J. Physiol.,' vol. **108**, p. 428.
Tileston, W., and Comfort, C. W. (1914). 'Arch. Intern. Med.,' vol. **14**, p. 620.
Van Slyke, D. D. (1923–24). 'J. Biol. Chem.,' vol. **58**, p. 523.
Whipple, G. H., Cooke, J. V., and Stearns, T. (1917). 'J. Exp. Med.,' vol. **25**, p. 479.
Widdowson, E. M., and McCance, R. A. (1935). 'Lancet,' vol. **1**, p. 1437.
Winter, K. A. (1934). 'Klin. Wschr.,' vol. **13**, p. 1454.
—— (1934). 'Z. ges. exp. Med.,' vol. **94**, p. 663.