2. With β : α ratios varying from 4 : 1 to 1 : 4, the action of the two enzymes is truly additive up to 20–25% hydrolysis; beyond this point it may be equal to, greater than or less than the sum of individual actions, depending on substrate conditions.

3. The significance of these facts in the assessment of amylolytic activity is discussed.

REFERENCES

American Society of Brewing Chemists (1944). *Methods of Analysis, Malt*-6, 4th ed. Wisconsin: A.S.B.C.
Blom, J. & Rosted, C. O. (1947). *Acta chem. Scand.* **1**, 32.
Claus, W. D. (1947). *Cereal Chem.* **24**, 59.
Freeman, G. G. & Hopkins, R. H. (1936). *Biochem. J.* **30**, 446.
Graesser, F. R. & Dax, P. J. (1946). *Wallerstein Lab. Comm.* **9**, 43.
Hopkins, R. H., Murray, R. H. & Lockwood, A. R. (1946). *Biochem. J.* **40**, 507.
Institute of Brewing (1933). *J. Inst. Brew.* **39**, 517.
Kneen, E. & Sandstedt, R. M. (1941). *Cereal Chem.* **18**, 237.
Kneen, E., Sandstedt, R. M. & Hollenbeck, C. M. (1943). *Cereal Chem.* **20**, 399.
Lampitt, L. H., Fuller, C. H. F., Goldenberg, N. & Green, G. H. (1947). *J. Soc. chem. Ind., Lond.*, **66**, 68.
Preece, I. A. (1947). *J. Inst. Brew.* **53**, 154.
Preece, I. A. (1948). *J. Inst. Brew.* **54**, 141.

Changes in the Extracellular and Intracellular Fluid Phases of Muscle During Starvation and Dehydration in Adult Rats

By S. E. DICKER (Beit Memorial Fellow), *Department of Pharmacology, University of Bristol*

(*Received* 28 *July* 1948)

It has been shown in a previous paper (Dicker, 1948) that rats fed on a protein-deficient diet develop tissue oedema very rapidly, i.e. the extracellular fluid phase of muscle and liver, as estimated in terms of chloride space, increases. The oedema starts at a time when the plasma protein concentration and the plasma colloid osmotic pressure are still normal. It seemed, therefore, of interest to investigate changes in the extracellular fluid phase of muscle in rats undergoing inanition or dehydration over short periods in which the plasma protein concentration was not decreased.

METHODS

Experimental animals. Adult male and female albino rats were used, of body wt. 250–320 g.

Diet. Some weeks before the experiment the animals were fed on a commercially prepared diet containing wheat offal 17·7, ground barley 8·8, white-fish meal 4·5, meat and bone meal 8·8, dried skimmed milk 14·0, dried yeast 1·2, salt 0·4, and cod-liver oil 0·4%. The total N content of the diet amounted to 3·26%, and its water content to 10·5%. The mineral content by analysis was 0·344 g. Cl, 0·210 g. Na, 4·100 g. K/100 g. and the calorific value 306·0 cal./100 g. During the period of experimentation the same type of food, but completely dehydrated, was given to one series of rats.

Analytical procedures. The following data were determined in each rat: (*a*) the content of water, chloride, sodium, potassium and nitrogen of heparinized plasma (in some cases, the urea concentration of plasma was also estimated); (*b*) the content of water, chloride, sodium and potassium of muscle samples (the muscle used was the rectus abdominis); (*c*) the concentration of Cl^-, Na, K and N in the urine; urea and NH_3 in the urine were estimated in some cases.

Water content, Cl^-, Na and K concentrations were estimated in tissue and plasma samples, in the same manner and by the same methods as described in a previous communication (Dicker, 1948). Urea in plasma was estimated colorimetrically according to the method of Lee & Widdowson (1937), and the N content of plasma was determined by a micro-Kjeldahl method.

In the urine, Cl^- was estimated according to Volhard (1878), and Na and K by the method of McCance & Shipp (1933). NH_3 was adsorbed on permutit and estimated after nesslerization (Folin & Bell, 1917), and the urea determination followed the method of Scott (1940). The specific gravity of urine was determined in the apparatus of Heller (1940), using a mixture of carbon tetrachloride and light petroleum. All data for muscle are expressed per 100 g. fat-free tissue (see Hastings & Eichelberger, 1937).

Estimation of the extracellular and intracellular fluid phases of muscle. The extracellular and intracellular fluid phases of muscle were calculated on the assumption that all the Cl^- is extracellular, and that its concentration is that of an ultrafiltrate of serum (Fulton, 1947). The volume of the extracellular fluid phase was determined by calculation (Hastings & Eichelberger, 1937).

The amount of potassium in the intracellular fluid phase was calculated as follows:

$$\text{m-equiv. intracellular K/kg. muscle} = (K)_M - (H_2O)_F \times (K)_F,$$

where $(K)_M$ = m-equiv. K/kg. muscle, $(K)_F$ = m-equiv.

K/kg. extracellular water $= 0{\cdot}95 \times (K)_s$, where $(K)_s$ = m-equiv. K/kg. plasma water, $(H_2O)_F$ = g. extracellular water/kg. muscle $= 0{\cdot}99 \times F$, where F = extracellular fluid phase as calculated from the chloride space. The amount of Na in the intracellular fluid phase was similarly calculated (Fulton, 1947).

Response to water administration (water diuresis). Starved and dehydrated rats were given an amount of water equal to 5% of their body wt. by stomach tube, and their urinary excretion was compared with that of normal rats. The evening before the tests the normal rats were deprived of food and water. Urine was collected in graduated cylinders; the urinary volumes were recorded at 15 min. intervals and expressed as percentages of the amount of water administered. The urine collection was continued over a period of 2 hr.

General procedure. Two series of rats were investigated: (*a*) rats fed on a dry diet, without any supply of water; (*b*) rats which were allowed a free supply of water, but no food. No experiment lasted more than 6 days. During the period of observation rats were kept in individual metabolism cages. Urine was collected under paraffin, and the contamination of urine by faeces was avoided by using glass separators. At the end of the period of observation, the animals were either killed under ether anaesthesia, and blood and tissue samples were taken and analyzed, or they were given a standard amount of water to drink and killed after their water diuresis had been followed for 2 hr.

Statistical treatment. Results are given as means and standard error. Student's '*t*' test (Fisher, 1944) for small samples was used for estimating the significance of means. The probability P for t was obtained from the Tables of Fisher & Yates (1943).

RESULTS

Rats without food, but with free access to water

The intake of water and the urinary excretion were measured daily. The amount of water drunk varied from day to day and from animal to animal, but in spite of important individual variations (Tables 1 and 3), at the end of the 6 days of observation, the average amount of water drunk over 24 hr. had fallen from 4·5 ml./100 g. body weight in the controls to 3·3 ml./100 g. body weight. This finding agrees with that of Adolph (1947).

Table 1 shows a typical experiment on two rats starved for 6 days. It will be noted that the amounts of water drunk and the amounts of urine excreted were not only irregular, but did not seem to bear any relation to each other. It will also be noted that in spite of the water drunk, the urine remained more concentrated than in the controls where the sp.gr. was found to be $1{\cdot}017 \pm 0{\cdot}0016$ (Heller, 1949). Towards the end of the experiment there was a fall in the urinary concentration of sodium, chloride and potassium, but not in that of nitrogen.

After 3 days without food, but with free access to water, the plasma protein concentration and plasma water content, as well as the concentration of chloride, sodium, and potassium in plasma, were comparable with those of controls. The total muscle water content was normal, but the concentrations of chloride and sodium in muscle were increased ($t = 4{\cdot}761$, $P < 0{\cdot}001$ and $t = 2{\cdot}020$, $P < 0{\cdot}1 > 0{\cdot}05$ respectively); this resulted in an increase of the extracellular fluid phase; it amounted to $20{\cdot}3 \pm 1{\cdot}41$ ml./100 g. fat-free tissue, as compared with $16{\cdot}7 \pm 0{\cdot}50$ ml./100 g. in controls ($t = 2{\cdot}978$, $P < 0{\cdot}01$). Chloride and sodium space, however, remained comparable in size ($t = 0{\cdot}585$, $P > 0{\cdot}5$), as in normal animals. Concurrent with the increase of the extracellular fluid phase of muscle, there was a decrease of the intracellular fluid phase ($t = 2{\cdot}403$, $P < 0{\cdot}05$), though its concentration of water remained unchanged (Table 2).

After 6 days with water, but without food, the plasma concentrations of proteins, chloride, and sodium were still in the normal range (Table 2), but there was an increase in the plasma concentration of potassium as compared with that of

Table 1. *The effect of withdrawing food from rats with free access to water on body weight and volume and composition of urine*

Rat no.	Day	Wt. of rat (g.)	Water drunk (ml./100 g./24 hr.)	Urine excreted (ml./100 g./24 hr.)	Urine (sp.gr.)	Urine (g./100 ml.) Cl⁻	Na	K	N
23	1	272	—	—	—	—	—	—	—
	2	268	2·61	1·86	1·049	0·290	0·150	1·056	0·55
	3	246	0·00	2·00	1·047	0·272	0·131	1·006	0·56
	4	231	3·00	2·16	1·046	0·187	0·110	1·420	0·75
	5	228	4·02	1·91	1·040	0·049	0·043	0·900	—
	6	220	5·45	2·70	1·040	0·045	0·045	1·100	0·54
	7	210	2·86	1·52	1·038	0·101	0·079	0·718	0·59
24	1	355	—	—	—	—	—	—	—
	2	340	0·88	1·76	1·056	0·309	0·196	1·030	0·80
	3	315	0·00	2·06	1·057	0·256	0·158	1·230	0·64
	4	297	1·68	1·69	1·051	0·320	0·125	1·600	0·77
	5	286	1·71	2·45	1·046	0·129	0·122	1·520	1·25
	6	262	1·14	3·00	1·049	0·142	0·114	0·940	0·82
	7	245	0·81	2·98	1·043	0·187	0·100	0·703	0·53

Table 2. *Effects of dehydration and of inanition on the composition of plasma, liver and muscles in rats*

(Number of rats indicated in brackets.)

	Plasma					Liver
	Water content (%)	Proteins (g./100 ml.)	Cl^- (m-equiv./l. plasma water)	Na (m-equiv./l. plasma water)	K (m-equiv./l. plasma water)	water content (%)
Normal rats	91·4±0·13 (27)	7·0±0·09 (27)	93·2±2·34 (27)	135·3±7·05 (18)	5·6±0·29 (17)	71·4±0·31 (27)
Rats deprived of food but with free access to water:						
(*a*) For 3 days	91·8±0·41 (9)	7·1±0·21 (9)	95·1±4·68 (9)	142·1±5·92 (9)	5·5±0·18 (9)	71·0±0·49 (9)
(*b*) For 6 days	92·1±0·14 (12)	7·0±0·17 (11)	93·1±4·24 (12)	141·7±2·64 (12)	6·4±0·18 (12)	72·0±0·54 (12)
Rats allowed dry food but no water for 6 days	91·1±0·10 (12)	7·6±0·08 (12)	103·1±3·24 (12)	156·9±6·83 (12)	10·0±0·52 (12)	71·6±0·39 (12)
Rats with free access to dry food for 5 days, and allowed water on the 6th day	92·1±0·04 (9)	7·2±0·07 (9)	84·5±1·91 (9)	104·3±0·75 (9)	5·8±0·22 (9)	75·0±0·53 (9)

	Muscle							
	Water content (%)	Cl^- (m-equiv./l. muscle water)	Na (m-equiv./l. muscle water)	K (m-equiv./l. muscle water)	Extracellular fluid phase (ml./100 g.)	Intracellular fluid phase (ml./100 g.)	Water content/100 g. intracellular fluid phase	Intracellular K (m-equiv./kg. muscle)
Normal rats	75·7±0·32 (27)	20·2±0·47 (27)	28·8±1·48 (17)	130·2±7·73 (17)	16·7±0·50 (27)	59·3±0·50 (27)	71·2±0·36 (27)	97·2±4·2 (17)
Rats deprived of food but with free access to water:								
(*a*) For 3 days	76·5±0·11 (9)	25·2±1·06 (9)	33·7±3·20 (9)	113·3±4·50 (9)	20·3±1·41 (9)	56·2±1·44 (9)	70·5±0·56 (9)	90·0±3·1 (9)
(*b*) For 6 days	76·7±0·44 (12)	28·0±1·56 (12)	47·5±3·09 (12)	110·6±2·60 (12)	22·3±3·40 (12)	54·8±1·14 (12)	69·5±1·00 (12)	83·7±2·15 (12)
Rats allowed dry food but no water for 6 days	73·3±0·20 (12)	23·2±1·96 (12)	42·6±7·64 (12)	117·4±3·20 (12)	15·6±0·31 (12)	57·5±0·53 (12)	68·2±0·33 (12)	87·3±3·88 (12)
Rats with free access to dry food for 5 days, and allowed water on the 6th day	78·7±0·35 (9)	27·1±1·88 (9)	41·5±2·25 (9)	117·0±8·83 (9)	22·4±1·85 (9)	56·3±1·70 (9)	72·5±0·90 (9)	91·2±1·20 (9)

controls ($t = 2{\cdot}045$, $P = 0{\cdot}05$). The total amount of muscle water was not significantly different from that of normal animals, but the extracellular fluid phase, expressed in terms of chloride space, was markedly increased; it amounted to $22{\cdot}3 \pm 3{\cdot}40$ ml./100 g. fat-free tissue instead of 16·7 ml./100 g. in controls. In contrast with normal rats, in which chloride and sodium occupied a comparable space (Dicker, 1948), the sodium space in this series of rats was greater than that of chloride ($t = 2{\cdot}028$, $P < 0{\cdot}1 > 0{\cdot}05$), indicating that sodium had penetrated into the muscle cells. The amount of muscle potassium decreased significantly as compared with controls ($t = 3{\cdot}023$, $P < 0{\cdot}01$).

There was thus clear evidence of tissue oedema in the starved rats with access to water. It will be noted, however, that the standard error of the calculated mean value for the extracellular fluid phase was much greater than that of controls (Table 2): the coefficient of variation of the extracellular fluid phase in the series of rats starved for 6 days amounted to $52{\cdot}8 \pm 10{\cdot}80$ %, as compared with $15{\cdot}0 \pm 2{\cdot}04$ % in normal rats (standard error of difference = 3·44). This significant increase of the coefficient of variation was correlated with the variability in the amount of water drunk by these rats. Table 3 shows changes in the body weight of two rats compared with the amount of water drunk and that of urine excreted per 100 g./24 hr. during 6 days of starvation. It will be seen that the amounts of urine/100 g. body weight/24 hr. were comparable, but that the amounts of water drunk were very different: they averaged 3·1 ml./100 g./24 hr. in one rat, and only 0·6 ml./100 g./24 hr. in the other. This resulted in a discrepancy in the decrease of body weights. The loss of body weight amounted to 24·8 and 33·0 % respectively. Concurrently with the discrepancy in the water load, the plasma ionic concentration, the muscle water content and the extracellular fluid phases of these two rats were markedly different (Table 3). These differences suggested that there was a failure in the mechanism of water excretion. To investigate this hypothesis, a standard amount of water was administered to rats which, though starved, had free access to water. Table 4 shows the renal response to water

Table 3. *The effect of withdrawing food from rats with free access to water on body weight, plasma and muscle composition*

(All values for muscle are expressed/100 g. fat-free tissue.)

Rat no.	Day	Decrease in body wt. (%)	Water drunk (ml./100 g./24 hr.)	Urine excreted (ml./100 g./24 hr.)	Plasma concentration (m-equiv./l.) Cl⁻	Na	K	Muscle (ml./100 g.) Water content	Extracellular fluid phase	Intracellular fluid phase
17	1	7·5	Nil	2·2						
	2	6·5	1·0	1·8						
	3	6·0	0·8	2·3						
	4	6·0	0·7	2·0	112·7	146·5	6·3	75·6	17·3	58·3
	5	7·0	0·5	2·7						
	6	6·5	0·6	2·3						
18	1	8·2	Nil	2·2						
	2	6·1	3·0	2·1						
	3	2·0	3·9	2·0						
	4	3·5	5·5	2·7	92·4	130·0	5·1	77·0	23·7	53·3
	5	3·0	3·0	1·5						
	6	3·0	4·3	2·1						

Table 4. *Water diuresis in starved and dehydrated rats*

(Each dose of water was 5 % of the body wt. and was administered by stomach tube.)

Time after water administration (min.)	Urine output (% of administered dose) 60 normal rats	10 starved rats with water *ad lib.* After 3 days	After 6 days	12 rats dehydrated for 6 days First dose of water	Second dose of water	Third dose of water
15	Nil	Nil	Nil	Nil	Nil	Nil
30	1·9 ± 0·50	,,	,,	,,	,,	,,
45	10·0 ± 0·82	1·9 ± 0·71	,,	,,	,,	,,
60	22·4 ± 1·54	7·4 ± 2·59	6·4 ± 1·31	,,	,,	8·0 ± 2·53
75	37·0 ± 1·83	17·4 ± 4·00	15·2 ± 2·45	,,	,,	16·0 ± 4·36
90	52·4 ± 2·00	29·0 ± 7·02	22·1 ± 3·55	,,	,,	19·0 ± 5·02
105	69·4 ± 1·81	37·9 ± 7·13	30·3 ± 5·70	,,	,,	26·0 ± 5·20
120	81·9 ± 0·94	44·0 ± 8·24	37·5 ± 7·09	,,	,,	30·0 ± 7·57

administration in rats starved for 3 and 6 days: after only 3 days of starvation, and in spite of the fact that the animals had water *ad lib.*, there was a delay in the onset of the diuresis, and a very marked decrease of the urinary volume. Ninety minutes after water administration the amount of urine excreted amounted to $29{\cdot}0 \pm 7{\cdot}02\,\%$ of the water given as compared with $52{\cdot}4 \pm 2{\cdot}00\,\%$ in controls (Table 4).

This finding and the fact that the samples of urine excreted during the period of experimentation had a relatively high specific gravity (Table 1) suggest that the water retention, and hence the tissue oedema might be, directly or indirectly, of renal origin.

Rats fed on a dry diet, without access to water

During the first 24 hr. the intake of food was normal, i.e. between 15 and 20 g./animal, yielding between 50 and 60 cal./day, which compared well with controls; but from the second day, the amount of food eaten fell sharply, and from the third to the sixth day all the animals refused to eat. During the last 2 days the rats became extremely nervous and restless, biting the wiring of their cage and trying to escape.

The urinary volume excreted/24 hr. decreased progressively from the first to the sixth day. Table 5 shows a typical experiment on two rats. The analyses of the urine samples reported in Table 5 are those of the second, fourth and sixth days. The most striking feature was the disappearance of chloride in the urine, in spite of the fact that the specific gravity remained high (Table 6). This disappearance of urinary chloride cannot be explained by a decrease in the concentration of plasma chloride (Table 5), nor is it the result of a failure in the ability of the kidney to concentrate (Tables 5 and 6). However, from the fact that on the sixth day the concentrations of chloride, sodium, potassium and urea were higher in the plasma of these rats than in controls, it may be assumed that the glomerular filtration rate of the kidneys was decreased.

After 6 days without water, the plasma water content of rats amounted to $91{\cdot}1 \pm 0{\cdot}10\,\%$ (Table 2), and their chloride, sodium and potassium concentrations were significantly higher than in controls ($t = 7{\cdot}630$, $P < 0{\cdot}001$; $t = 2{\cdot}037$, $P = 0{\cdot}05$ and $t = 6{\cdot}738$, $P < 0{\cdot}001$, respectively). The total water content of muscles in this series of thirsting rats amounted to 73·5 ml./100 g. fat-free tissue, which was

Table 5. *Effects of withdrawal of water on the composition of urine and plasma of rats*

(On the second, third and fourth days the urine was collected over a period of 24 hr.)

Day of observation		Vol. (ml.)	Cl^- (g./100 ml.)	Na (g./100 ml.)	K (g./100 ml.)	NH_3 (g./100 ml.)	Urea (g./100 ml.)
			Male rat (initial wt. 298·5 g., final wt. 260·0 g.)				
Second	Urine	6·65	0·283	0·332	1·023	0·889	2·705
Fourth	Urine	2·90	0·064	0·115	1·421	2·370	9·350
Sixth	Urine	1·80	0·011	0·086	1·591	5·785	9·075
Sixth	Plasma	—	0·327	0·318	0·028	—	0·108
			Male rat (initial wt. 272·5 g., final wt. 190·0 g.)				
Second	Urine	4·5	0·476	0·290	0·984	1·036	2·857
Fourth	Urine	2·6	0·429	0·447	1·635	1·580	11·475
Sixth	Urine	2·5	0·015	0·190	1·787	4·350	8·325
Sixth	Plasma	—	0·312	0·307	0·030	—	0·129

Table 6. *Effects of dehydration and of subsequent administration of water on the body weight and volume and composition of urine of rats*

(The rats were deprived of water from day 1 to day 6, and free access to water was allowed on day 7.)

Day	Average body wt. (g.)	Average wt. of food eaten (g.)	Average vol. of water drunk (ml.)	Urine				
				Average vol. excreted (ml./24 hr.)	Sp.gr.	Cl^- (g./100 ml.)	Na (g./100 ml.)	K (g./100 ml.)
1	200	—	—	—	—	—	—	—
2	181	19·0	Nil	8·8	1·060	0·390	0·304	0·749
3	171	6·5	,,	2·5	1·060	0·224	0·201	0·806
4	159	Nil	,,	2·6	1·060	0·171	0·103	0·826
5	154	,,	,,	1·2	1·060	0·045	0·036	0·944
6	149	,,	,,	0·8	1·059	0·043	0·020	0·713
7	164	,,	21·0	3·5	1·042	0·021	0·017	0·720

significantly lower than that of controls ($t = 3{\cdot}818$, $P < 0{\cdot}001$). The loss of muscle water affected both the extracellular and the intracellular fluid phases, but not in the same manner. In spite of an increase in its ionic concentration (Table 2), the extracellular fluid phase, expressed in terms of chloride space, decreased from an average of 16·7 to 15·6 ml./100 g. fat-free tissue ($t = 2{\cdot}026$, $P < 0{\cdot}05$). The hypertonicity of the extracellular fluid was checked by the concurrent loss of water from the intracellular fluid phase, which fell from an average of 59·3 ml. in control animals to 57·5 ml./100 g. fat-free tissue ($t = 2{\cdot}015$, $P = 0{\cdot}05$). The magnitude of this water loss can be best estimated by comparing the concentration of water of the intracellular phase in the present series of rats with that of normal rats; in normal animals, the intracellular water concentration amounted to 71·2 ml./100 g. but was only 68·2 ml./100 g. in dehydrated rats ($t = 4{\cdot}225$, $P < 0{\cdot}001$). The concentration of intracellular potassium followed that of water and decreased from 97·2 to 87·3 m-equiv./kg. muscle (Table 2). It is thus likely that while the extracellular fluid phase constituted the first line of defence against dehydration, intracellular water must have been made available to avoid an increase in the ionic concentration of the body fluid which would have been fatal to the animal.

The extent of the state of dehydration in this series of rats could be indirectly assessed by observing the amount of water needed to induce a water diuresis. Table 4 shows that in a series of 12 rats, which had been dehydrated for 6 days, the administration of 5 % of their body weight of water failed to produce a water diuresis. Three hours later a second administration of the same amount of water also failed. A third administration of the standard amount of water, however, produced a moderate urinary excretion.

This finding led to the question: Was all that water (15 % of their body weight) used to reduce the increased osmotic pressure of the body fluids, or was its retention in the tissues partly the result of the failure of the kidneys to excrete it? In the following experiment a group of six rats, each weighing about 200 g., was kept in a metabolism cage for 6 days. During the first 5 days they were given dry food but no water; on the sixth day food was withheld but water allowed. During the first 24 hr. of observation, they ate an average of 19·0 g. of dry food/rat; on the second day, the average quantity of food eaten amounted to 6·5 g. only; from then on, all the animals refused to eat (Table 6). The urine was collected daily. Table 6 shows the average volume of urine excreted/24 hr./animal, its specific gravity and the concentration of chloride, sodium and potassium; it gives also the average amount of water drunk/rat during the last 24 hr. In spite of an average amount of 21·0 ml. being drunk (representing 13 % of the body weight) the average urinary volume excreted did not exceed 3·5 ml., i.e. 2·1 % of the body weight (Table 6). Furthermore, there were no signs of increased excretion of chloride, sodium or potassium. The sudden increase of the average body weight indicated clearly that most of the water drunk had been retained by the tissues (Table 6). When killed, a gross post-mortem examination showed that the intestinal tract was oedematous, and that the muscles and liver were abnormally 'wet'.

Further investigations showed that the plasma water content and plasma protein concentration were normal (Table 2), but that the plasma concentration of chloride and of sodium were significantly decreased when compared with normal ($t = 2{\cdot}060$, $P < 0{\cdot}05$ and $t = 2{\cdot}988$, $P < 0{\cdot}01$), while the plasma concentration of potassium had returned to normal values (Table 2).

The total water content of liver and of muscle was markedly increased and exceeded significantly that of normal rats ($t = 5{\cdot}164$, $P < 0{\cdot}001$ and $t = 6{\cdot}533$, $P < 0{\cdot}001$). Examining the partition of the water in muscle, it could be shown (Table 2) that the increase of the total muscle water was mainly the result of an increase of its extracellular fluid phase. The intracellular fluid phase, however, remained comparable to that of dehydrated animals, though its concentration of water returned to normal values (Table 2). It can thus be concluded that the increased osmotic pressure of the body fluid in dehydrated rats was not the only factor opposing further loss of body water; when these animals were allowed water, the amount of water drunk exceeded that required to bring the enhanced ionic concentration back to normal values. As this excess of water load was not excreted by the kidneys it resulted in a marked tissue oedema. It is, therefore, likely that during dehydration of animals the renal mechanism of water excretion intervenes directly or indirectly in the preservation of body water.

DISCUSSION

In the two series of rats investigated, those which were starved but had ample supplies of water, and those allowed a dry diet but no water, the first developed an early tissue oedema, the others did not. Though some food was eaten during the 2 first days, the rats of the second series soon refused to eat and starved for the last 3–4 days of the experiment. It may, therefore, be assumed that both series suffered from a comparable degree of starvation, but that they differed in that one was allowed access to water and the other not.

According to accepted theories, the mechanism of water preservation in dehydrated animals can be

represented as follows. At the beginning, the extracellular fluid phase is kept normal by transfer of water made available from the intracellular phase, where it has been released by the consumption of protoplasm incidental to fasting (Gamble, 1947). This shift of water to the extracellular fluid phase is accompanied by an extrusion of the intracellular base, potassium (Elkinton & Winkler, 1944; Heller, 1949). As the dehydration proceeds, water is drained from the extracellular fluid phase, with the result that the latter tends to become hypertonic (Elkinton & Taffel, 1942; Winkler, Elkinton, Hopper & Hoff, 1944). This in turn produces more transfer of water from the intracellular into the extracellular fluid phase. The maintenance of the extracellular fluid phase, within limits compatible with the survival of the organism, is thus ultimately provided at the expense of the intracellular phase (Table 2). This interpretation, derived from the estimation of body fluid lost, and calculated from changes in body weight and from the urinary excretion of sodium, potassium and nitrogen, is based entirely on the assumption that the renal function remains normal.

It has repeatedly been demonstrated that during advanced dehydration there is a functional renal failure (see McCance, 1936) leading to retention of crystalloids. The results of the present series of experiments show that dehydration in rats produced ultimately a retention of urea, potassium, chloride and sodium in the blood, suggesting impairment of renal function. Furthermore, Gilman & Goodman (1937) have shown that the urine of dehydrated rats contains significant amounts of an antidiuretic substance, which they assumed to be similar to 'vasopressin'. It seems likely, therefore, that the attempt to explain the shifts of water during dehydration by a purely physical mechanism, like osmotic pressure, omits one important factor, viz. the influence of the kidney.

This hypothesis of a renal intervention is supported by the results of the present experiments where water was allowed to rats which had been so deprived for 5 days. Adolph (1947) claimed that, when water was again offered to rats which had been entirely deprived of it for several days, only a 'small excess' of water was ingested. In contrast with these findings it could be shown that the amount of water drunk in 24 hr. by rats which had been previously dehydrated for 5 days amounted to 13% of their body weight. (Controls drink on the average 4·5% of their body weight of water in 24 hr.) The corresponding urinary volume, however, amounted only to just over 2% of their body weight as against 5% in normals. The marked discrepancy between the amount of water drunk and that of urine excreted resulted in a sudden increase of body weight of nearly 10% (Table 6). Furthermore, it could be shown that the total water content of the liver and muscle of the test rats exceeded significantly that of normal rats, and that there was a marked increase of the extracellular fluid of muscle, i.e. clear symptoms of tissue oedema (Table 2).

Had the osmotic pressure been the only factor responsible for the regulation of the volume of body fluid in dehydrated animals, it would be difficult to understand why the regulation failed when the rats were allowed to drink. Besides the mechanism of hypertonicity, there must have been one which opposed the renal excretion of the excess water load. From the results presented there is evidence that, both the rate of glomerular filtration, and that of tubular water reabsorption were affected. It was, however, outside the scope of this work to determine the factor that produced both a decrease of the rate of glomerular filtration and an increase of that of tubular water reabsorption.

In contrast with dehydrated animals, rats which were starved, but allowed free access to water, developed signs of tissue oedema. As early as 3 days after the beginning of the experiment, the chloride space of muscle was significantly greater than that of normal rats (Table 2). The objection might be raised that to equate chloride space with extracellular fluid phase holds only for normal rats, where chloride and sodium occupy a comparable volume of distribution (Dicker, 1948), and that in starved animals there might be changes in the cell permeability which would account for changes in the chloride distribution. However, the fact that chloride occupied a comparable fraction of the muscle water in normal and starved rats suggests that chloride space can be assumed to give some measure of the extracellular fluid phase in muscle. It must be remembered, however, that chloride space is likely to be somewhat larger than the true value of the extracellular space, even in normal animals (Fulton, 1947).

As these rats were deprived of food, it could be assumed that the decrease of the intracellular fluid phase observed in this series (Table 2) was the result of a loss of water, released from the cells as a consequence of the degradation of proteins incidental to fasting. This loss of intracellular water was accompanied by a loss of cell potassium, which proceeded in spite of the fact that these animals could, by drinking, maintain an apparently normal state of hydration. Furthermore the loss of intracellular water seemed to be independent of the amount drunk.

The extracellular fluid phase, on the other hand, was not only increased, but the magnitude of the increase was directly correlated with the amount of water drunk (Table 3). This suggests at once a failure in the regulation of water excretion in these

rats, as is also indicated by the following facts: (*a*) the urinary volume did not bear any relation to the amount of water drunk; (*b*) in spite of the fact that water was freely obtainable, the specific gravity of the urines remained much higher than in normal rats which had free access to food and water; (*c*) following the administration of a standard amount of water, the onset of urinary excretion was delayed and its total volume diminished.

Comparing the results obtained in starved rats having free access to water with those in rats without water the following conclusions could be reached: (*a*) prolonged starvation, up to 6 days, leads to tissue oedema when water is drunk; (*b*) in starved animals, allowed free access to water, there is a direct relation between the amount of water drunk and the magnitude of the extracellular fluid phase, expressed in terms of chloride space; (*c*) the onset of tissue oedema in starved rats with access to water does not bear any relation to the plasma protein concentration, and seems mainly to be the result of a failure in the mechanism of water excretion.

SUMMARY

1. Total water content of plasma, muscle and liver and the chloride and the sodium space of muscle were estimated in two series of rats: (*a*) those kept for 3 or 6 days without food but with free access to water; (*b*) those allowed dry food but no water for 6 days.

2. Group (*a*) developed an early tissue oedema, and after only 3 days the extracellular fluid phase of their muscle, expressed in terms of chloride space, was significantly increased. When water equal to 5% of their body weight was administered, the onset of the water diuresis was delayed, and the volume of urine excreted in 2 hr. was lower than in normal rats.

3. Rats allowed a dry diet but no water for 6 days (group (*b*)) showed retention of chloride, sodium, potassium and urea in the plasma, accompanied by a decrease of the intracellular and the extracellular fluid phase of the muscles. Administration of water (as above) failed to produce a water diuresis. When offered water *ad lib.* after 5 days of water deprivation, an amount equal to 13% of their body weight was drunk and the animals developed tissue oedema; the total water content of the liver and muscle was significantly increased over that of normal animals, and so was the extracellular fluid phase of muscle. The plasma protein concentration, however, remained normal.

The expenses of this investigation were partly defrayed by a grant from the Colston Research Committee, whose help is gratefully acknowledged. The author expresses his appreciation of the technical assistance of Miss P. A. Ashby.

REFERENCES

Adolph, E. F. (1947). *Amer. J. Physiol.* **151**, 110.
Dicker, S. E. (1948). *Biochem. J.* **43**, 444.
Elkinton, J. R. & Taffel, M. (1942). *J. clin. Invest.* **21**, 787.
Elkinton, J. R. & Winkler, A. W. (1944). *J. clin. Invest.* **23**, 93.
Fisher, R. A. (1944). *Statistical Methods for Research Workers*, 9th ed., p. 114. London: Oliver and Boyd.
Fisher, R. A. & Yates, F. (1943). *Statistical Tables for Biological, Agricultural and Medical Research*, 2nd ed., p. 30. London: Oliver and Boyd.
Folin, O. & Bell, R. D. (1917). *J. biol. Chem.* **29**, 329.
Fulton, J. F. (1947). *Howell's Textbook of Physiology*, 15th ed., p. 940. London: W. B. Saunders.
Gamble, J. L. (1947). *Chemical Anatomy, Physiology and Pathology of Extracellular Fluid.* Cambridge, Mass.: Harvard University Press.
Gilman, A. & Goodman, L. (1937). *J. Physiol.* **90**, 113.
Hastings, A. B. & Eichelberger, L. (1937). *J. biol. Chem.* **117**, 73.
Heller, H. (1940). *J. Physiol.* **98**, 1*P*.
Heller, H. (1949). *J. Physiol.* In the Press.
Lee, M. H. & Widdowson, E. M. (1937). *Biochem. J.* **31**, 2035.
McCance, R. A. (1936). *Lancet*, i, 37.
McCance, R. A. & Shipp, H. L. (1933). *Spec. Rep. Ser. med. Res. Coun., Lond.*, **187**, 35.
Scott, L. D. (1940). *Brit. J. exp. Path.* **21**, 93.
Volhard, J. (1878). *Z. anal. Chem.* **17**, 482.
Winkler, A. W., Elkinton, J. R., Hopper, J. & Hoff, H. E. (1944). *J. clin. Invest.* **23**, 103.